UNIVERSITY OF TROMSØ UIT

INSTITUTE OF COMMUNITY MEDICINE, UNIVERSITY OF TROMSØ
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Functional ultrasound of the anal canal

The Effect Of Pregnancy And Childbirth

Ingrid Petrikke Olsen

A dissertation for the degree of
Philosophiae Doctor

2011

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HELSE FINNMARK
FINNMÁRKKU DEARVVAŠVUOHTA

HELSE NORD

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Conflicts of interest: B-K Medical lent me the 10 MHz endoanal transducer 2050.

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Acknowledgements

The seed for this study was sown during my meetings with women whom had been secretly suffering from anal incontinence for many years. As a young resident, these women's stories made a strong impression on me. Even though this study does not solve the problems of anal incontinence, I hope it can be a contribution towards removing the taboo in society regarding understanding the continence mechanism and development of diagnostic tools.

This work has been carried out parallel to my specialist training to be a gynaecologist. I am extremely grateful to my supervisor Professor Torvid Kiserud, Department of Obstetrics and Gynecology, Haukeland University Hospital, whose skills are numerous. During the years we have worked together, he has demonstrated qualities as patience and flexibility, as well as the ability to supervise me at my current scientific level. My co-supervisor and statistician Tom Wilsgaard, Institute of Community Medicine, University of Tromsø, has had the difficult job of helping me understand basic statistics; very often by telephone and always at short notice. Thank you!

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This work was generous supported financially by the research grant of Helse Finnmark and Helse Nord. Though, I have to admit that there have been major problems related to lack of compatible software between the University of Tromsø and Helse Finnmark, stealing time from research.

My family explicitly told me not to mention them in this acknowledgement. They claim that they have seen more of me in periods of research than in periods of clinical work. Their statement reinforces how my colleagues Julia, Jes, Kevin and Siv-Elin have been very flexible and given me their support every day. In addition, Kevin has kindly revised my English spelling. I also want to thank fellow researchers at Hammerfest Hospital for valuable discussions and suggestions for improvements.

Finally, my most sincere "thank you" goes to the participants of these studies. You have actually travelled 10 876 kilometres along the roads in Finnmark, winter and summer, your main purpose being to help me with my research. Some of you travelled these great distances over perilous roads during pregnancy, completing up to four visits. The greatest distance made by one traveller alone was an amazing 1744 kilometres!

Hammerfest, March. 16th. 2011, Ingrid Petrikke Olsen

Abbreviations

ARC	Ano-rectal curvature
ATLA	Arcus tendineus levator ani
AVD	Ano-vaginal distance
AVA	Ano-vaginal angle
BMI	Body mass index
cm	centimeter
CTP	Centrum tendineum perinei
ECMUS	European committee for medical ultrasound safety
EAS	External anal sphincter
IAS	Internal anal sphincter
ICM	Iliococcygeal muscle
IS	Internal anal sphincter
2D	Two-dimensional
3D	Three-dimensional
M	Anal mucosa
MHz	Megahertz
mm	millimetre
MRI	Magnetic resonance imaging
PAM	Puboanal muscle
PB	Perineal body
PBR	Puborectal muscle
PPM	Puboperineal muscle
PRM	Puborectal muscle
PVM	Pubovaginal muscle
R	Rectum
SAC	Sacral promontory
SD	Standard deviation
TPS	Transversus perinei superficialis
VT	Vaginal transducer

Terms of direction and positions

The standard anatomical position, i.e. the human body standing erect, feet together and arms at sides with thumbs pointing away from the body, is used when anatomical structures and their relationship to each other are described.

Midsagittal plane:
The plane through the middle of the body dividing it into a right and left half.

Coronal/frontal plane:
The plane that divides the body in a ventral and dorsal part.

Axial/transverse plane:
The plane that divides the body into an upper and lower part.

Distal/proximal:
Usually used for descriptions of the limbs, but can also be used about different canal systems in the body where it refers to the beginning of (proximal) and end of (distal) the canal. In this study the expressions are used in interpretations of the structures in the ultrasound acquisitions.

Anterior/posterior:
In front and behind, respectively.

Cranial/caudal:
Towards the head and towards the feet, respectively.

(Dahl H 1976; Taylor A.N 1990)

Abstract

The anatomy and mechanism of anal continence is not fully understood, but the ability to squeeze is an important voluntary component of continence. Pregnancy affects continence, but the natural development of the anal canal during pregnancy and childbirth is not known. Compared with endoanal ultrasound, the hypothesis was that the transvaginal technique adds new information on structures and possibility of testing functions. **Aims:** 1) Assess the reproducibility of 3D vaginal and 3D endoanal ultrasound and compare the two techniques. 2) Determine the dimension and direction of the anal canal, including the anal mucosa during rest and voluntary squeeze. 3) Determine the development of the anal canal during pregnancy and the postpartum period, during rest and squeeze. **Material and methods:** 30 women with varied obstetrical backgrounds were recruited to a cross-sectional study of the anal canal using 3D endoanal ultrasound (study I and II). Another group of 20 healthy nulliparous women were recruited for a cross-sectional study comparing 3D endoanal and 3D vaginal ultrasound techniques (study III). Finally a group of 23 nulliparous women were examined at 18, 28 and 36 weeks of pregnancy, and three months postpartum using 3D vaginal ultrasound (study IV). Age, BMI and anal incontinence score were noted for all participants at all visits, and information of the delivery was noted. The ethical committee had approved the studies. Statistical analysis included methods for intra- and inter-observer variation, t-tests and linear mixed models. In all analyses a two-tailed p-value less than 5 was considered statistically significant. **Results:** Endoanal- and vaginal ultrasound techniques assessed anal length and sphincter volumes differently ($p<0.001$). The intra-class correlation coefficients for the intra-observer variation were 0.60 – 0.96 for both techniques while inter-observer variation showed higher variability (study II and III). In the women with varied obstetrical backgrounds, the anal length and the external anal sphincter length and volume were less in the subgroups who had delivered. During voluntary squeeze the volume of the internal anal sphincter became smaller in the subgroup that had had a complicated vaginal delivery. Endoanal ultrasound visualised the mucosa as a thin layer around the transducer, and direction and curvatures of the anal canal could not be appreciated (study I). Using vaginal ultrasound, the anal mucosa was measured to constitute 40% of the total anal complex. In addition, the variation of the anal direction and bowel curvatures was assessed. Voluntary squeeze caused a distorted mucosa at the ano-rectal junction, increased bowel bend, increased angle between the anal canal and vagina, and elongation of the canal (study III). The impact of pregnancy was an elongation of the canal and 20 % increase in volume and much of the effects of voluntary squeeze were maintained. Post partum the length and volumes returned to the level found at 18 weeks of pregnancy (study IV). **Conclusion:** While endoanal ultrasound is a well-established method of assessing the anal sphincters, we have shown that the transvaginal technique provides valuable additional information. It presents less distorted details of the shape, position and dimensions of the anal structures in standard rest position, and permits functional studies such as during squeeze. In contrast to the endoanal technique the transvaginal scan provides a complete record of the volumes of the anal canal, including the mucosa that constitutes 40 % of the anal structures. When using this technique, we also demonstrated a transient elongation of the anal canal, together with a 20 % increase in volume during the second half of pregnancy. During squeeze, the bending and angulation were less prominent than in the nulliparous women. Vaginal 3D ultrasound is a promising method for functional studies, beyond the capacity of the 3D endoanal ultrasound technique.

List of articles

This dissertation is based on the following articles:

I: Olsen I.P, Augensen K, Wilsgaard T, Kiserud T. "Three-dimensional endoanal ultrasound assessment of the anal sphincters during rest and squeeze." *Acta Obstet et Gynecol* 2008; 87: 669-674.

II: Olsen I.P, Augensen K, Wilsgaard T, Kiserud T. "Three-dimensional endoanal ultrasound assessment of the anal sphincters: Reproducibility." *Acta Obstet et Gynecol* 2008; 87: 675-681.

III: Olsen I.P, Wilsgaard T, Kiserud T. **"**Transvaginal three-dimensional ultrasound: a method of studying anal anatomy and function" *Ultrasound Obstet Gynecol* 2011; 37: 353-360.

IV: Olsen I.P, Wilsgaard T, Kiserud T. "Development of the anal canal during pregnancy and the postpartum period – a longitudinal and functional ultrasound study." Submitted February 2011.

1. Introduction

> *"There is no considerable muscle in the body whose form and function are more difficult to understand than those of the levator ani, and about which such nebulous impressions prevail. "* Dickinson R.L, 1889

Ultrasound of the genital structures was introduced in the end of the 1950's and beginning of 1960's but the quality did not allow detailed imaging (Donald I 1958 and 1963). Defecography was first described in 1964 (Burhenne H.J 1964) and the ano-rectal angle in 1985, using radiology (Bartram C.I 1985). Computer tomography was introduced in the late 1970's (Steinbrich W 1979) and imaging of the soft tissues in the pelvic became possible. The first indication for pelvic floor ultrasound was the assessment of bladder neck mobility and details about the urethral sphincter related to urine incontinence (Kohorn E.I 1986; Grischke E.M 1986; Gordon D 1989; Schaer G.N 1995). Since this early beginning, description of ultrasound imaging of the pelvic floor has been published, detailing anatomical structures and emphasizing functional aspects of the anterior compartment (Dietz H.P 2004 a, b and 2010). MRI was introduced in 1991 (Hugosson C 1991). Endoanal ultrasound or MRI of the pelvic floor is today a recommended diagnostic tool in evaluations after birth-associated sphincter tears (Royal College of Obstetricians and Gynaecologists 2007). Recently developed high–resolution probes have further enhanced the possibility to study anatomical structures in a more detailed level.

This thesis aims to amplify the present knowledge about the anatomy of the female posterior pelvic floor as it is visualised on endoanal- and vaginal ultrasound acquisitions, and describe the natural development in the posterior compartment during pregnancy and after childbirth. The functional aspects are studied in acquisitions assessed during the voluntary squeeze-manoeuvre.

1.1 Anatomy of the female pelvic floor

Dissection studies have shown that the pelvic floor has the shape of a funnel (Gray H 2000; Fritsch H 1994). However, in vivo MRI studies describe it more as vault shaped, as seen in the diaphragm dividing the chest from the abdomen (Hugosson C 1991) and change form during contraction and valsalva (Hjartardottir S 1997).

1.1.1 Anterior compartment

The female pelvic floor consists of two muscle layers, which are inserted to the bony pelvic ring, and converge around the natural openings of the urethra, the vagina and the anal canal. The caudal, and rather thin, muscle layer is named the urogenital diaphragm (Figure 1). As the name implies, it is sited in the anterior compartment of the pelvic and surrounds the urethra and vagina. It consists of transverse perinei- superficialis and profundus muscles.

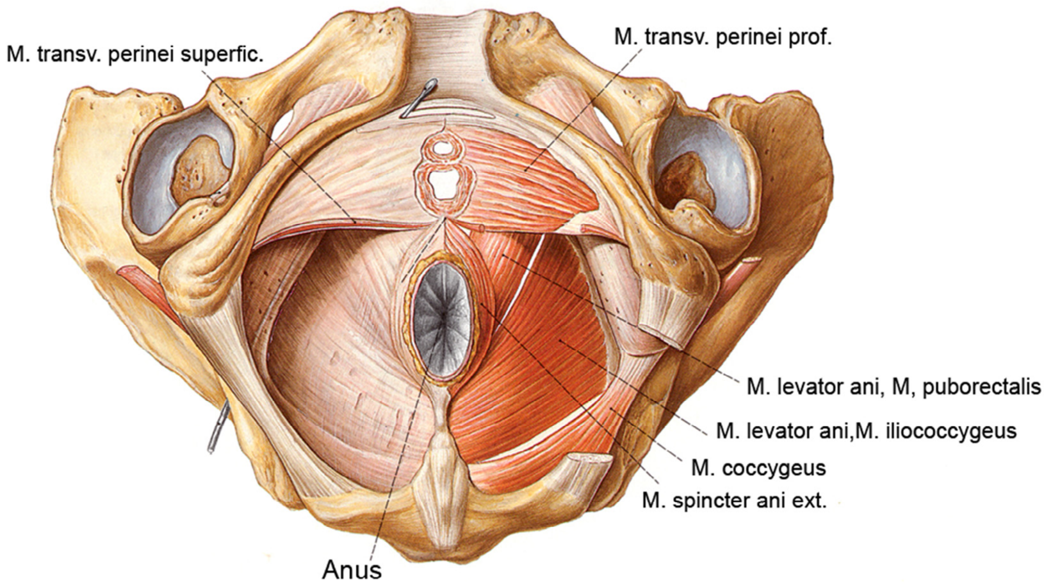

Figure 1: *The female pelvic floor from below. (Copyright requested)*

1.1.2 Posterior compartment

The more cranial layer is named the pelvic diaphragm and consists of the coccygeus and levator ani muscles. The terminology of the levator ani muscle is a challenge. In 2004 Kearney performed a literature study and found 16 different and overlapping names on the different parts of the levator ani muscle (Kearney R 2004). Despite this divergence in terms, the agreement of the offspring and insertion of the structures was found to be rather good, and Kearney introduced a terminology after origin and insertion (Figure 2).

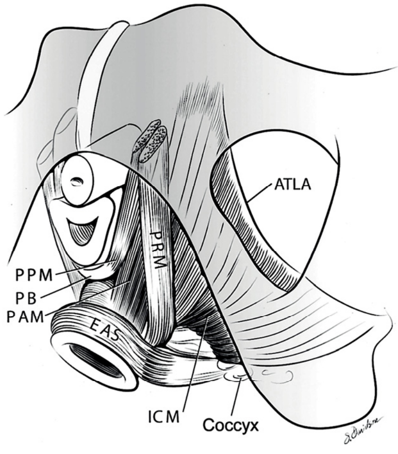

Figure 2: *Schematic view of the levator ani muscles from below after the vulvar structures and perineal membrane have been removed showing the arcus tendineus levator ani (ATLA); external anal sphincter (EAS); puboanal muscle (PAM); perineal body (PB) uniting the 2 ends of the puboperineal muscle (PPM); iliococcygeal muscle (ICM); puborectal muscle (PRM). Note that the urethra and vagina have been transected just above the hymeneal ring.*

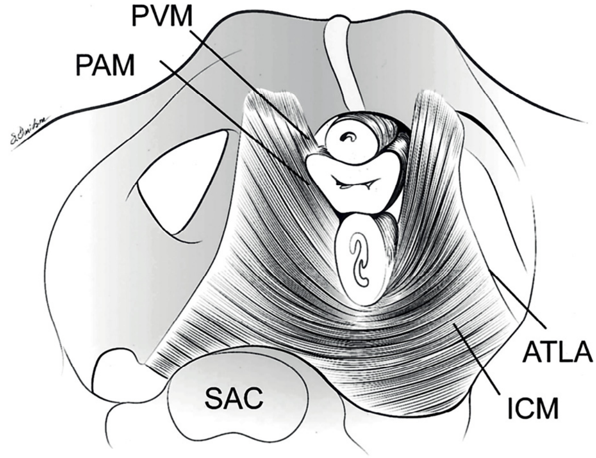

Figure 3: *The levator ani muscle seen from above looking over the sacral promontory (SAC) showing the pubovaginal muscle (PVM). The urethra, vagina, and rectum have been transected just above the pelvic floor. PAM = puboanal muscle; ATLA = arcus tendineus levator ani; and ICM = iliococcygeal muscle. (The internal obturator muscles have been removed to clarify levator muscle origins.)*

The nomenclature given in Sobotta (figure 1) and the terminology from Kearney (figure 2) merge well. According to Sobotta, the levator ani muscle consists of three muscles: the iliococcygeal-, the puborectal- (PBR) and the pubococcygeal-muscle. Kearney proposed a division of the pubococcygeal muscle into puboperineal-, pubovaginal- and puboanal- muscle, the latter described as an inter-sphincteric groove between external- and internal sphincter, figure 3.

The puborectal muscle is open anteriorly and has a left and right arm that continues on either side of the bowel, vagina and urethra, to be inserted in the symphysis pubis as the anterior part of the arcus tendineus levator ani (Whitehead W.E 1987; Hjartardottir S 1997) and figure 4. Inside the lateral and lower section of the pelvic ring, the obturatorius muscle is sited on both sides. The space between the levator ani muscle, the posterior bony pelvic and musculus gluteus maximus is called fossa ischiorectalis and is filled with fat (Taylor A.N 1989; Gray H 2000). Women have different strengths in the pelvic floor muscles and its supportive function varies between individuals and thereby interferes with the position of the anal canal (Jorge J.M 1993; Hjartardottir 1997).

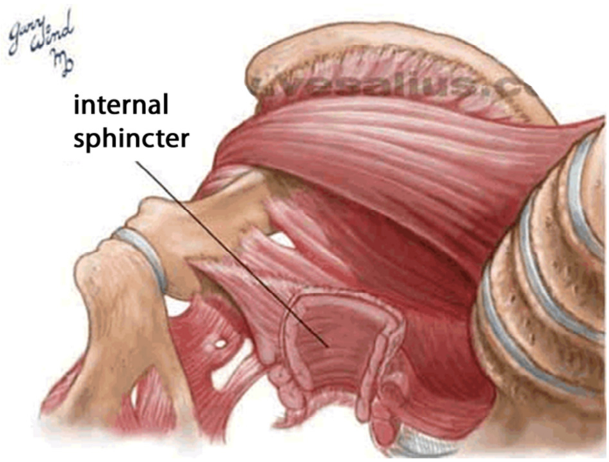

Figure 4: *A sagittal left view that illustrates how the anal canal is anchored in the female pelvic floor muscles (www.vesalius.com 2011).*

The external anal sphincter (EAS) is the caudal and outermost part of the striated muscles in the pelvic floor. In the posterior and cranial direction it merges with the puborectal muscle and is attached to os coccygeus. Anteriorly it integrates to the lower level of the urogenital diaphragm and the muscles around the vaginal wall; the bulbospongiosus muscle, according to Sobotta, or the puboperineal muscle, according to Kearney. This meeting point is named centrum tendineum perinei (CTP) or perineal body, respectively (figure 1 and 2).

1.1.3 The anal canal

The anal canal consists of the external anal sphincter (EAS), which is a striated muscle with somatic nerve supply, and the internal anal sphincter (IAS) which is a smooth muscle with autonomous nerve supply. It is controversial whether EAS consists of two or three bundles; hence an agreement exists that the most cranial bundle blends with the puborectal muscle (Oh. C 1972; Taylor A.N 1989). The IAS continues as a smooth muscle layer that surrounds the wall of the bowel into the rectum and beyond. Both the IAS and EAS are circular and overlap each other such, so that only the EAS is most caudal in the canal, and opposite only the IAS in the cranial part of the canal. Innermost and surrounded by the IAS and EAS is the anal mucosa with longitudinal folds. Looking at the cylindered anal canal from below, a description according to a clock's dial can be given. If the patient lies in a standard gynaecological position 12 o'clock is anteriorly towards the vaginal opening, 6 o'clock against the os coccygeus and 3 and 9 o'clock at left and right side, respectively.

1.1.4 Blood- and nerve supply

The blood supply to the pelvic floor is mainly by the internal pudendal artery, but branches from the rectalis inferior artery supply the levator ani muscle. Next to the veins and arteries, branches from the pudendal nerve (S2-S4) run and innervate the striated pelvic muscles. In addition, the sphincters are innervated from Onuf's nucleus, probably with both somatic and autonomic nerve fibers (Dubrovsky B 1989). Branches from the hypogastric plexus (the autonomic nervous system) innervate the IAS (Kneist W 2010) and it is believed that parasympathetic nerve fibers stimulate relaxation of the IAS, allowing bowel content to enter into the anal canal (Zbar A.P 2000).

In this thesis the further anatomy discussions are based on the nomenclature of Sobotta's Atlas of Human Anatomy (Taylor A.N 1989).

1.2 The continence mechanism

The pelvic floor and the structures in the anal canal are important for maintaining continence for faecalia and flatus (Nelson R 1995). The reservoir function of the rectum secures the emptying of the bowel in a socially acceptable situation. The smooth IAS muscle amounts for 80 % of the pressure found in the continent anal canal (Freckner B 1975; Schweiger M 1979). When stretching on the bowel wall exceeds a certain level, the recto-anal inhibition reflex releases and the IAS relaxes (Mavrantonis C 1998; Örnö A-K 2005). EAS is only partly contracted during rest. Every 15 minutes it contracts and a reversed peristaltic movement arises (Mavrantonis C 1998). During defecation the contraction of EAS is inhibited and the bowel content can pass. In situations where the abdominal pressure rises, for instance when coughing, there is a reflex from the spinal cord which maintains the contraction of the EAS (Pemberton J.H 1986; Sun W.M 1990; Madoff R.D 1992). The anal reflex mechanism is released if the skin around the anal entrance is touched (Whitehead W.E 1999).

The puborectal muscle lies like a sling beneath the anal canal in the level where the anal canal continues into the rectum. The angle that appears in this junction between the rectum and anal canal, the ano-rectal angle, has been measured on proctograms. In resting position it was 92-103°. If voluntary squeeze was performed, the angle became steeper (figure 5), and obstruction of the outlet of bowel content is seen. In defecation situations (straining) the ano-rectal angle rises to 123-137°, due to the relaxation of the puborectal sling (Mahieu P 1984 a, b; Beer-Gabriel M 2004) and the anal tube becomes straighter making it easier for content to pass. The same tendency was found in MRI-studies and in defectograms (Kruyt R.H 1991; Hjartardottir S 1997). Hence, the ability to squeeze seems to be an important contribution to the mechanism of faecal continence (Bharuda A.E 2006). Amount of content, the consistence, bowel motility, neurological status, inflammatory status of the bowel, effects from medicaments and psychological status are all factors that influence a person's ability to maintain continence.

1.2.1 Role of the anal mucosa in the continence mechanism

The focus on the anal mucosa as a structure of the anal canal is almost absent. A theory that the anal mucosa, as a highly vascular organ, may act as a cushion when the pressure in the anal canal is low in the relaxation state, has been published (Gibbson C.P 1986) but not further explored. There are two reports about the possibility to measure the anal cushions – also called the haemorrhoidal plexus- using vaginal ultrasound (Timor-Tritsch I.E 2005a; Nicholls M.J 2006). In two studies using 2D vaginal - or perineal ultrasound, comments about the mucosal columns filling the anal lumen are aired (Sultan A.H 1994; Örnö A-K 2005).

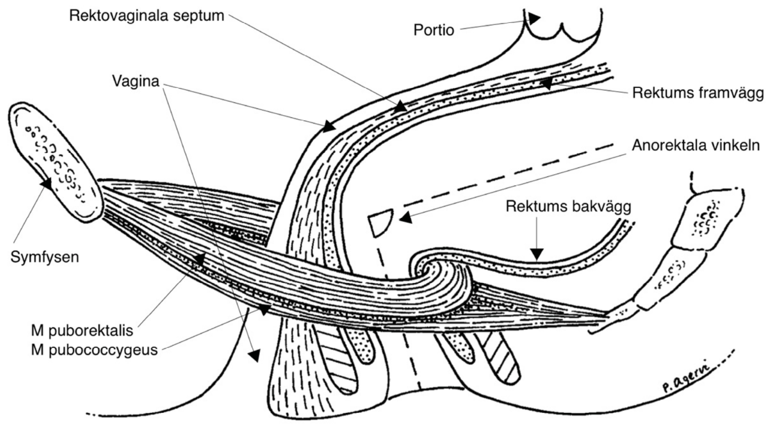

Figure 5: *Illustration of how the puborectal muscle bends the bowel at the ano-rectal junction and consequently the ano-rectal angle varies between contraction and relaxation of the muscle.*

1.3 Alterations of the pelvic floor during pregnancy.

The knowledge of how the pelvic floor is altered during pregnancy and after delivery is limited and usually based on cross-sectional studies. It is known that there is a connection between experienced urine incontinence during pregnancy and later in life, maybe due to denervation of the pelvic floor after delivery (Dolan L.M 2003). Another plausible factor could be increased bladder neck mobility after vaginal delivery, especially after a long second stage and/or an operative vaginal delivery (Peschers U 1996; Dietz H.P 2002; Dietz H.P 2003). A descent of the pelvic floor is common (14 - 46 %) late in pregnancy and indicates that the delivery alone does not constitute the whole risk of genital prolapse (Sze E.H 2002; O`Boyle A.L 2002; Dietz H.P 2006). Hence, vaginal deliveries increase the prevalence of prolapse (Dannecker C 2004; Dietz H.P 2004c) and weaken the lift of the levator ani muscle (Dietz H.P 2004d). In a Best Practice review, Dietz and Wilson state that vaginal delivery can cause damage to the pudendal nerve, the inferior aspects of the levator ani muscle and fascial pelvic organ supports. However, whether such trauma is clinically relevant and important for development of incontinence and prolapse later in life, is uncertain (Dietz H.P 2005).

Epidemiological studies in this field are difficult to perform as the aetiology of the pathophysiological findings of prolapse, urinary- and faecal incontinence is multifactorial. In addition, women’s exposure to risk factors vary during a woman’s life, between delivery units, between age-cohorts and other confounders.

Notwithstanding these variations, pregnancy and childbirth are well documented as major risk factors for prolapse (Carley M.E 1999; Olsen A.L 1997). However, whether this is due to pregnancy and/or delivery is not unequivocal. For urine incontinence, large studies have shown that caesarean section provides only partial protection against stress-urine incontinence (MacLennan A.H 2000; Wilson PD 2002; Rørtveit G 2003) and that this protection may fade with time (Rørtveit G 2003).

2. Ultrasound of the posterior pelvic floor

In contrast to the anterior compartment, where bladder neck mobility and prolapse are explored, the posterior compartment of the pelvic floor has been investigated to a lesser extent as a functional unit. The possibility of using ultrasound for real-time functional tests as demonstrated by Örnö A-K and Mârsál K in 2005 and 2007a, b, is still awaiting further exploration.

However, publications describing the EAS and IAS in detail are numerous (Gold D.M 1999a; Konerding M.A 1999; Williams A.B 2000 and 2001a; Bollard R.C 2002; Williams A.B 2002c; West R.L 2005a; West R.L 2005b; Starck M. 2005). From ultrasound acquisitions, the anal canal in women is found to be 4.2 cm long and shorter than in men, where it is 5.2 cm. The EAS in women is 2-2.5 cm long and shorter at 12 o'clock than at 6 o'clock (Williams A.B 2000; Bollard R.C 2002; Starck M 2005). In men EAS is 3.5 cm in the whole circumference (Williams A.B 2000), and this disparity between genders explains the shorter anal canal in women. Both the EAS and IAS are studied in different groups of women and description of the structures in still-picture are detailed (Williams A.B 2001b; Williams A.B 2002a, b, c; Starck M 2003; Starck M 2005, Timor-Tritsch I.E 2005a). Synonymous for these studies are the usage of MRI or endoanal ultrasound - either 2D or 3D - to visualize the anatomy. In general, the reproducibility studies for these methods report of acceptable intra-observer data but poor inter-observer data (Enck P 1997; Gold D.M 1999b; Williams A.B 2002a; West R.L 2005a,b; Cazemier M 2006; Gregory W.T 2006).

2.1 Ultrasound of the pelvic floor during and after pregnancy

Because ultrasound beams do not harm the foetus and ultrasound equipment is a widely available and low-cost tool, ultrasound examination is excellent for visualisation of the pelvic floor. In a longitudinal study using a perineal ultrasound transducer, the excursion of the ano-rectal angle and the mobility of the puborectal sling between resting and squeeze positions was 20° and 35°, respectively at 38 weeks of pregnancy. The angles were significantly reduced to 10° and 18° three months postpartum (Constantini S 2006). An increased mobility of the urethra after delivery was also seen. Another longitudinal study using perineal ultrasound found no effect on bladder neck mobility before and after postpartum training of the pelvic floor (Meyer S 2001). In contrast, a Norwegian cross-sectional study in pregnant women at 20 weeks and in nulliparous women, found a strong correlation between urine incontinence and a thin diaphragma urogenitalis using a perineal transducer (Mørkved S 2004). Örnö and Dietz studied the effect of the valsalva maneuver, and how co-activation of the levator muscle interfered, in a group of nulliparas at 37 weeks of gestation (Örnö A-K 2007b). Using 3D vaginal transducers, IAS was found to be thinner after completed first-time pregnancy (Ochsenbein N 2001), and suitable for evaluation of perineal anatomy and extent of perineal tears provided that both longitudinal- and transverse sections are available (Örnö A-K 2008). Finally, the incidence of levator avulsions after the first vaginal delivery is found 13 % and 31 % in studies using 3D translabial or perineal ultrasound (Dietz H.P 2010; Blasi I 2011).

> *”The issue of levator trauma, one of the most significant developments in clinical obstetrics since the introduction of fetal monitoring, will take pelvic floor ultrasound from a niche application into the mainstream and speed the convergence of clinical specialities dealing with pelvic floor disorders.”* Dietz H.P 2010

2.2 Vaginal ultrasound

The use of a vaginal probe to visualise the pelvic floor structures is an available and technically easy procedure. It is suitable for visualisation of the anal sphincters, figure 6 and (Stewart L.K. 1999; Timor-Tritsch I.E 2005b).

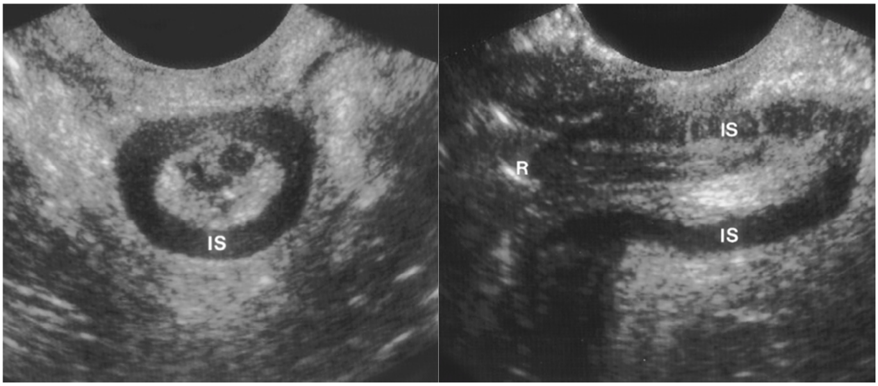

Figure 6: *2D vaginal ultrasound visualising the anal mucosa, IAS (IS), EAS and the ano-rectal junction. The vaginal transducer is placed in the posterior vaginal fourchette. A coronal section from the mid canal level (left) and a sagittal section (right) where also rectum (R) visualise.*

In study III and IV a 7 MHz vaginal biplanar transducer with both a 2.2 cm transverse and 6 cm longitudinal array head were used to achieve 3D acquisitions. The transducer was connected to a handheld mover to avoid artefacts from an unstable hand and sampled a 180° view from right to left (Figure 7).

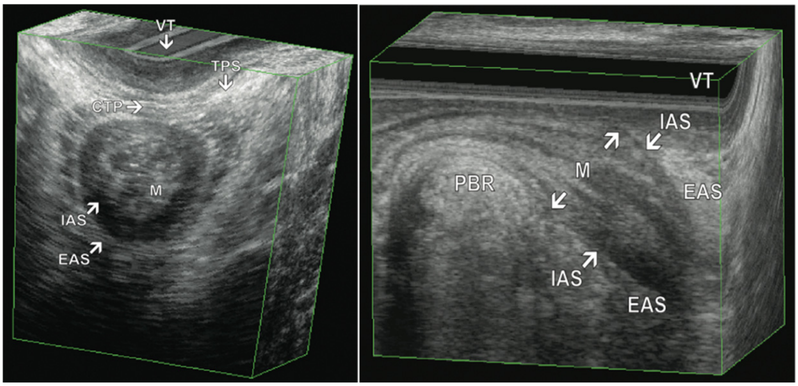

Figure 7: *3D vaginal ultrasound acquisitions seen in a transverse section (right) and in a sagittal section (left). VT: vaginal transducer. TPS: transversus perinei superficialis. CTP: centrum tendineum perinei. M: Anal mucosa. IAS: internal anal sphincter. EAS: external anal sphincter. PBR: puborectalis.*

2.3 Endoanal ultrasound

A transducer is introduced into the lower end of the rectum (at the most 6 cm). For 3D acquisitions, a motorized pullback (study I and II) is connected to the transducer or a transducer with an integrated handheld mover (study III), providing a 360° view (Figure 8). In this study the endoanal transducer had a diameter of 1.7 cm.

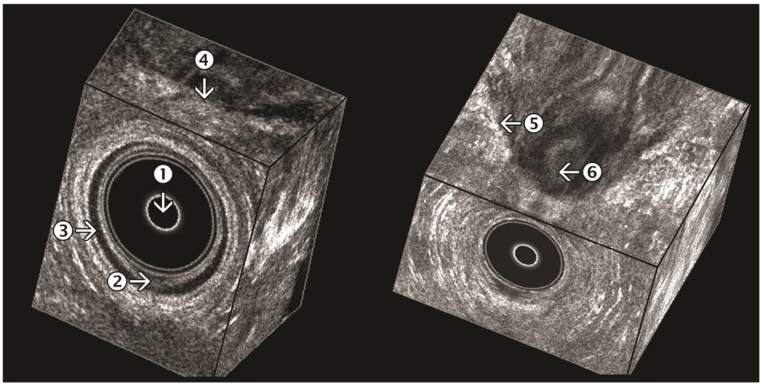

Figure 8: *3D endoanal images of the anal canal in different sections. Axial section: 1) Endoanal probe. 2) Anal mucosa. 3) IAS. Coronal section: 4) Anterior and proximal limit of EAS. 5) Right side of the puborectal sling and 6) IAS.*

2.4 Perineal ultrasound

A transducer, usually with a curved array, is placed on the perineum in the mid sagittal plane. The field of view, angulation of the probe and depth of the acquisitions may vary (Dietz H.P 2004a, b). As with vaginal ultrasound, the technique is a readily available and a low cost procedure.

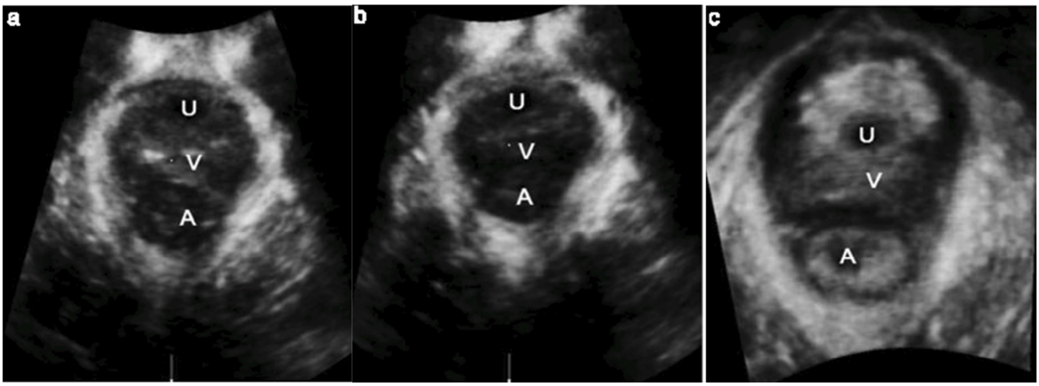

Figure 9: *Axial images of the levator hiatus at rest (a), during contraction (b) and during valsalva manoeuvre (c). A) anal opening; U) urethra; V) vagina.*

2.5 Safety

As safety of ultrasonography will always be a concern (Salvesen KA 2009), international guidelines have been proposed (ECMUS 2010). As a general rule, the tissue absorbs a portion of the energy from the ultrasound beams and converts it to heat. The amount of generated heat depends both on the characteristics of the tissue and the transmitted ultrasound (Bartram C.I 1997). B-mode ultrasound operates at an output level that is incapable of producing harmful increases in temperature. However, the operator should exert caution, especially when examining foetuses, and follow the principles of ALARA (as low as reasonably achievable).

3. The present studies

3.1 Hypothesis for the studies

- 3D vaginal ultrasound is suitable for structural and functional studies of the pelvic floor.
- There is an alteration in length, diameter and volumes of the anal canal during voluntary squeeze.
- The direction of the anal canal changes during voluntary squeeze.
- Pelvic floor muscles and anal canal structures change during pregnancy and after childbirth.
- Pregnancy and childbirth alters the effect of squeeze on the anal canal.

3.2 Aims of the studies

- To compare measurements of the anatomical structures achieved by 3D endoanal ultrasound and 3D vaginal ultrasound, and report on the reproducibility of the methods.

- To determine the dimension and direction of the structures in the anal canal and posterior pelvic floor in: nulliparous women, parous women and women through their first complete pregnancy and childbirth.

- To assess the effect of voluntary squeeze in the same groups of women.

3.3 Material and Methods

Study I and II

3.3.1 Design and setting

Cross-sectional studies. All the participants were examined in the period between December 2002 and October 2003 at the Department of Gynaecology at Klinikk Hammerfest, Hammerfest, Norway.

3.3.2 Study population

According to a protocol approved by the Regional Committee for Medical Research Ethics in Northern Norway (P REK V 75/2001), 30 women were recruited. There were three groups: Nine in the "0-gravida group", 10 in the "normal vaginal delivery group", and 11 in the "complicated delivery group". The women were recruited from a group of nursing students and women referred to our unit for other gynaecological problems. No power calculation was performed due to lack of reported volume measures of the sphincters in the literature at the time.

The participants filled in a questionnaire concerning anal incontinence very similar to the validated St. Mark's incontinence form, but with two additional questions about influence on spare time activities and sexual life, (Appendix 1). From medical records we collected data on parity, method of delivery, duration of the second stage and perineal status after delivery.

3.3.3 Ultrasound equipment

An endoanal 7 MHz rotating ultrasound transducer Brüel & Kjær, type 1850 with diameter of 1,7 cm and a 360° view, covered by a sheath filled with boiled water, was used. The participant was placed in the left lateral position with thighs retracted upwards when the transducer was introduced into the lower end of the rectum and positioned above the upper level of the anal canal. Serial 2D-images were taken while the transducer retracted by a pullback device at the speed of 0.25 mm/second until it had passed the distal edge of the external anal sphincter. 3D ultrasound volumes were reconstructed and analysed using the software program Imaging System 2000, L3Di version 6.0.0.140, connected to the ultrasound machine.

3.3.4 Anatomical definitions

The most proximal point of the IAS was defined as the level where the typical irregular pattern of the rectal mucosa ended and the regular appearance of both the circular IAS and the striated puborectal muscle started (six o'clock according to clockwise orientation, twelve being anterior), figure 10. This level also defined the proximal end of the anal canal. The distal end of the IAS was defined as the lowest section where the low echogenic ring could be visualised. The distal edge of the EAS was visualised as the start of a continuous ring of hyperechogenic tissue. This was also defined as the distal end of the anal canal, figure 10. The proximal level of the EAS was best identified in a coronal plane (figure 8 and 10).

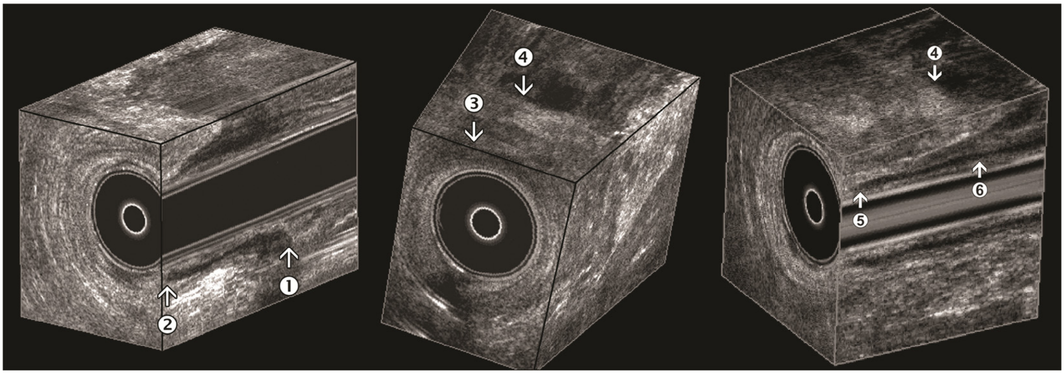

Figure 10: *3D endoanal images of the anal canal: Sagittal section: 1) proximal and 2) distal level of the internal anal sphincter. Coronal section: 3) Distal and 4) proximal end of the external anal sphincter. The distance between 5) and 6) is the part of IAS that overlaps the EAS. Notice the straight transducer in the sagittal views.*

3.3.5 Measurements

All the area measurements were done in the axial plane, except for the lengths, which was measured in the coronal plane. Finally, the outer and inner areas of the

EAS and IAS were traced at 0.30 mm intervals defining the areas of an outer cylinder and an inner cylinder. The volume of each 0.30 mm cylinder was calculated from these areas. The total volume of each sphincter is the sum of the inner cylinders subtracted from the sum of the outer cylinders (Figure 11).

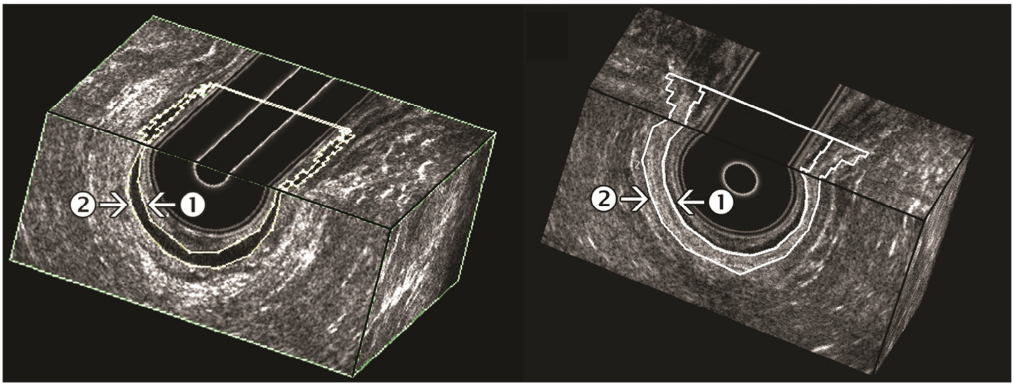

Figure 11: ***Left:*** *Volume measurement of the internal anal sphincter. 1) inner cylinder volume (10.2 cm^3), 2) outer cylinder volume (12.8 cm^3) and the difference 2.6 cm^3 representing the volume of the internal anal sphincter.* ***Right:*** *Volume measurement of the external anal sphincter. 1) inner cylinder volume (5.0 cm^3), 2) outer cylinder volume (8.7 cm^3) and the corresponding volume of the muscle 3.7 cm^3.*

The anal length, EAS volume and length, IAS volume and length were measured. The overlap between IAS and EAS were measured as illustrated in figure 10. All the measurements were performed in both rest and squeeze position = 60 acquisitions, all performed by one examiner.

For the reproducibility study we used the acquisitions achieved from the nine 0-gravida women and the 11 women from the complicated delivery group (study II). The length of the anal canal and the volume of the EAS and IAS were determined by two observers. Observer 1 repeated the measurements three times for all 20 women both in rest and squeeze positions, while observer 2 repeated the measurements twice for the nine 0-gravida.

3.3.6 Acceptable Scans

We achieved acceptable sets of endoanal 3D ultrasound scans in all 30 participants in both rest and squeeze positions.

Study III and IV

3.3.7 Design

Study III: Cross-sectional study conducted during September 2008 - February 2009 at the Department of Gynaecology at Klinikk Hammerfest, Hammerfest, Norway.

Study IV: A longitudinal study conducted during March 2007 - July 2009 at the Department of Gynaecology at Klinikk Hammerfest, Hammerfest, Norway.

3.3.8 Study population

Study III: In accordance with a power calculation (section 3.4), 20 nulliparous women gave written consent in according to a protocol approved by the Regional Committee for Medical Research Ethics in Northern Norway (REK Nord 64/2006). The participants' age and BMI were noted and St Mark's incontinence questionnaire (Appendix II) for anal incontinence was completed.

Study IV: In accordance with the same power calculation and the same REK Nord 64/2006 approval, 23 nulliparous women gave written consent. They were scheduled for examination three times in pregnancy, at 18, 28 and 36 weeks of gestation, and 3 months postpartum. BMI and St. Marks incontinence questionnaire for faecal incontinence were completed for all four visits. Age, mode of delivery and perineal tears was registered at first and fourth visit, respectively.

3.3.9 Ultrasound equipment

A Profocus 2202-3D, the 7 MHz biplane vaginal transducer 8658 with a hand held mover and the 10 MHz endoanal transducer 2050 with integrated pullback - all equipment from B-K Medical, Copenhagen - were used. The vaginal transducer had a diameter of 2.2 cm and a 6 cm long linear array with a capacity to produce sagittal and transverse images. The transducer was positioned in the vagina making sure that the posterior compartment was visualised. When activating the mover, it could sample a volume that covered a 180° view from right to the left (Figure 7). Once the vaginal examination had been completed, the endoanal transducer 2050 (not 1850 with boiled water as described in 3.3.3, and used in study I and II) was introduced into the lower end of the rectum and a handheld motorized pullback device was activated.

Volume acquisitions of both transducers were repeated during rest and voluntary squeeze of the pelvic floor in study III. In the longitudinal study IV, only vaginal acquisitions in rest and during squeeze, were obtained. The analysing software B-K Medical 3D viewer version 7.0.0.300 was used, and all acquisitions were retrieved and analysed by a single observer (I.P.O).

3.3.10 Anatomical definitions

The same anatomical definitions as described in 3.3.4 were used for images from both the vaginal- and endoanal transducer. For the 3D vaginal acquisitions it was necessary to define additional anatomical structures. The IAS encircled the cylinder of the anal mucosa that had a low echogenic star-shaped central structure in the transverse section (Figure 7). Also the mid-sagittal section visualised the shape and direction of the canal (Figure 7). To describe the dimension of the anal mucosa and the shape of the anal canal we produced new anatomical expressions and definitions, as will be explained in section 3.3.12.

3.3.11 Acceptable scans

Study III: We achieved acceptable sets of both 3D vaginal- and 3D endoanal ultrasound images, in all 20 women and in rest and squeeze position = 80 acquisitions.

Study IV: We achieved acceptable sets of ultrasound scans in all 23 women. Three women dropped out after the first visit, while 20 women completed all four visits. This gave 166 acquisitions left for analyses. Out of these, it was not possible to complete the EAS volume measurement in 11 images in rest and eight in squeeze position due to unsatisfactory visualisation of the posterior limitation of EAS.

3.3.12 Measurements

Due to the application of the transducer in the vagina, all the examinations were carried out with the participants in the lithotomy position. The tracing interval for the volume measurements of the EAS and IAS, as explained in section 3.3.5, was set to 0.25 mm. From the vaginal acquisitions, the volume of the anal mucosa (figure 12), in addition to volumes of the IAS and EAS, was measured.

To describe the bending of the rectum, the ano-rectal curvature (ARC) was assessed using kappa, i.e. the angle (in degrees) divided by the length of the curvature (in mm). Lines were drawn 90° to the endpoints of the bend so that the crossing of these lines represented the angle (Figure 12).

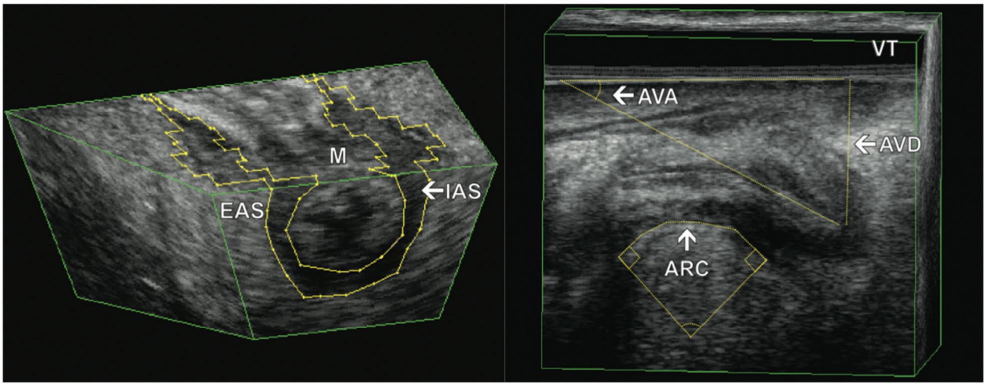

Figure 12: *Additional measurements performed in the 3D vaginal acquisitions.* ***Right:*** *Volume measurements of the anal mucosa (M) and the IAS.* ***Left:*** *The anal canal visualised in longitudinal section: ultrasound transducer (VT). The ano-vaginal angel (AVA) is measured by drawing a line along the mucosa until it crosses the vaginal wall. The ano-vaginal distance (AVD) is the distance from the outermost part of the anal lumen and the vagina. The length of the ano-rectal curvature (ARC) and the angle between the two lines erected perpendicular to the mucosa are used in the ratio (kappa) that quantifies the bend.*

The anal canal also forms an angle with the posterior wall of the vagina; the ano-vaginal angle (AVA), which is open towards the perineum (Figure 12). In addition to measuring this angle, we determined the distance between the posterior vaginal wall and the lumen of the canal at its distal end, the ano-vaginal distance (AVD).

The kappa of the ano-rectal curvature (change of direction in degrees divided by the length of the bend), ano-vaginal angle and ano-vaginal distance was measured during rest and squeeze positions in the 3D vaginal acquisitions, figure 13a and b. Also the lateral diameter and cross-section of the mucosa at the upper and lower level of the anal canal was obtained in study III, figure 13c and d.

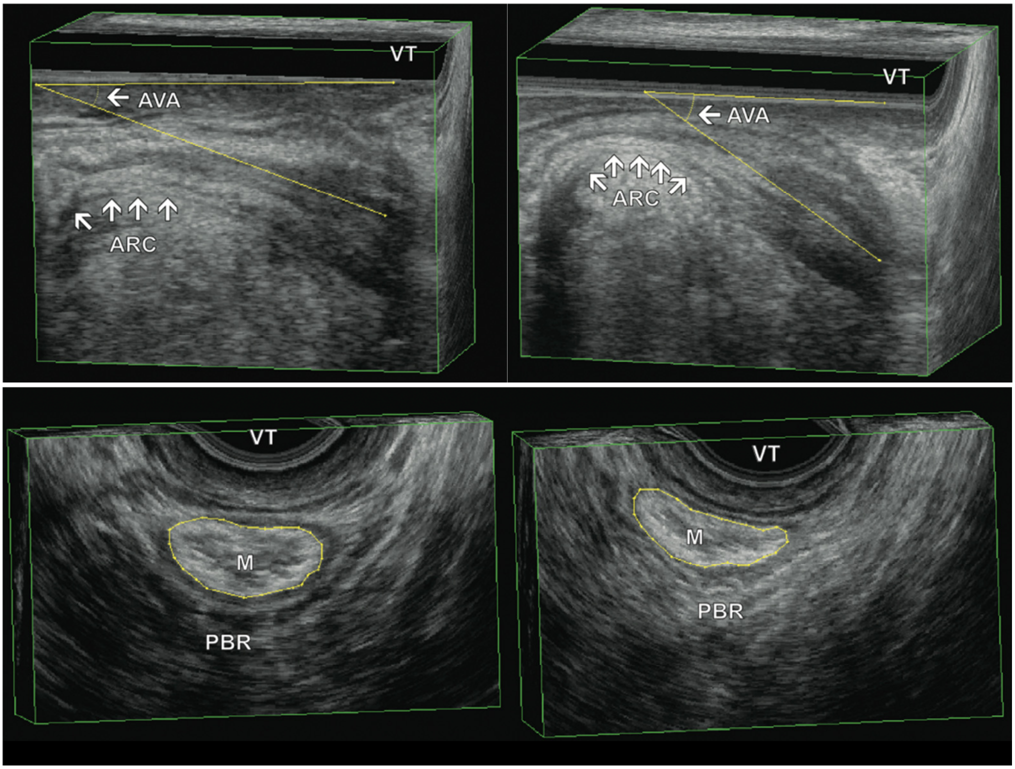

Figure 13: *The ano-vaginal angle (AVA) and ano-rectal curvature (ARC) visualized in a sagittal scan during rest (a) and squeeze (b). The puborectal muscle (PBR) dislodges the bowel in the anterior direction squeezing the mucosa (M). This is correspondingly visualised in the transverse section, i.e. rest (c) and squeeze (d).*

Study III: Intra-observer agreement was assessed in 20 women using the vaginal acquisitions during rest, while 10 randomly chosen acquisitions were used for the inter-observer study, which involved an additional observer.

3.4 Statistics

Data was analysed using SPSS for Windows version 14.0 (studies I and II) and 16.0 (Statistical Package for Social Sciences, Chicago, IL; USA) and SAS system version 9.2 (SAS institute Inc., Cary, NC, USA) (studies III and IV).

Power calculation

A power calculation of the ano-rectal angle measurement in rest and squeeze position was performed on data from a pilot study. Here the standard deviation was 10.66 and the clinically relevant difference of interest (angle in rest – angle in squeeze) was set to 7.5°. Given a power of 80 % and 5 % significance level the formula (Altman D.G 1991):

N > = (u+v)**2 x s**2 / diff**2 was applied: n = 16 in each group.

Thus, 20 participants were included in both the cross-sectional study (study III) and the longitudinal observational study (study IV).

T-tests
Differences between groups were assessed by independent samples t-tests, and differences within the same individual were assessed by paired samples t-test. A two-tailed p-value < 0.05 was considered statistically significant.

Linear mixed-models
The normal linear regression model is specified as

$$y_i = \beta_0 + \beta_1 x_{1i} + \beta_2 x_{2i} + \ldots + \beta_p x_{pi} + \varepsilon_i$$

where y_i = the response variable, (x_{1i}, x_{2i}, … , x_{pi}) = the independent variables, i = subject, (β_1 , β_2 , … , β_p) = the regression coefficients and ε_i = error term. The errors are normally independently distributed.

Linear Mixed Models is an extension to the normal linear model. The extension includes random effects and errors that are multivariate normally distributed.

Longitudinal designs that have repeated observations may not be analysed using a normal linear regression model because there might be dependence between the repeated observations within each subject. A linear mixed model controls for this dependence structure by including random effects or by including a covariance matrix for the residual errors.

Hence, linear mixed models were used to assess the association between total volume, and anal length, during rest or squeeze position with the following variables: time point during or after pregnancy (four time points) and the covariates BMI, faecal incontinence score, ano-vaginal angel and ano-rectal curvature (total volume and anal length were also included when appropriate). Dependence between the four time points was controlled for by including an unstructured covariance matrix to the model. Model assumptions were assessed by visual inspection of residuals. Scatter plots and box plots were used to present the longitudinal association between measurements in the anal canal and gestational age. Reference lines in the scatter plots were estimated with linear mixed models with gestational age modelled using fractional polynomials as outlined by Royston (Royston P 1995). A two-sided p-value <0.05 was considered statistically significant.

Reproducibility
Intra- and inter-observer agreement, based on the same volume acquisitions, was assessed using repeatability coefficient, coefficient of variation, intra-class correlation coefficient (Bland J.M 1996), Bland Altman plots, and 95% limits of agreement (Bland J.M 1999).

4. Synopses of papers with main results

4.1 Study populations

Study I –II: The median age for the three groups was 31 years (range 21-40). The incontinence scores of the three groups are presented in table I.

Table 1: *Anal incontinence reported by nine 0-gravida, 10 women with normal and 11 women with traumatic deliveries.*

	0-gravida delivery (n=9)		*Vaginal delivery (n=10)*		*Complicated (n=11)*	
	flatus	stool leak	flatus	stool leak	flatus	stool leak
never	6	8	5	8	3	9
seldom*	2	1	2	2	7	1
daily	1	0	3	0	1	1

**Seldom = leakage once a month to six times a week.*

Study III: The mean age was 26 years (range 20-32), mean BMI 24 kg/m^2 (range 19-39) and faecal incontinence score 0.

Study IV: The participants BMI and faecal incontinence score are presented in table 2, together with age at inclusion.

The foetuses were delivered at day 280 (SD 9.6), range 260-294. None had a birth weight > 4500 grams. For 16 women the delivery was classified as normal, three underwent acute caesarean section and one a vacuum extraction. After the delivery the perineum was intact in nine women, nine had grade 1-2 tears and two women grade 3 tears.

__Table 2:__ The score for faecal incontinence and BMI at four visits during and after pregnancy, as well as age and pregnancy history registered at the first visit in gestational week 18. 23 women were enrolled and 20 completed all four visits.

	Mean (SD) or Median	*Range*
Age (years)	26.7 (5.2)	19-37
Gravida	1	0-4
St Mark's score		
18 weeks	1.26 (2.60)	0-11
28 weeks	1.30 (2.72)	0-11
36 weeks	1.70 (2.92)	0-10
12 weeks postpartum	1.15 (2.18)	0- 8
BMI (kg/m^2)		
18 weeks	25 (3.66)	21-35
28 weeks	28 (4.02)	22-37
36 weeks	29 (3.95)	24-41
12 weeks postpartum	25 (4.24)	20-36

4.2 Anal length

Using the endoanal transducer the anal length measured 3.28 (SD 0.63) cm in a group of nine women and 2.57 (0.57) cm in a group of 20, both groups consisting of nulliparous women. Compared with the 21 women with a mean parity of 1.76 (range 1-3), a significant decrease to 2.30 (0.77) cm was found after delivery, p = 0.002. The anal length did not alter during the squeeze manoeuvre.

Using the vaginal transducer the anal length was measured to 3.64 cm (0.48), 1 cm longer than in the endoanal acquisitions, previously achieved from the same 20 nulligravid women. It also became significantly longer, 3.86 (0.39) cm during the squeeze manoeuvre, p = 0.007.

During pregnancy, the anal length increased to 4.21 (0.67) cm at 36 weeks of gestation, $p < 0.05$. Three months after delivery it was reduced to the same length as found at 18 weeks of gestation, 3.91 (0.54) cm. When the pregnant women were asked to squeeze the anal length became significantly longer than in rest position, at all 4 time measures, p = 0.007, 0.007, 0.022, 0.004 and figure 14.

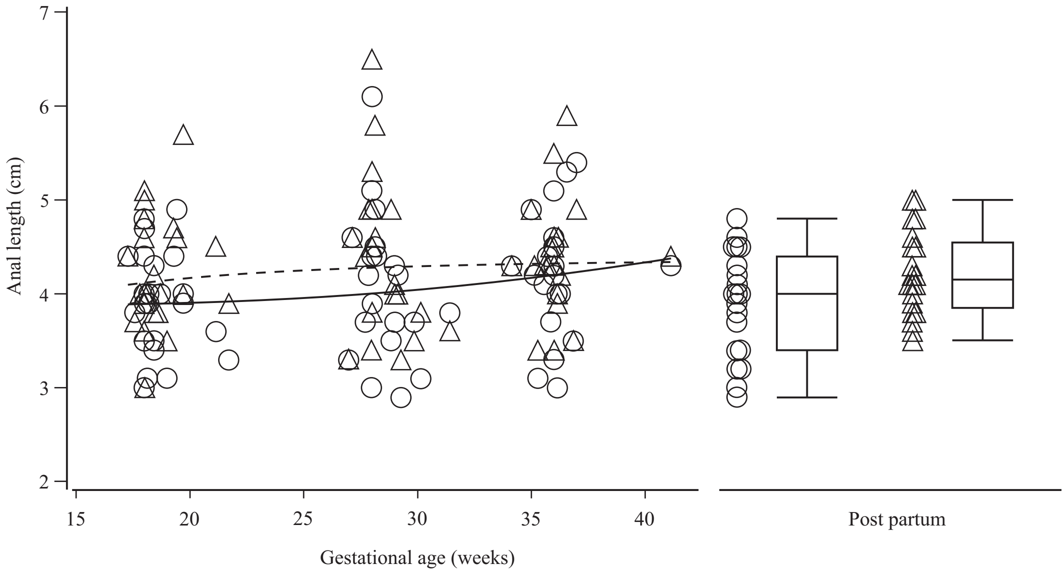

Figure 14: *Development of anal length during pregnancy and three months post partum, and the effect of voluntary squeeze (Δ) compared to rest position (O), presented with regression lines (dashed line, squeeze) and box plots with median, 1st and 3rd quartile, and range.*

4.3 The shape and curvature of the anal canal

The vaginal acquisitions visualised an undisturbed posterior compartment, including the anal canal. As described in section 3.3.12, the scans achieved with the vaginal transducer revealed that the shape of the anal canal was curved and angled, figure 13a and b. During voluntary squeeze, the position of the anal tube could be shifted laterally, figure 13c and d. These findings were in contrast to the acquisitions obtained by the stiff endoanal transducer that dislodged and distended the anal mucosa and the sphincters, and in addition distorted the natural position and curvature of the anal canal complex, figure 10. Hence, the natural shape and position could only be evaluated from the vaginal scans (study III and IV).

The squeeze manoeuvre bent the anal tube in the upper level and made the ano-rectal curvature (ARC) more acute (p=0.031). At the same time the anal tube rotated anteriorly and the measured ano-vaginal angle (AVA) became wider from 32° to 36° (p = 0.010). The dynamics during squeeze were reduced in the group of women who underwent pregnancy and delivery. The longitudinal measures of the ARC did not differ between gestational weeks or between rest and squeeze position. The kappa ranged from 3.22 (SD 0.94) - 3.76 (1.22) and was equally curved as found in the nulligravida group (kappa 3.46 (1.08) in rest position).

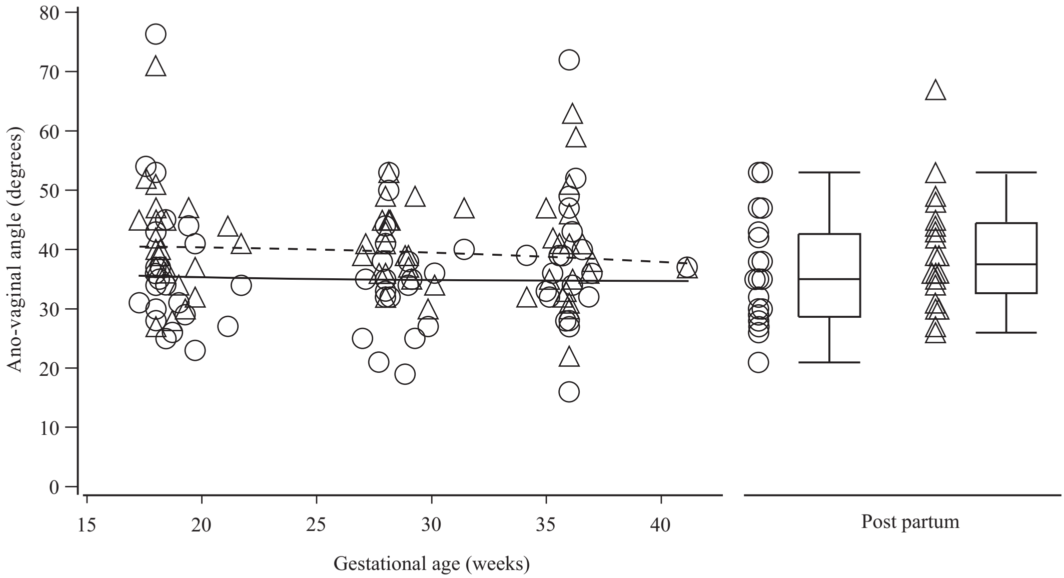

Figure 15: *Development of the ano-vaginal angle during pregnancy and three months post partum, and the effect of voluntary squeeze (Δ) compared to rest position (o), presented with regression lines (interrupted line, squeeze) and ± 2SD or box plot with mean, SD and range.*

However, the distal tilt of the anal canal (AVA) increased during squeeze until 28 weeks of gestation. Later in pregnancy and after delivery, it ceased, figure 15.

The ano-vaginal angel (AVD) did not change between rest and squeeze position for the nulliparous women, or women during pregnancy. To retrieve a selection of women having had a normal pregnancy and delivery, four women who underwent an eventful delivery were excluded. We found that the AVD became shorter and the AVA increased post partum (supplementary I).

4.4 Measurements of the Anal Mucosa

The mucosal volume was only possible to measure in the vaginal acquisitions where distortion from the transducer did not interfere (study III and IV). In rest it was 3.12 (SD 0.79) cm^3 in the nulliparous group with no alteration during squeeze. A volume increase to 4.42 (1.44) cm^3 was found at 28 weeks of gestation, table 3. No further increase in the mucosal volume was found at 36 weeks of gestation and or 3 months post partum, where it remained 4.03 (0.89) cm^3, although significantly higher (p=0.004) than in the 0-para group.

Table 3: *Volume of the anal mucosa. Measurement was performed longitudinally in 23 pregnant women, and during rest and squeeze position.*

	Rest	*Squeeze*	*p*
Anal mucosa volume (cm^3)			
18 weeks	3.86 (1.22)	3.90 (1.09)	0.90
28 weeks	4.42 (1.44)*	4.44 (1.45)	0.93
36 weeks	4.19 (1.68)	4.32 (1.29)	0.67
12 weeks post partum	4.03 (0.89)	4.10 (0.79)	0.75

** a significant increase in anal mucosa volume between 18 and 28 weeks , p=0.01.*

Comparing measurements during squeeze position to those in rest, the lateral diameter increased and the cross-section was reduced in the upper level over the puborectal sling. In the lower level, where the EAS is active during squeeze position, no effect on the diameter or cross-section of the mucosa could be measured, table 4, figure 16.

Table 4: *Volume, cross-section and diameter of the anal mucosa. Measurements were performed in 20 nulliparous women during rest and squeeze position. The results are presented with mean (standard deviation).*

	Rest	*Squeeze*	*p*
Anal mucosa volume (cm^3)	3.12 (0.79)	2.94 (0.67)	0.193
upper cross-section (cm^2)	1.20 (0.33)	0.90 (0.16)	<0.001
upper diameter (cm)	0.94 (0.23)	0.68 (0.22)	<0.001
lower cross-section (cm^2)	1.22 (0.36)	1.13 (0.36)	0.160
lower diameter (cm)	1.16 (0.21)	1.09 (0.21)	0.065

4.5 IAS volume

In acquisitions enhanced with the endoanal transducer, the IAS volume was ~2.65 cm^3 in rest position, in the 30 women with different parity and history of delivery. The only change in IAS volumes were seen in squeeze position in the group that had undergone a complicated delivery, 2.09 (SD 1.11) cm^3, p = 0.01.

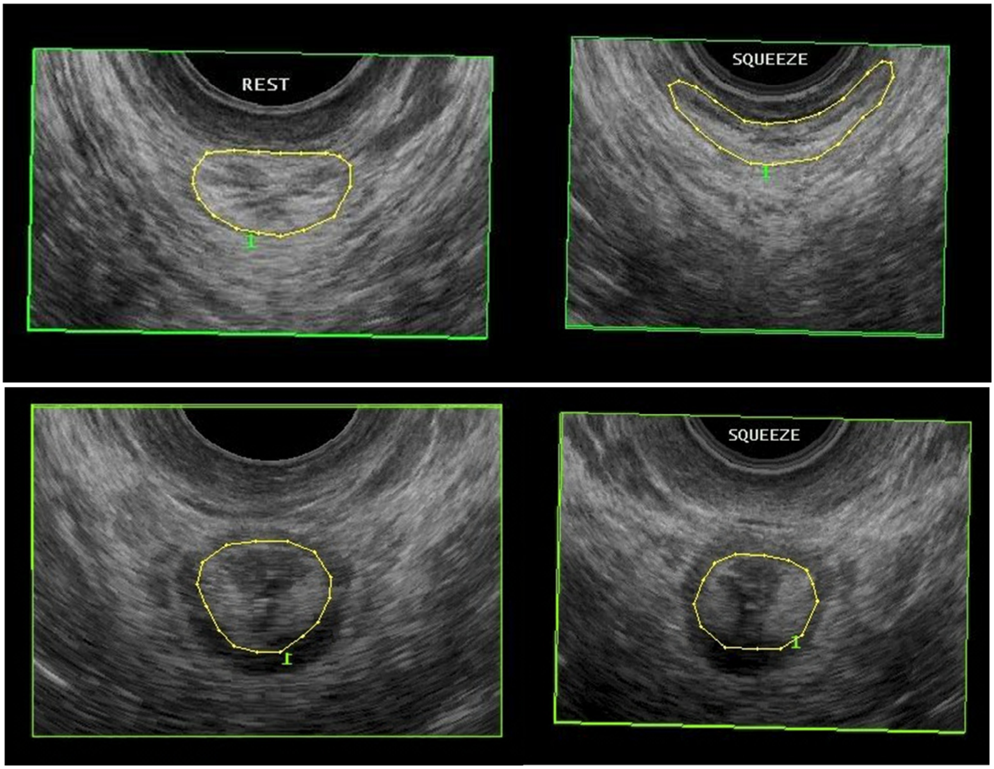

Figure 16: *The effect of squeeze on the anal mucosa.* ***Above:*** *In the upper-level where the puborectal muscle is active the cross-sectional area, lateral diameter and shape of the mucosa are altered during squeeze.* ***Beneath:*** *In the lower level where the EAS is active no change of the area, diameter or shape were seen.*

In study III, volume measurements of the IAS were performed in acquisitions from both transducers and from the same participants. The volume obtained with the vaginal transducer was 2.97 (1.05) cm^3 and with the endoanal transducer 2.08 (0.92) cm^3, a difference of almost 1 cm^3, $p = 0.003$. No change in IAS volume during squeeze position was seen, though the 1 cm^3 size difference between the two ultrasound techniques maintained also in squeeze position, $p < 0.001$.

During pregnancy the IAS volume increased by 1 cm^3 (25 %) from 18 to 36 weeks of gestation and was reduced again by ~ 1cm^3 three months post partum. This pattern did not repeat during the squeeze manoeuvre. Hence, the post partum volumes of 3.84 (1.85) cm^3 and 4.01 (1.71) cm^3 in rest and squeeze, respectively, differed by ~ 1 cm^3 from the nulliparous women's group, (p= 0.078 and 0.018).

4.6 EAS volume

In contrary to the anal length and IAS volume measures, the EAS volume was found to be lesser in the vaginal acquisitions, table 5. The reduction of size by 60 % is considerable and consistent for both rest and squeeze position in study III. In the longitudinal study of the pregnant women, the EAS volume increased from 2.71 (1.74) cm^3 at 18 weeks of gestation to 3.24 (2.63) cm^3 at 28 weeks of gestation, $p < 0.05$. At 36 weeks of gestation the volume was similar as in week 18. In accordance with the other volume measurements, no change between rest and squeeze position was measured.

Table 5: *EAS volumes using 3D vaginal or 3D endoanal ultrasound techniques in a group of 20 nulligravida. Presented with mean (SD).*

	Vaginal US mean	*Endoanal US mean*	*p-value between groups*
EAS volume (cm^3)			
Rest	1.85 (1.31)	3.96 (1.73)	<0.001
Squeeze	1.47 (0.87)	3.86 (1.50)	<0.001
p-value within groups	0.045	0.70	

The EAS volume measured by the endoanal technique in study I and II was 7.61 (2.63) cm^3 in the nine nulliparous women in rest position, almost twice the volume presented in table 5 and what was subsequently found in the longitudinal study IV.

4.7 Faecal incontinence score and BMI

There was a significant inverse association between the anal canal volume and incontinence score ($p = 0.016$). The volume decrease 1.12 cm^3 per standard deviation increase in score. A standard deviation (0.5 cm) longer anal canal indicated a 1.41 cm^3 increase in anal canal volume, $p = 0.000$. An inverse association between ARC and anal length in rest and squeeze, $p = 0.0001$ and $p = 0.001$, respectively, were found. No association between the total volume of the anal canal and BMI ($p = 0.73$) was found.

4.8 Reproducibility

Agreement between the endoanal- and vaginal methods was poor with significant difference between all measured variables, both in rest and squeeze, $p < 0.001$. This was also the case for some of the inter-observer measurements performed in acquisitions from both transducers (anal length and EAS volume in the vaginal scans, p = 0.036 and 0.001) and IAS volume, p = 0.006 in the endoanal scans). The repeatability data was acceptable as the intra-class correlation coefficient were >75%, and coefficient of variation were <15% for observer I, for all variables measured in both ultrasound methods, except for the EAS volume measured in the vaginal acquisitions.

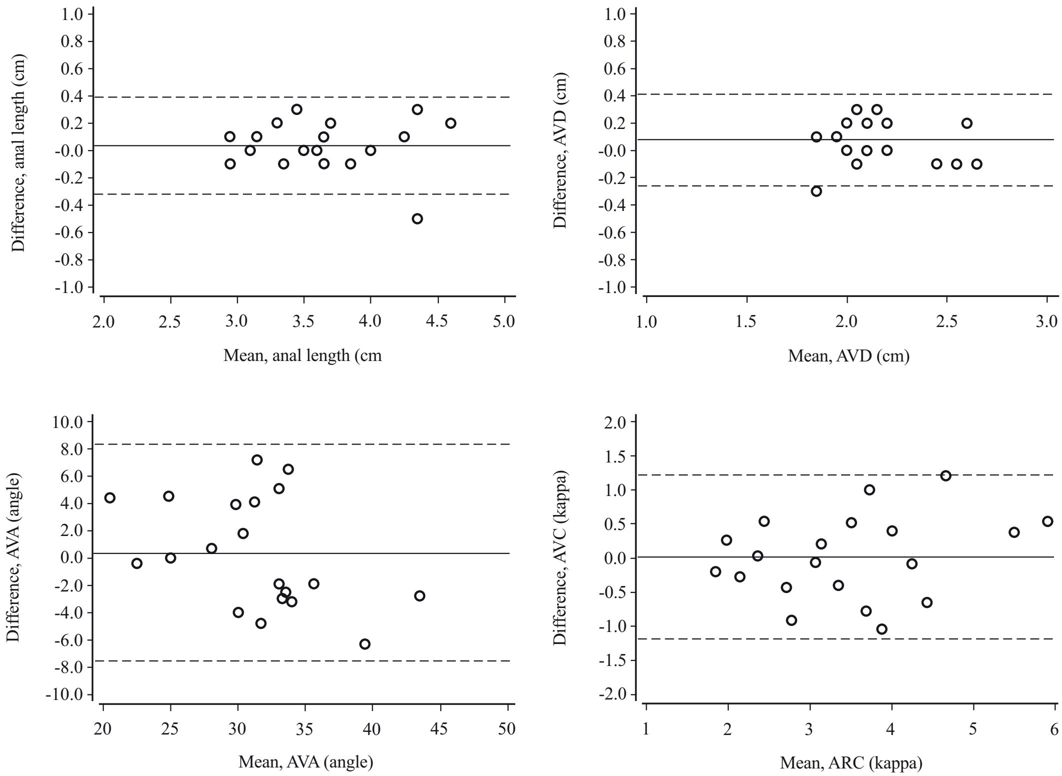

Figure 17: *Bland-Altman plots showing intra-observer variation for 3D vaginal ultrasound, i.e. the difference between the first and second measurement for the length of the anal canal (a), ano-vaginal distance (b), ano-vaginal angle (c), and ano-rectal curvature (d). The solid lines represent the mean difference and the dashed lines represent the 95 % limits of agreement.*

Bland-Altman plots of the variables anal length, AVD, AVA and ARC are presented in figure 17. The repeatability coefficient relates to the actual size of the measurement. For the mucosal volume and anal length the intra-observer variation were ±22% and ±9.6 % from the mean (study III). For the anal length obtained in the endoanal acquisitions (study II), the variation was ±27 %.

5. Discussion

The anal mucosa and its possible role in the continence preserving mechanism is discussed in relation to its dimensions and how it appears in ultrasound images, in both nulliparous and pregnant women. The effects of voluntary squeeze on the structures in the anal canal could be visualised and studied. New variables: ano-rectal curvature (ARC), ano-vaginal angel (AVA) and ano-vaginal distance (AVD) were defined for the purpose to describe the altered bend and angulations of the anal canal seen during squeeze.

Endoanal ultrasound was found to distend and distort the anatomy when the straight rod was inserted to the anal canal. This major disadvantage could be overcome using vaginal 3D ultrasound. In addition to measurements of the anal sphincters, the anal mucosa and the natural bends and angles of the anal tube could be quantified since the transducer is outside the target organ.

5.1 3D endoanal versus 3D vaginal transducer

The diameter of the anal mucosa was approximately 1 cm. The endoanal transducer is 1.7 cm in diameter and when inserted into the anal canal it compressed the anal mucosa and widened the surrounding IAS and EAS. The widening of the canal might have been at the expense of the anal length which was >1 cm shorter when comparing endoanal- and vaginal acquisitions assessed from the same group of nulliparous women. In recordings performed with the endoanal technique, both the IAS and EAS protrude as regular and circular structures (figure 8). From scans assessed with the vaginal technique, the structures are visualised undisturbed and protrusions are more irregular and individual in shape (figure 7). An explanation might be that the IAS and EAS are forced to enclose the endoanal transducer due to its wider diameter, and depict it as being more circular than it really is.

Other studies that have compared endoanal- and vaginal ultrasound techniques have found that the IAS is thicker and the EAS thinner in vaginal scans, probably due to absence of the endo luminal distension (Sultan A.H 1994; Poen A.C 1998). This is also true for trans-perineal versus endoanal scans (Cornelia L 2002). In this work the IAS had a greater volume and the EAS a smaller volume in the vaginal acquisitions compared to the measurements performed in the endoanal scans. Since both length and thickness of the cylindered sphincters affect volume-measurements, the volume result suits the overall picture.

The 3D vaginal recordings especially visualised the centrum tendineum perinei clearly (Figure 7). In this junction between the striated proximal EAS, the transversus perinei superficialis muscle and bulbospongiosus muscle, it was difficult to distinguish the exact limitation of the anterior part of the EAS from ten to two o'clock. Also, the sagittal view of the 3D vaginal scans confirmed the anatomical integration of these three structures. In the endoanal scans it is easier to define the anterior-proximal limitation of the EAS (figure 8). Again, we speculate if the wide diameter of the endoanal transducer compresses the three layers of muscles joining in centrum tendineum perinei together, and that a misinterpretation of it as only EAS is made. Support for the theory of anatomical integration of the structures was found in a Swedish study using the same 3D vaginal transducer to image perineal structures (Örnö A-K 2008).

Since the form of the anal canal changes during voluntary squeeze and the anal mucosa adapts to this (figure 13), it is important to be aware of the lack of such information in scans from the endoanal transducer. It is equally important to acknowledge that the endoanal technique is at present the best technique for detection of sphincter lesions, though it might overestimate them (Poen A.C 1998; Bollard RC 2002; Cornelia L 2002).

5.1.1 Reproducibility

The difference of the measured anal length both between transducers and between observers explains the poor agreement. The anal length influences the volume measurements considerably, as the traced cylinders become longer or shorter. Bland-Altman plots of the important anal length reveal that the reproducibility of observer I was very good for vaginal acquisitions, hence the basis for the volume measures were good. Overall, the acceptable intra-observer data and the poor inter-observer data found in this study are in line with other reports (Enck P 1997; Gold D.M 1999b; Williams A.B 2002a; West R.L 2005a,b; Cazemier M 2006; Gregory W.T 2006). Though the inter-observer data of this study is based on few patients, the consistent similar results in the literature probably reflect a long apprenticeship. This is a disadvantage for implementation in clinical settings, for both the endoanal- and vaginal technique.

5.2 Anal length, EAS and IAS

Consistently, a longer anal canal was found in the 3D vaginal scans in both the nulliparous women and the pregnant women groups. Also, an elongation during squeeze position was seen, including the four points of measures during pregnancy and after childbirth. Equally consistently, no elongation of the anal length was found during the squeeze-manoeuvre in neither of the groups where endoanal acquisitions were assessed.

In the cross-sectional group of women with varied parity, a shortening of the anal length by almost 1 cm was found after delivery. This was in contrast to the group of pregnant women, which was followed longitudinally and by vaginal ultrasound. Here the length was found to increase by 0.3 cm throughout pregnancy. After delivery it decreased to the same level as seen at 18-weeks of pregnancy and within the nulliparous women. The higher parity of the group examined with the endoanal transducer could be a reason for the shorter length. High parity could initiate a looser connection between the distal anal canal and the centrum tendineum perinei after stretching from the born foetuses. When the 1.7 cm endoanal transducer was introduced it widened the anal tube at the expense of the anal length, which was less anchored.

For the low echogenic IAS the volume became $1cm^3$ larger in the vaginal acquisitions, probably due to the 1cm length difference between ultrasound methods. As previously mentioned, the IAS was visualised thicker in volume in the vaginal scans than in endoanal scans (Sultan A.H 1994; Poen A.C 1998). This could have been contributing factor to the larger volumes. Unfortunately, IAS thickness was not measured in this study. Another author has found an even smaller IAS volume than the present, using endoanal ultrasound (West R.L 2005a). From volume reconstructions in MRIs, IAS was found 4-5 times more voluminous (Cornella J.L 2003). Even though it seems easy to identify IAS in ultrasound acquisitions, the variety in volume measurements in different techniques must be due to different anatomical definitions between authors and to how the ultrasound beams insonate the fibers (Santoro G.A 2004).

The EAS was difficult to define using both ultrasound techniques. Anteriorly, the possibility of misinterpretation due to the endoanal transducer width and the integration with the transversus perinei superficialis muscle and bulbospongiosus muscle seen in the vaginal scans, made the limitations difficult. Posteriorly, a 5-6 cm distance between the array of the transducer and the EAS interfered with the quality of the acquisitions and it was a major challenge to define the posterior limitation. Laterally, there was an overall problem with the limitations for both techniques.

There was a 50 % reduction in the measured volume using the endoanal method from study I and II versus study III, and a 60 % divergence between the vaginal and endoanal method. Others that have used the endoanal technique to assess EAS volumes found sizes in line with the result in study I and II (West R.L 2005a; Gregory W.T 2006). A shift in the observer I's interpretation of the EAS limitations in the endoanal images in study III and IV might have occurred after working in the vaginal acquisitions. Despite the fact that EAS appears thinner in volume in both vaginal- and perineal ultrasound, compared to endoanal acquisitions (Sultan A.H 1994; Poen A.C 1998; Cornelia L 2002), the 60 % divergence in EAS volumes between the ultrasound methods seems considerable and the problem of anatomical definition is reflected in the reproducibility data. In addition, correlations between measurements of the EAS performed in 3D endoanal ultrasound pictures and MRI were poor (West R.L 2005b).

5.3 Anal mucosa and shape of the canal

3D vaginal ultrasound gave the opportunity to study undisturbed the posterior compartment of the pelvic floor. The volume of the mucous cylinder in the anal canal, turned out to be 3 cm^3 in the 0-gravida and 4 cm^3 in the pregnant group of women, 40 % and 30 % of the entire anal apparatus. Voluntary contraction of the pelvic muscles was associated with squeezing of the proximal, but not the distal ends of the mucosal cylinder of the anal canal. Also, the anal canal became additionally 2-3 mm longer while the volumes remained constant, implying that the flexible anal mucosa and IAS adapted to the longer canal, more or less acting as a dynamic plug. This pattern was seen in both the nulligravidae, pregnancy and three months post partum groups.

Örnö A-K may have observed some of these dynamics when she showed that the columns of the anal mucosa decreased during the rectoanal inhibitory reflex, allowing the content of the ampulla to descend into the anal canal (Örnö A-K 2005) and also during rectal sensations (Örnö A-K 2007a). In a recent study, combined tears involving IAS, EAS and/or the anal mucosa are where anal incontinence persists (Roos A.M 2010). This emphasizes that the anal mucosa cylinder is functionally too important to be neglected, and is in accordance with the conclusion in a study looking at the anal cushions using a 2D vaginal transducer (Nicholls M.J 2006) and the theory of Gibbons C.P, as mentioned in section 1.2.1.

The increased angle between the anus and the vagina seen during voluntary squeeze in both nulliparous and pregnant women may be an expression of increased folding and could be another possible augmentation of the mechanism of continence. It supports the concept that the distal end of the anal canal is fixed to the pelvic floor near the centrum tendineum perinei muscle while the proximal end is tilted forward under the influence of the puborectal sling augmenting the natural curvature of the bowel (figure 13). After a normal delivery the anchor mechanism of centrum tendineum perinei muscle might be looser, as the angle increased during squeeze position while as the ano-vaginal distance became shorter. The proximal tilting due to contraction of the puborectal sling could not be reproduced in the longitudinal study. This was in line with another longitudinal study that observed a reduced bend in the ano-rectal junction after delivery of the first child when voluntarily activating the puborectal sling (Constantini S 2006).

5.4 Alterations during pregnancy and after childbirth

The elongation of the anal canal and the corresponding 20 % rise in anal canal volume during pregnancy, probably contributes to the anal continence mechanism. The associations between anal length, total anal canal volumes and incontinence scores, confirms this. An elongation of a tube represents a corresponding increase in flow resistance. The elastic mucosa acts as a plug supported by the surrounding IAS. These factors may especially be a valuable reinforcement of the mechanism of continence during a period of pregnancy with generally increased intra-abdominal pressure. After childbirth the mechanism seems to be returned.

The main focus of the few ultrasound studies of the pelvic floor in pregnancy is on the anterior compartment of the pelvic floor and the relationship between urethral mobility and development of urine incontinence (Meyer S 2001; Mørkved S 2004; Ochsenbein N 2001). However, the group of A-K Örnö have concentrated on the posterior compartment when evaluating the extent of perineal tears before surgical repair (Örnö A-K 2008). The reduced mobility of the puborectal sling three months after delivery (Constantini S 2006) is also in line with the result in this study. The recent publications describing 13 % levator avulsions three months after first vaginal delivery and 31 % 12 hours after, by 3D trans- labial or perineal ultrasound (Dietz H.P 2010; Blasi I 2011) adds another perspective to the alterations in the pelvic floor during pregnancy and after childbirth.

6. Conclusions

While endoanal ultrasound is a well-established method for assessing the anal sphincters, we have shown that the vaginal technique provides valuable additional information. It presents less distorted details of the anal shape, position and dimensions in standard rest position and permits functional studies, such as voluntary squeeze. In contrast to the endoanal technique the vaginal scan provides a complete accord of the volumes of the anal canal, including the mucosa that constitutes 40% of the anal structures.

During voluntary squeeze an augmented bend of the anal canal at the level of the puborectal muscle, together with compression of the mucosal cuff, were observed. A tilt in an anterior direction at the proximal level and an increased angle between the vagina and distal part of the anal canal, were seen. The altered form of the anal canal and its effect on the anal mucosa that possibly act as a dynamic cuff is probably a part of the continence preserving mechanism. The activity of the puborectal sling seems to be of no less importance in pregnancy where the anal unit increased 20% in volume (mainly by elongation, which also represents increased resistance) during the second half of pregnancy, and later reduced to postpartum level and physiology.

7. Clinical relevance and future aspects

From study I we learned that the anal sphincters were well described according to different gender, age, history of birth and incontinence score. Study II showed that the reproducibility of 2D endoanal ultrasound did not improve in 3D acquisitions. In study III and IV we concentrated on the total anal complex and regarded it as a functional unit. We believe our findings (shape, dimension, angulations and bending) must be of importance in the complicated anal continence mechanism. The knowledge of increased size of the anal structures during pregnancy should be provided to all personnel involved in suturing of perineal tears immediately after delivery. Description of the normal anatomical development during a pregnancy is a basic platform for understanding and further research of pathological conditions (such as anal incontinence) after delivery.

2D vaginal- or perineal ultrasound also visualises the structures in the anal canal. The transducers are placed in the posterior fourchette of the introitus vaginae or on the perineum, allowing visualization of an undisturbed canal and how functional tests initiate movements of it. 2D ultrasound is a more available technique than MRI or endoanal ultrasound and a potential low-cost tool in the diagnosis of anal disturbances. Functional studies with 2D vaginal transducers should be performed and the reproducibility of the method reported.

We are currently conducting a study on anally incontinent women and will compare their anatomy and physiology with a group of continent women, matched in age. This study may add valuable insight as to whether the focus on the anal complex as a unity should be further developed and used in diagnostic evaluation situations. In this context the role of the anal mucosa as a barrier for bowel content entering into the anal canal is of interest.

Some authors have already advertised that perineal ultrasound will replace defecografi (Perniola G 2008; Steensma A.B 2007). In a recent study perineal ultrasound was found to be a promising diagnostic tool for patients with obstructed defecation (Martellucci J 2010).

There is a need for functional studies that visualise the anal mucosa and the shape, dimensions and interactions between the structures, rather than isolated evaluations of the anal sphincters, especially after severe sphincter defects.

Also, the role of grade 1 and 2 perineal tears/episiotomies and the adaptation of transversus perinei superficialis and profundus muscles and bulbospongiosus muscle and association to later perineal hyper mobility, ano-vaginal angulations and anal incontinence should be explored.

Cooperation between professions are already established in this field and might be the future way forward in order to understand the mechanism of continence and develop soundly founded treatments.

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Article I

Article II

Article III

Article IV

Appendix I

Skjema nummer

SPØRRESKJEMA TIL KVINNER SOM SKAL FÅ UTFØRT ULTRALYDSUNDERSØKELSE AV ENDETARMENS LUKKEMUSKEL VED GYNEKOLOGISK AVDELING, HAMMERFEST SYKEHUS

Navn: **Født:**

Hvor ofte har du ufrivillig lekkasje av luft eller avføring fra endetarmen ? Sett kryss i ruten som passer.

Lekkasje av	Aldri	1-4 ganger per måned	1-6 ganger per uke	1 gang per dag	Mer enn 1 gang per dag
Luft					
Løs avføring					
Fast avføring					

Bruker du bind på grunn av avføringslekkasje ? Sett kryss ☐ ja nei ☐

Må du skifte undertøy på grunn av avføringslekkasje ? Sett kryss ☐ ja nei ☐

Hvor ofte har du vanligvis avføring ? Sett kryss

Sjeldnere enn 1 gang per dag	1-3 ganger per uke	4-6 ganger per uke	1-4 ganger per uke	Mer enn 4 ganger per dag

Påvirker lekkasje fra endetarmen (luft og avføring)

- Dine fritidsaktiviteter (ferie, hobby, trening, friluftsliv)? Ja ☐ Nei ☐ Ikke aktuelt ☐

- Ditt sosiale liv (gå ut, treffe venner)? Ja ☐ Nei ☐ Ikke aktuelt ☐

- Ditt seksualliv ? Ja ☐ Nei ☐ Ikke aktuelt ☐

Appendix II

Appendix II

REK: 5.2006.2024

Spørreskjema for forskningsprosjektet

Bekkenbunnstukturer, endringer i svangerskap og ved inkontinens

En ultralydstudie

NAVN: FØDT:

ADR:____TLF:________________

Antall svangerskap ☐

Antall barn ☐

Vekt

Høyde

BMI ☐

Tidligere planlagt keisersnitt ☐ eller ☐ vaginal fødsel

	JA	NEI
Ble barnet forløst med sugekopp/tang ?	☐	☐
Veide minst ett av barna over 4500 gr ?	☐	☐

Opplysninger som innhentes av legen:

	1.barn	**2.barn**	**3.barn**
Tid fra barnets hode stod på bekkenbunnen til barnet var forløst: 1t >			
1t <			
Grad I-II rift (inkl episiotomi) :			
Grad III-IV rift:			

1/2

Inkontinensvurdering:

	Aldri	**Sjelden**	**Av og til**	**ukentlig**	**Daglig**
Inkontinens for fast avføring	0	1	2	3	4
Inkontinens for flytende avføring	0	1	2	3	4
Inkontinens for luft	0	1	2	3	4
Endring av livsstil	0	1	2	3	4

	Nei	Ja
Må bruke bleie/bind eller plugg mot avføringslekkasje.	0	2
Bruker forstoppende medisin	0	2
Kan ikke utsette avføring i 15 min etter følelse av trang	0	4

St Marks inkontinensskjema. Maks poengscoore er 24 poeng.

Dato:

Poengscore

Table Supplemental 1: Comparison between measurements at 18 weeks of pregnancy and 3 months post partum after exlusion of the six women who underwent caesarean section, vacuum extraction or third-grade perineal tears in addition to the dropouts. Measurements of the anal length, volume of the anal mucosa, internal anal sphincter (IAS), external anal sphincter (EAS), ano-vaginal angle (AVA), ano-vaginal distance (AVD) and ano-rectal curvature (ARC)* at rest and voluntary squeeze.
* Angle (°) divided by the length of the curvature (in mm).

		18 weeks	3 months post partum	P-value*
Total volume anal canal (cm^3)	Rest	9.46 (2.66)	10.89 (4.31)	0.088
	Squeeze	10.07 (4.23)	10.94 (3.41)	0.37
	P-value**	0.22	0.90	
Anal length (cm)	Rest	3.81 (0.49)	3.97 (0.47)	0.40
	Squeeze	4.06 (0.51)	4.11 (0.40)	0.69
	P-value**	0.060	0.11	
Mucosa volume (cm^3)	Rest	3.56 (1.19)	4.01 (0.90)	0.30
	Squeeze	3.73 (1.14)	4.02 (0.79)	0.41
	P-value**	0.66	0.94	
IAS volume (cm^3)	Rest	3.59 (1.57)	3.96 (2.05)	0.57
	Squeeze	3.69 (1.68)	4.13 (1.99)	0.13
	P-value**	0.83	0.54	
EAS volume (cm^3)	Rest	2.65 (1.59)	3.02 (2.27)	0.35
	Squeeze	2.67 (2.54)	2.82 (1.86)	0.95
	P-value**	0.43	0.47	
Ano-vaginal angle (°)†	Rest	38.9 (13.8)	34.4 (8.32)	0.21
	Squeeze	42.1 (10.9	38.2 (6.65)	0.14
	P-value**	0.12	0.020	
Ano-rectal curvature (° /mm)	Rest	3.61 (1.05)	3.36 (0.91)	0.52
	Squeeze	3.92 (0.79)	3.53 (0.82)	0.19
	P-value**	0.23	0.56	
Ano-vaginal distance (mm)	Rest	24.6 (7.80)	25.3 (4.79)	0.71
	Squeeze	26.8 (4.82)	24.0 (4.02)	0.028
	P-value**	0.29	0.23	

† Angle (in degrees) divided by the length of the curvature (in mm).
* P- value, post partum compared with measurements at 18 weeks
** P-value, rest versus squeeze.

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