

Structural and functional ultrasound imaging of the pelvic floor during pregnancy and postpartum

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Structural and functional ultrasound imaging of the pelvic floor during pregnancy and postpartum

Structurele en functionale echografie van de bekkenbodem
tijdens zwangerschap en na de bevalling
(met een samenvatting in het Nederlands)

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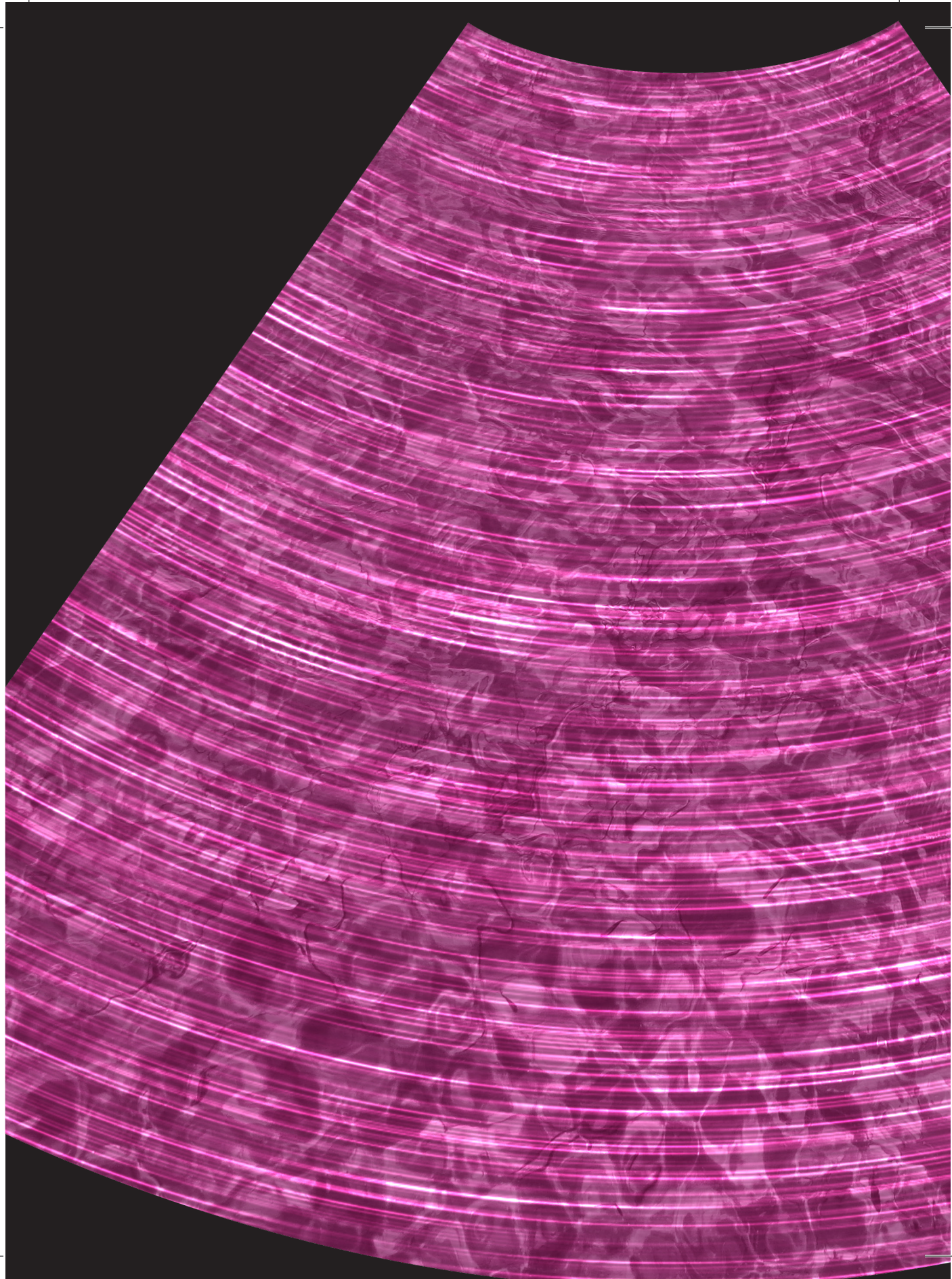
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CHAPTER 1

General introduction

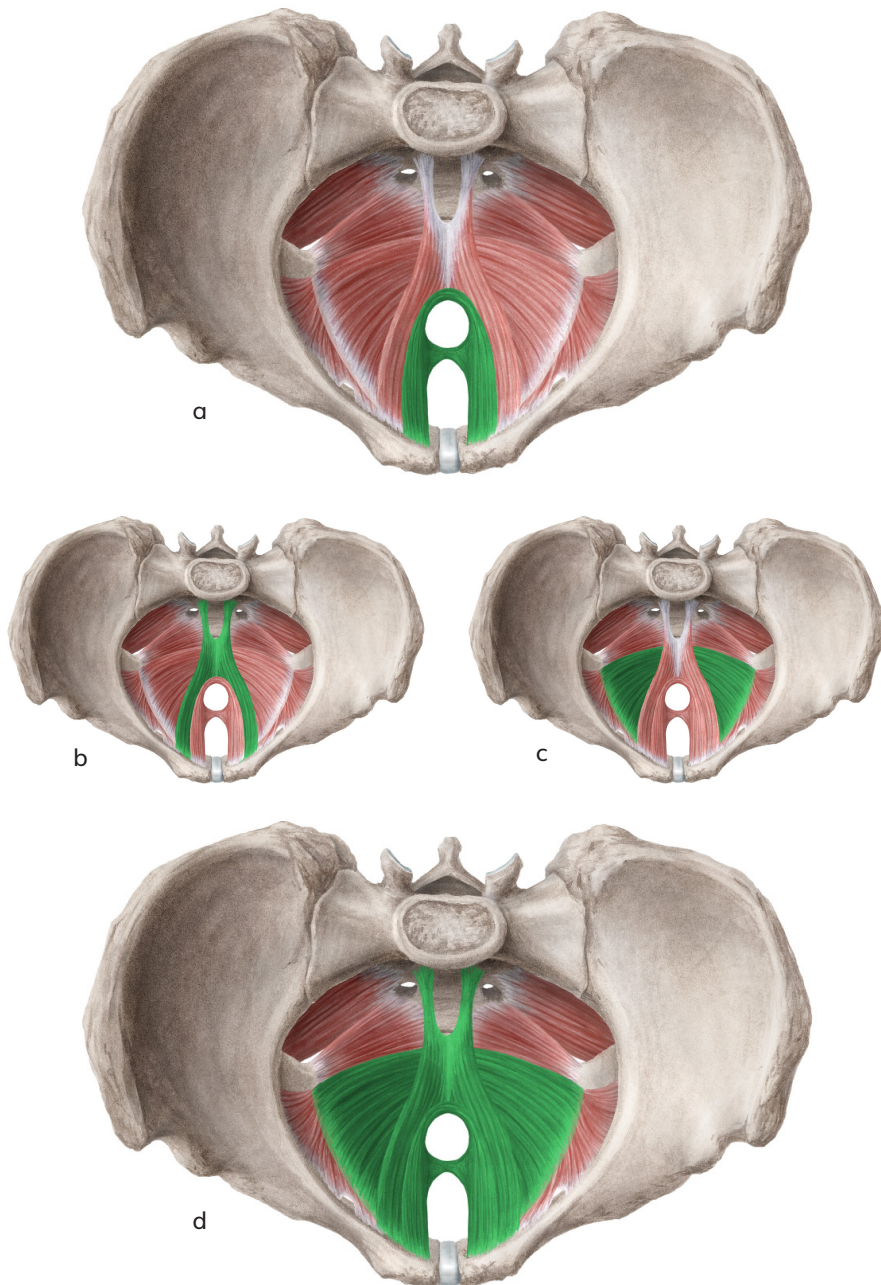


FIGURE 1 Anatomy of the pelvic floor
a Puborectalis **b** Pubococcygeus **c** Iliococcygeus **d** Levator Ani
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Anatomy of the pelvic floor

"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"¹. Dickinson made his observation over a century ago, however the function of the levator ani muscle remains somewhat of a mystery. A study by Kearney and co-workers in 2004 clearly showed that part of the difficulty in understanding the pelvic floor musculature can be found in the description and terminology of the levator ani muscle².

The pelvic floor is formed by the diaphragm pelvis, which consists of the coccygeus muscle, the levator ani muscle (LAM) and fascia covering the muscles. The levator ani muscle is the biggest muscle of the pelvic floor, from its attachment at the pubic symphysis to its sling shape surrounding and supporting the female pelvic organs (the bladder, vagina and rectum). Standardized terminology identifies three major components of the levator ani muscle: pubococcygeus (also known as pubovisceralis), iliococcygealis and puborectalis, as visualized in Figure 1³⁻⁶.

There is a discrepancy in anatomical terms, dividing the levator ani muscle in different parts, more specifically the puborectalis part of the levator ani. Some authors use the term puborectalis muscle to describe all the muscles arising from the pubic bone while others divide it into two components, the pubococcygeus and puborectalis. The discrimination between the muscles can be done based on the orientation of their fibers retrieved from Magnetic Resonance Imaging (MRI) (Figure 2)⁷. Following the differentiation between the pubococcygeus and puborectalis in muscle fiber direction as well as the international standard for anatomical terms (Terminologia Anatomica), we consider this description of muscle anatomy to be the most accurate.

The pubococcygeus and puborectalis muscle originate from either side of the pubic bone and unite with its partner to form a U-shaped sling. The pubococcygeus lies lateral to the puborectalis, arises high on the pubis near the superior pubic ramus and inserts into the vagina, perineal body and anus. The puborectalis arises near the inferior pubic ramus and passes dorsal to the rectum at the anorectal angle

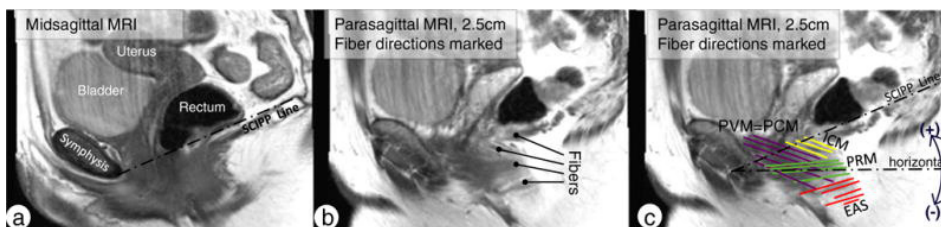


FIGURE 2 **a** Midsagittal MRI view of the muscles from the left side of the pelvis. The sacrococcygeal inferior pubic point (SCIPP) line is drawn in the midsagittal plane and transposed to all parasagittal slides. **b** Fibers are demonstrated (lines with round tips) on a parasagittal slide. **c** Fiber directions were marked and evaluated in respect of the individual SCIPP line and expressed as the angle to the average horizontal line. PVM pubovisceral muscle, PCM pubococcygeal muscle, ICM iliococcygeus muscle, PRM puborectalis muscle, EAS external anal sphincter muscle⁷

just cephalad to the anal sphincter⁸. The iliococcygeus muscle forms a horizontal sheet that spans the pelvic outlet.

Function of the pelvic floor

It is its specific orientation that allows the LAM to provide its two functional characteristics. First, its ability to lift (vertically in the standing posture) the pelvic organs and second its action in closing the levator hiatus (horizontally in standing posture). Betschart and co-workers studied the orientation of the muscle fibers in the different parts of the levator ani⁷. They found that the pubococcygeus has a substantial lifting component while the puborectalis component actually acts in a downward (caudal) direction so that it has no "lifting" action. This would indicate that both the pubococcygeus and the puborectalis contribute to the hiatal closure, but only the pubococcygeus contributes to perineal elevation in normal women. The LAM is thus the main reason that we can stay continent, while standing up right.

The complex interaction between the LAM and intra-pelvic connective tissue in maintaining a normal orientation and function of the pelvic organs has been described as the "ship on the dock" effect. Imagine the bony pelvis is the dock, and the pelvic floor is the ocean, hence the pelvic organs (uterus, bladder, anorectum) the ship, and the ropes tying the ship/bladder to the dock/pelvis are the ligaments and fascia. If there is a weak and loose pelvic floor, so the ocean is resting lower than it should, it is not holding up the ship/bladder. As a consequence, the ship/bladder is just hanging off the dock/pelvis by its ropes/fascia. Over time the ropes/fascia stretch and the ship/bladder sinks lower onto the ocean/pelvic floor, until the support completely fails (Figure 3).

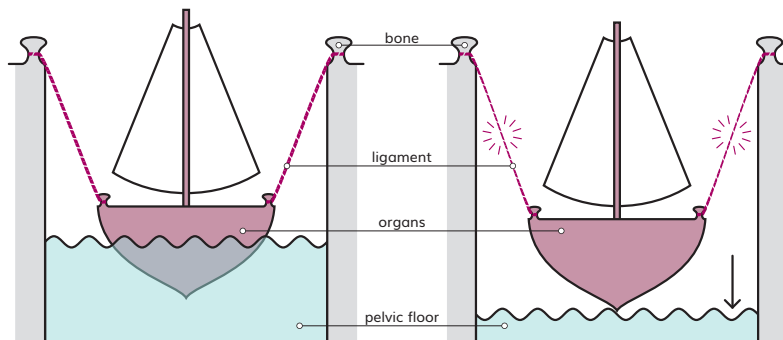


FIGURE 3 Graphic representation of ship on dock effect related to the pelvic floor.

Pregnancy and child birth and the pelvic floor

Dysfunction of the pelvic floor muscles can be caused by instant trauma or long term deterioration. Several studies have shown that aging increases muscle degeneration in general. It has not been described in the pelvic floor muscles, but in other muscles it can be shown by means of ultrasound⁹⁻¹¹. The most common

reason for instant trauma to the pelvic floor muscles is vaginal child delivery. In contrast to its supportive role in non-pregnant women, the LAM needs to relax substantially during childbirth. During pregnancy the LAM, more specific its medial border the puborectalis muscle, needs to prepare for vaginal childbirth. It needs to stretch up to 250% its original length¹² in order to allow passage of the child. This passage of the fetal head could lead to direct crush trauma. Incomplete or insufficient healing of this trauma may lead to persistent dysfunction causing urinary incontinence (UI) or pelvic organ prolapse (POP). However, instant trauma to the muscles does not necessarily directly leads up to POP or UI. This is most likely caused by a compensation mechanism or spare capacity which is lost with aging. The difference in incidence of urinary incontinence between cesarean delivery (no fetal passage) and vaginal delivery has been demonstrated in primiparous women. However a study by van Veelen and co-workers also showed that cesarean delivery does not completely protect against changes in pelvic floor anatomy and function¹³⁻¹⁵.

Prevalence of Urinary Incontinence (UI) and Pelvic Organ Prolapse (POP).

Various definitions of incontinence, measures to quantify urine leakage, severity of incontinence and different populations under study have been described^{16,17}. As a result, a wide range in prevalence urinary incontinence has been reported in literature^{18,19}. In the Netherlands, reports of urinary incontinence indicate a prevalence from 23.5% up to 57% in the age range 45-70 years²⁰.

Comparable to the definition of UI, the prevalence of pelvic organ prolapse also varies widely across studies, depending on the population studied and entry criteria. Women of all ages may be affected, although it is, as is UI, more common in older women. In the Women's Health Initiative study, investigators found a 41.1% prevalence of pelvic organ prolapse at a standard physical assessment in postmenopausal women older than 60 years who had not had a hysterectomy²⁴.

To fully appreciate its societal and economical context, 20% of women will have surgery for one (or both) of these symptoms during her lifetime²¹. In the Netherlands in 2011 a total of 155 million euro is spend each year on incontinence pads²². In addition, UI and POP have been shown to have a profound negative impact on daily functioning and health perception²³.

Risk factors for urinary incontinence and pelvic organ prolapse

The most frequently reported risk factors for developing incontinence and pelvic organ prolapse are: pregnancy, mode of delivery, parity and Body-Mass-Index²⁵⁻²⁷. Increasing parity and vaginal delivery increase the risk for stress urinary incontinence in later life. Especially when assisted vaginal birth (vacuum and forceps extraction) has been performed^{28,29}.

Diagnostics of pelvic floor function

Given the important role of the levator ani muscle in the normal function of the pelvic floor, information on damage and recovery of the muscle seems crucial. On the one hand to investigate whether you can limit damage around childbirth, and on the other hand to promote early recovery after injury. Additionally, more and better information on structure and function of the muscles (e.g. new parameters) may enable clinicians to choose the best treatment possible from a variety of conservative and surgical treatments. Individualizing treatment is more likely to be of benefit as compared to the "one size fits all" approach.

In order to improve our understanding of the structure and function of the levator ani muscle (more specific the puborectalis muscle) we decided to study it during and after first pregnancy. As stated before, during pregnancy and delivery major changes occur in a relatively short period of time. So pregnancy seems to be the ideal time to obtain and analyze information on changes in the levator ani muscle. For this purpose the use of ultrasound imaging has rapidly gained popularity. Since the imaging tool to determine the integrity of the pelvic floor needs to be (repeatedly) usable during pregnancy both CT-scanning (ionizing radiation) and MRI (movement of fetus) have their practical limitations. Pelvic floor ultrasound scanning systems have a short acquisition time, are readily available, relatively cheap, easy to operate, safe, and allow easy acquisition of images over time.

Over the last years transperineal ultrasound has rapidly evolved as a method to evaluate pelvic floor anatomy and function. With the introduction of 3D volume ultrasound scanning, the symphysis pubis, urethra, bladder, vagina, anal canal and parts of the levator ani muscle can be visualized in specific 2D planes, called Tomographic Ultrasound Images (TUI's)³⁰. This was not possible with 2D recordings, given the perpendicular orientation of the muscle to the ultrasound beam. The



FIGURE 4 Tomographic Ultrasound Image (TUI) of the puborectalis muscle. Red arrows indicate the attachment of the muscle to the pubic symphysis

ultrasound examination can be performed with the pelvic floor muscles at rest, on maximum pelvic floor muscle contraction and on maximal Valsalva maneuver. Additionally, the puborectalis muscle can be clearly identified on the ultrasound image (Figure 4), surrounding the hiatal area (rectum, vagina and urethra) and attached on both sides to the dorsal surface of the pubic bone, indicated by the red arrows in Figure 4.

However, the fiber directions of the muscles surrounding the puborectalis muscle, as visualized in Figure 2 cannot be seen on ultrasound. Therefore, it is very difficult to segment the puborectalis muscle on ultrasound with absolute certainty that none of the other muscles of the levator ani muscle are partially included. Nevertheless, even though we cannot segment the puborectalis muscle with 100% certainty, the bright sling on ultrasonography is named the puborectalis muscle.

The puborectalis muscle can be visualized well on ultrasound images, enabling clinicians and researchers to study several aspects. Measurements of the levator hiatus on three/four-dimensional (3D/4D) transperineal ultrasound, and especially changes in these measurements (the genital hiatus and bladder neck mobility^{15,30,31}) during pelvic floor contraction and Valsalva maneuver, have been regarded as measures of levator ani muscle anatomy and function. However, these measurements will only provide us with indirect functional information of the LAM.

Since 2003, 568 articles have been published describing the clinical value of ultrasound when studying the pelvic floor. Although transperineal ultrasound has thus been widely used over the last decade, no systematic review has been published on the diagnostic accuracy of pelvic floor ultrasound imaging. Ultrasound has to be compared to various gold standards, physical examination (POP-Q), urethrocystography, magnetic resonance imaging, defecography, (surface) electromyography and pressure testing^{32–37}. This thesis therefore starts with a systematic review to determine the quality of papers describing the diagnostic accuracy of pelvic floor ultrasound.

Structural (echogenicity) and functional (strain) parameters

Most research on the levator ani muscle provides us with indirect functional information. In the search for more direct information on the structure of the LAM and function of the LAM, we explored two new parameters. We conducted several studies to determine the possibilities of a structural and functional parameter. To define whether the parameters truly can be considered as new added value we tested them for correlations to demographics such as age and Body Mass Index (BMI).

The first parameter studied is the echogenicity of the puborectalis muscles. Echogenicity is based on the greyscale of an image. Every pixel of an ultrasound image represents a value between 0 (black) and 255 (white). Muscle cells are echolucent and appear dark, while connective and fatty tissue appears bright. Echogenicity is used in clinical practice to diagnose neuromuscular diseases in children without the need for tissue biopsy to discriminate between myopathies and neuropathies, in orthopaedics to analyse supraspinatus tendon tears, and

in animal studies as an indicator of muscle healing processes^{10,11,38,39}. To increase our understanding of normal physiological changes in the structure of the pelvic floor, the possibilities of echogenicity as a clinical parameter are defined in a reproducible and quantified way. Since differences in echogenicity caused by probe pressure were described by Odegaard in a different clinical setting before⁴⁰, it is important to study the relation between perineal ultrasound probe pressure and the echogenicity of the puborectalis muscle.

The second parameter is strain. Muscle trauma during delivery is described as a possible cause of postpartum pelvic floor muscle dysfunction (e.g. stress urinary incontinence and pelvic organ prolapse)⁴¹⁻⁴³. Assessing muscular function, like contractility, in a quantitative way is possible by measuring its strain^{44,45}. Strain, in case of muscle tissue, represents the amount of deformation during contraction or relaxation⁴⁶. In cardiology, strain measurements provide important clinical functional information. For instance, longitudinal strain of the left ventricle has proven to be an adequate parameter to diagnose ventricular function⁴⁷. Measuring strain in the puborectalis muscle during and after pregnancy may add to our understanding of the physiological and functional effect of pregnancy and delivery on the pelvic floor.

Aims and outline of this thesis

The studies presented in this thesis focus on composition and functionality of the puborectalis muscle based on calculating echogenicity and strain from transperineal ultrasound recordings. We studied these parameters in a dataset of nulliparous pregnant women as well as postpartum, as a representation of one specific group.

STARD

In Chapter 2 we described our study on determining the compliance of diagnostic accuracy studies investigating pelvic floor ultrasound, with Standards for Reporting of Diagnostic Accuracy (STARD) guidelines by means of a systematic review.

MEAN ECHOGENICITY PUBORECTALIS

In Chapter 3 we evaluate the possibilities of developing a semi-automated method to assess puborectalis muscle echogenicity on 3D/4D volume transperineal ultrasound images using 4D View and Matlab® software and evaluating its intra- and interobserver reliability.

The study in Chapter 4 was designed to investigate the effect of probe pressure, body mass index, age and sport activity on the puborectalis muscle echogenicity and area

In Chapter 5 the results of our study to assess the changes in the Mean Echogenicity of the Puborectalis muscle (MEP) and the Puborectalis Muscle Area (PMA) during first pregnancy and after childbirth are described.

The results on our study evaluating the association between mean echogenicity of the puborectalis muscle, measured using transperineal ultrasound, in women during their first pregnancy and the subsequent mode of delivery are described in Chapter 6.

STRAIN

The results of our study to evaluate the effect of pregnancy and child-delivery on global strain of the puborectalis muscle are described in Chapter 7.

The results of this thesis are summarized and discussed in Chapter 8.

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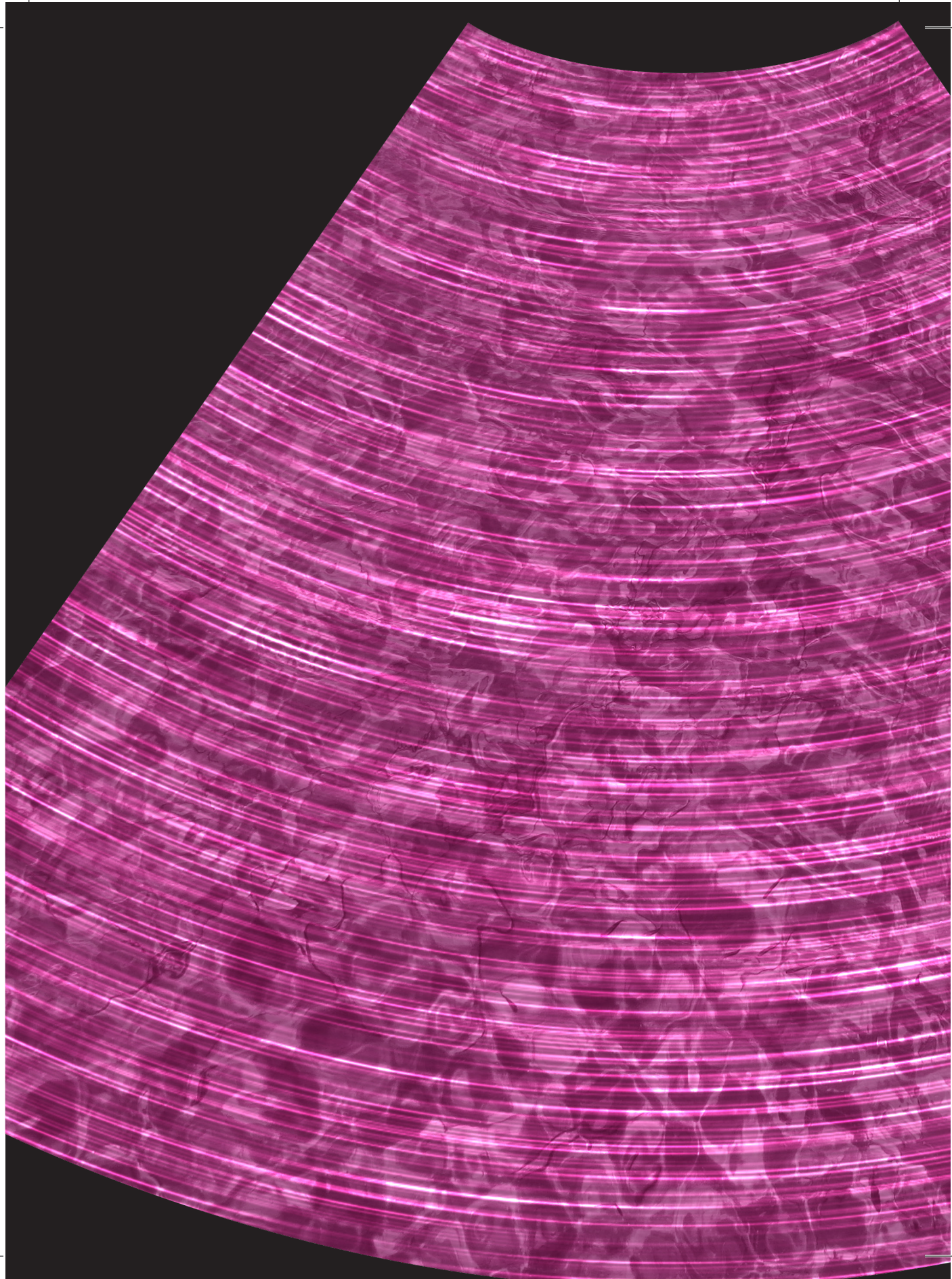
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General introduction







CHAPTER 2

Compliance with STARD checklist among studies of pelvic floor 3D transperineal ultrasound: A systematic review

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Submitted for publication

Abstract

INTRODUCTION In recent years, a large number of studies have been published on the clinical relevance of pelvic floor three-dimensional (3D) transperineal ultrasound. Several studies compare ultrasonography to other imaging modalities or clinical examination. The quality of reporting these studies is not known.

OBJECTIVE To determine the compliance of diagnostic accuracy studies investigating pelvic floor 3D ultrasound, with Standards for Reporting of Diagnostic Accuracy (STARD) guidelines by means of a systematic review.

METHOD This study reviewed published articles on pelvic floor 3D ultrasound identified by means of a systematic literature search that included the MEDLINE database. Prospective and retrospective studies that compared 3D pelvic floor ultrasound with other clinical and imaging diagnostics were included in the analysis. STARD compliance was assessed and quantified by two independent investigators, using 22 of the original 25 STARD checklist items. Items with the qualifier "if done" (items 13, 23 and 24) were excluded because they were not applicable to all papers. Each item was scored as reported (score=1) or not reported (score=0). We calculated observer variability, the total number of reported STARD items per article and summary scores for each item. We also statistically tested the difference in total score between STARD adopting and non-adopting journals, as well as the effect of year of publication.

RESULTS Thirty-two studies published in 12 scientific journals were included in the analysis. The mean score (SD) of articles included was 16.2 (2.4) out of a maximum of 22 points. The lowest scores, below 55%, were found on quality of reporting on handling of indeterminate results or missing responses, adverse events and time interval between tests. Interobserver rating agreement of STARD items was substantial (ICC 0.76). The independent t-test showed no significant mean differences (\pm SD) in total score between the adopting 15.9 (\pm 2.7) and non-adopting 16.3 (\pm 2.3) journals. The mean STARD score for the period 2003–2009 (15.1 ± 2.2) was significantly lower ($p=0.035$) as compared to the period 2010–2015 (16.9 ± 2.3).

CONCLUSION The overall compliance with reporting guidelines of diagnostic accuracy studies of pelvic floor 3D transperineal ultrasound is relatively good, compared to other fields. However specific items should require more attention when reported.

Introduction

In everyday clinical practice history taking and physical examination are often followed by the execution of diagnostic tests. These tests aim to provide additional information on the nature and severity of the disease, and to reduce uncertainty about the diagnosis. In the end, the information gathered by diagnostic tests should improve outcome for the patient to an extent that would not have been reached without the test.

To improve awareness of the quality of reporting and to overcome over- or underestimating the outcome under study, a group of methodological researchers and editors developed the Standards for Reporting of Diagnostic Accuracy (STARD) statement¹. The STARD checklist and the corresponding flowchart, which were published in 2003, are intended to support authors in reporting essential study elements of diagnostic research².

To evaluate the efficacy of an index test in comparison with the reference standard, studies on diagnostic accuracy are executed⁹⁻¹⁵. These reports help clinicians to decide if a diagnostic test is suitable for the disorder of interest. In order to be able to assess the value and to interpret the results of such studies, a clinician should be able to rely on accurate reporting of relevant study characteristics. In addition, a poorly reported article on diagnostic accuracy limits the possibility to identify potential bias and value the report.

Ultrasound as an imaging and diagnostic tool has become more and more important in the field of urogynecology in the recent years. Its clinical ability and added value is used and described in many studies³⁻⁸. Since ultrasound imaging is a diagnostic instrument, information on the sensitivity and specificity of the images and retrieved parameters is crucial, to judge its clinical application

To our knowledge, no evaluation has been performed to investigate STARD compliance in reporting diagnostic studies on 3D ultrasound of the pelvic floor. The objective of this systematic review was to determine the compliance of diagnostic accuracy studies of pelvic floor ultrasound with the STARD guideline.

Materials and Method

DATA SOURCES

To identify papers, a systematic literature search was performed. We searched MEDLINE (by using PubMed), by using a combination of the search terms for the index test (ultrasound), and the anatomy being investigated (pelvic floor). Additional search limits applied were "human", "female", "English" and "01-01-2003–12-31-2015". We only included publications after 2003 since this was the first year that the guidelines were reported. An article was excluded if children were studied, the study was a systematic review, the abstract or manuscript were missing or the technology (3D transperineal ultrasound) was incorrect..

PAPER SELECTION

We first screened titles and then abstracts and in a second step, read all full-text articles to evaluate all remaining potentially eligible studies for inclusion and exclusion criteria. Articles had to meet the following inclusion criteria: the study

focus needs to be on one or more of the four major pelvic floor lines of interest (bladder neck mobility, genital hiatus, avulsion or prolapse), or related medical term. Studies with a predictive design were excluded since these manuscript should be checked on quality of reporting following the TRIPOD (Transparent Reporting of a multivariable prediction model for Individual Prognosis Or Diagnosis) guideline. TRIPOD as a guideline is comparable to STARD, but with a focus on reporting full information on all aspects of a prediction modelling study, risk of bias and potential usefulness of the prediction model. When the study only focused on reliability of one technique, or the primary aim was to determine the clinical value of ultrasound imaging (e.g. "Is there an avulsion?"), the study was excluded.

DATA EXTRACTION

We evaluated the quality of reporting by using the standard 25 questions STARD checklist. STARD is designed as a checklist to improve the completeness and transparency of reporting studies of diagnostic accuracy, to allow readers to assess the potential for bias in the study (internal validity) and to evaluate its generalisability (external validity). In this study, as did Walther in 2014, we changed the "design" of STARD in two ways. First we eliminated three items compared to the original checklist and second, we used a checklist meant for author publishing guidelines as a retrospective scoring instrument. We eliminated the same (if done) checklist items from scoring (13, 23 and 24) as did Walther and Wilczynski^{16,17}. We did this given the same argument "If these items were not reported in the diagnostic accuracy papers evaluated. It would be impossible to determine whether this lack of reporting was because the item was not done or because it was not reported. Our design changes from the study by Walther and co-workers, since they additionally eliminated item 9 from analysis since all papers scored a full 100% on this item¹⁶. And our study differs from the study by Wilczynski and co-workers, because they only studied items "that have been empirically shown to have a potentially biasing effect on the results of diagnostic accuracy studies and those items that appear to account for variation between studies"¹⁷.

The STARD checklist is known to have good reproducibility^{16,18}. The included articles were independently scored by the same two reviewers blinded for each other's results. After scoring 12 papers, a consensus meeting was scheduled to make sure the perception of the STARD criteria context was aligned between reviewers, and to discuss potential discrepancies. After the consensus meeting both reviewers independently evaluated the remaining papers. Discrepancies in the analysis were resolved in consensus.

DATA AND STATISTICAL ANALYSIS

Each STARD item was scored as reported (score = 1) or not reported (score = 0). Equal weights were given to all items. The total score for each article according to the STARD checklist was calculated by summing the awarded points for the 22 items included. Items were considered well reported when they were found in more than 80% of the papers and were poorly reported when the items was found in less than 50% of the papers.

Disagreement between reviewers , as a measure of subjectivity of the assessment, was calculated by using Cohen k statistics. For calculating the Cohen k statistics, only the remaining papers after the consensus meeting were included. According

to Landis and Koch¹⁹, a k value of 0.41–0.60 indicates moderate agreement between the reviewers; a k value of 0.61–0.80 substantial agreement and a k value of 0.81–1.00, almost perfect agreement.

Normally distributed data are reported as means \pm standard deviations (SD), and percentages are reported with 95% confidence intervals (CI). To test for differences in total scores between STARD adopting and non-adopting journals, we performed an independent samples t-test. To determine whether a journal was STARD adopting, the online author guidelines per journal were checked in July 2016. To check for the correlation between year of publication or Impact Factor and total STARD score the Pearson correlation coefficient is calculated. In addition, an independent t-test is performed to compare the STARD score for the first period of the study (2003–2009) and second period (2010–2015). Statistical analyses were performed with statistical software (SPSS Statistics 20).

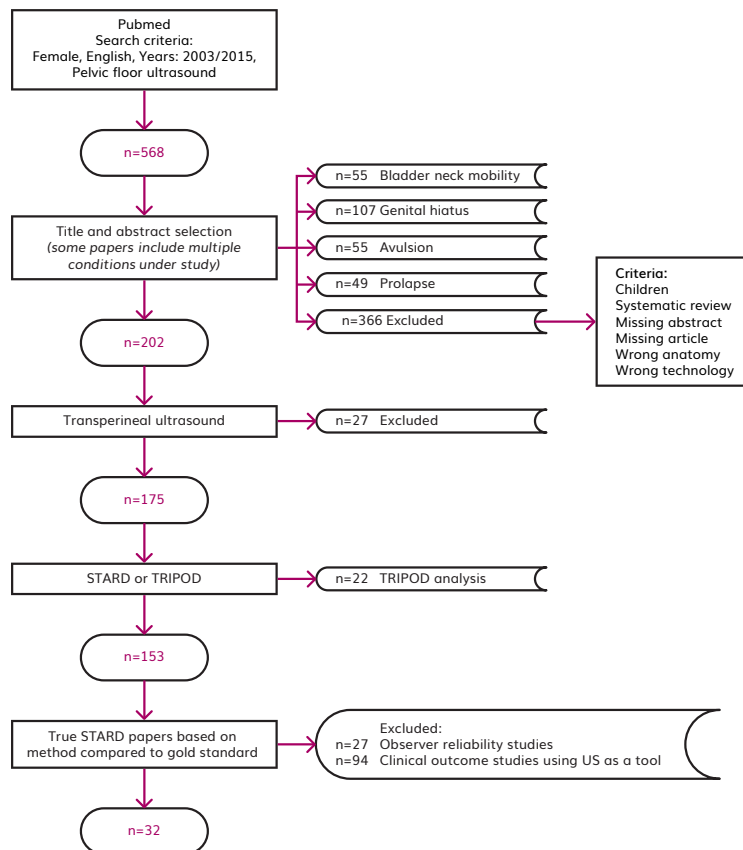


FIGURE 1 Flow diagram of manuscript selection proces

TABLE 1 The reporting of the STARD items

Item	Topic	Reported (%)
1	Identify the article as a study of diagnostic accuracy (recommended MeSH heading 'sensitivity and specificity')	31 (97)
2	State the research questions or study aims, such as estimating diagnostic accuracy or comparing accuracy between tests or across participant groups	31 (97)
3	Describe the study population: the inclusion and exclusion criteria, setting and locations where the data were collected	22 (69)
4	Describe participant recruitment: was recruitment: based on presenting symptoms, results from previous test, or the fact that the participants had received the index tests or the reference standard	28 (88)
5	Describe participant sampling: was the study population a consecutive series of participants defined by the selection criteria in items 3 and 4? If not, specify how participants were further selected	18 (56)
6	Describe data collection: was data collection planned before the index test and reference standard were performed (prospective study) or after (retrospective study)?	31 (97)
7	Describe the reference standard and its rationale	30 (94)
8	Describe technical specification of material and methods involved including how and when measurements were taken, and/or cite references for index tests and reference standard	31 (97)
9	Describe definition and rationale for the units, cutoffs and/or categories of the results of the index tests and the reference standard	28 (88)
10	Describe the number, training and expertise of the persons executing and reading the index tests and the reference standard	23 (72)
11	Describe whether or not the readers of the index tests and the reference standard were blind (masked) to the results of the other test and describe any other clinical information available to the readers	23 (72)
12	Describe methods for calculating or comparing measures of diagnostic accuracy, and the statistical methods used to quantify uncertainty (e.g. 95% CI)	28 (88)
14	Report when study was done, including beginning and ending dates of recruitment	22 (69)
15	Report clinical and demographic characteristics of the study population (eg age, sex, spectrum of presenting symptoms, comorbidity, current treatments, recruitment centers)	32 (100)
16	Report the number of participants satisfying the criteria for inclusion that did or did not undergo the index tests and/or the reference standard; describe why participants failed to receive either test (a flow diagram is strongly recommended)	21 (66)
17	Report time interval from the index test to the reference standard, and any treatment administered between	14 (44)
18	Report distribution of severity of disease (define criteria) in those with the target condition; other diagnoses in participants without the target standard	18 (56)
19	Report a cross tabulation of the results of the index tests (including indeterminate and missing results) by the results of the reference standard; for continuous results, the distribution of the test results by the results of the reference standard	30 (94)
20	Report any adverse events from performing the index test or the reference standard	1 (3)
21	Report estimated of diagnostic accuracy and measures of statistical uncertainty (eg 95%CI)	24 (75)
22	Report how indeterminate results, missing responses and outliers of the index tests were handled	0 (0)
25	Discuss the clinical applicability of the study findings	31 (97)

Results

The systematic literature search yielded 568 eligible papers. After scoring the abstracts on the conditions under study (bladder neck mobility, avulsion, prolapse and genital hiatus) 366 papers were excluded, leaving 202 papers for further analyses. Twenty-seven studies did not report on 3D/4D transperineal ultrasound and 22 studies were prognostic (TRIPOD scoring list) instead of diagnostic studies, leaving 153 papers. Of these 153 papers 27 studies were observer reliability studies, and 94 studies did not compare ultrasound to another diagnostic method (e.g. POP-Q, MRI), leaving 32 papers based on transperineal ultrasound imaging compared to a gold standard (STARD)^{20-43,43-51}. Figure 1 shows a flowchart representing the inclusion of papers.

Overall, the STARD score of the included papers ranges from 11 to 21 with a mean of 16.2 (SD=2.4). The reporting of each item is presented in Table 1. The best reported item was item 15, which refers to reporting demographic characteristics of the study population. All papers fulfilled the requirements for scoring on this item. Other well reported items (>80%) were item 1, 2, 4, 6, 7, 8, 9, 12, 19 and 25. Three items (17, 20 and 22) were especially poorly reported (<50%). None of the papers mentioned how indeterminate results were handled and only one paper describes the occurrence of an adverse event. Only 44% of all papers mentioned the time interval between tests, 56% reports on distribution of severity of disease and 56% describes whether the study population was a consecutive study or how patient were further selected.

The 32 papers were published in 12 different medical journals, of whom four journals (11 papers) advised the authors to use the STARD checklist in their author guidelines (Table 2). The independent t-test showed no significant mean differences (\pm SD) in total score between the adopting 15.9 (\pm 2.7) and non-adopting 16.3 (\pm 2.3) journals. We found no correlation between total STARD score and the Impact Factor (Pearson correlation 0.00). Over time the mean STARD score per year significantly increased (Pearson correlation coefficient 0.52, $p=0.002$) shown in Figure 2. The mean STARD score for the period 2003–2009 (15.1 ± 2.2) was significantly lower ($p=0.035$) as compared to the period 2010–2015 (16.9 ± 2.3).

Overall agreement of the reviewers in scoring the STARD items was 90.3% The k value was 0.76 (95% CI: 0.73–0.79), indicating substantial agreement between the reviewers. The items with the highest disagreement were items 5 (consecutive patient sampling), 17 (time interval reporting), 18 (distribution of severity of disease) and 19 (reporting of a the distribution of test results) with ICC values of 0.51; 0.62; 0.52 and 0.30 respectively.

The STARD checklist strongly recommends the use of a flow diagram to illustrate the key elements of the study design and the patient flow though the study setup, such a diagram was only provided in one paper.

Discussion

In this study we assessed the quality of reporting of pelvic floor 3D ultrasound. The results of our study indicate that the quality of diagnostics accuracy reports

is fair but not optimal. The mean score of 16.2 out of 22 points indicates that there is room for further improvement. However, within the timeframe we selected for our review we could demonstrate that the adherence to the STARD criteria is significantly improving. Interestingly, papers in journals who explicitly state in their guidelines to authors that diagnostic study reports should adhere to the STARD criteria, do not perform better as compared to journals who do not mention the STARD criteria. As it could be that a STARD adopting journal had only very recently become an adopter, and that the papers that were included in the analysis were published before that, it would have been even more interesting to check the author guidelines at time of the accepted studies. This however requires per journal an overview of changed author guidelines, we are not able to access this information.

To the best of our knowledge this is the first time that the reporting of quality of pelvic floor 3D ultrasound as a diagnostic tool has been studied. When we compare our findings to other papers on the quality of reporting diagnostic accuracy in other fields of medicine, we found that our average score of 16.2 out of 22 points (74%) was relatively high. In the paper by Zafar and co-workers a different scoring scale was used, but with a mean score of 19.8 out of a 50 points (40%) their relative score is poorer. The same accounts for the study performed by Areia and co-workers, who used the original, 25-points scale. They found a mean

TABLE 2 Medical journals included - representing number of papers included in STARD analysis, compliance to STARD in author guidelines and Impact Factor 2015

Journal	Frequency (%)	STARD compliance	Impact Factor
American Journal of Obstetrics & Gynecology	1 (3.1)	Yes	4.681
Australian and New Zealand Journal of Obstetrics and Gynaecology	3 (9.4)	No	1.738
BJOG: An International Journal of Obstetrics and Gynaecology	2 (6.3)	Yes	4.039
British Journal of Radiology	1 (3.1)	No	1.840
European Journal of Obstetrics & Gynecology and Reproductive Biology	1 (3.1)	No	1.662
Female Pelvic Medicine & Reconstructive Surgery	1 (3.1)	No	1.331
International Journal of Colorectal Disease	2 (6.3)	No	2.383
International Urogynecology Journal	10 (31.3)	No	1.834
Medical Ultrasonography	1 (3.1)	No	1.167
Neurology and Urodynamics	2 (6.3)	No	3.128
Obstetrics & Gynecology	1 (3.1)	Yes	5.656
Ultrasound in Obstetrics & Gynecology	7 (21.9)	Yes	4.254

score of 12.2 out of 25 points (49%)^{12,14}. Several explanations for this difference can be given. First the other studies were performed in 2010 (including published articles from 1998 to 2008) and in 2008 (including published articles from 1995 to 2006) and as we also have shown, the quality of reporting in the early period was significantly poorer as compared to the later period. The second explanation could be that we scored to liberal or other authors to strict. Since the STARD criteria leave room for interpretation this potential bias cannot be ruled out. Since our interobserver reliability was good we believe our results accurately represent the current status of reporting diagnostic studies in pelvic floor ultrasound. Finally, it is not always obvious, based on title or abstract, that the paper is a diagnostic study and therefore need to be checked for the STARD criteria. This is supported by the fact that journals who specifically state that STARD criteria needed to be used, did not perform better than other journals who did not mention STARD in the author guidelines.

When comparing the scores on individual items to the studies by Paranjothy (2007), Areia (2010) and Zafar (2008) we found that the items 17, 20 and 22 were consistently poorly reported (<50%)^{10,12,14}. Especially items 22 "Report how indeterminate results, missing responses and outliers of the index tests were handled", is one of crucial information since it is related to the potential risk of selection bias. Authors should inform their readers on this in a consistent way.

To reduce our own observer bias in our evaluation of the quality of reporting, each paper was independently evaluated by two reviewers. The interrater reproducibility indicated substantial agreement. This is in line with the results of Smidt and co-workers¹⁸, who investigated the interrater reproducibility of the STARD checklist for evaluating studies of diagnostic accuracy. However, certain items were more reliably scored than others. Items, that in itself contained multiple questions were found more difficult to score with a 0 or 1. These items (e.g. 3 and 17) needed more discussion to reach consensus between the reviewers.

Our analysis was based on the original STARD checklist that was published in 2003. However, last year, the STARD group published an updated STARD checklist and most STARD adopting journals now recommend to use the updated version⁵². We deliberately scored all papers based on the 2003 STARD guidelines. As we believe that scoring a paper based on a checklist that was not available at the moment of writing the original study is not valid.

Our study had some limitations. The first relates to the scoring method of granting 0 or 1 point per item. When multiple items were described within one STARD item a paper was granted 1 point when at least one of the items was described in the text. Following the scoring as described by Zafar and co-workers (score 2: completely reported; score 1: partly reported and score 0: not reported) might have provided more detail, however this approach reduces the observer reliability¹⁴. A second limitation of our study was that the exclusion of three items represents a deviation from the original checklist. Nevertheless, we believe the exclusion was justified, because the "if done" items 13, 23 and 24 were not applicable to all studies. A third limitation can be found in the definition of the STARD adopting journals. We checked the author guidelines, but not other parts of the journal's website. We defined this way, since this will be most likely be the path followed by future


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researchers. However the STARD guidelines could have been placed on other parts of the journal's website. A final limitation was our choice to limit our search to the English language report, although we believe that inclusion of studies published in other languages would not alter our conclusion.

In conclusion, our study demonstrates that the overall compliance with reporting guidelines of studies addressing diagnostic accuracy of pelvic floor ultrasound has improved over time and is relatively good compared to other fields of medicine. However specific items should require more attention when reported.


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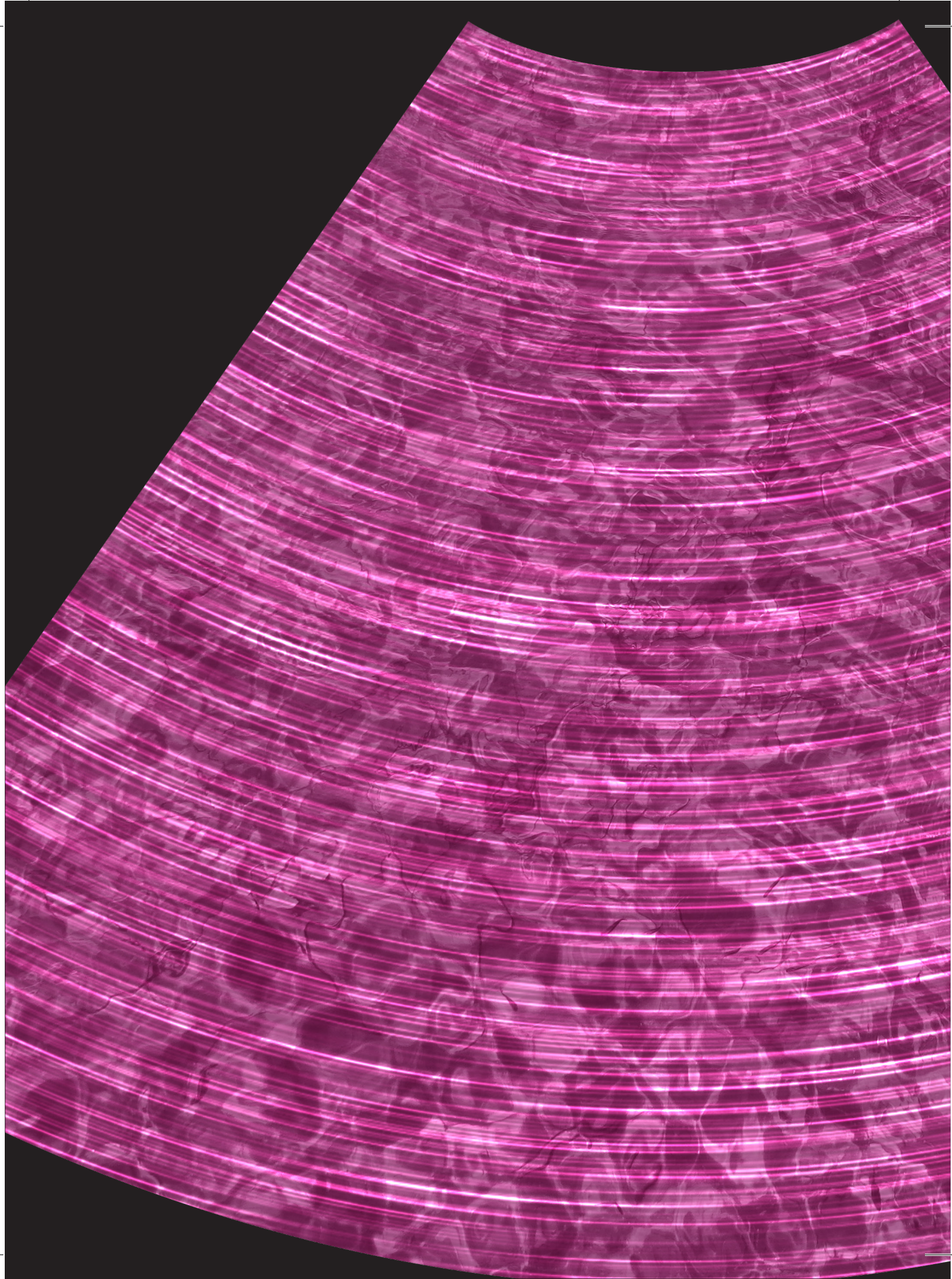
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CHAPTER 3

Measuring echogenicity and area of the puborectalis muscle: method and reliability

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Abstract

OBJECTIVES

To develop a semi-automated method to assess puborectalis muscle echogenicity on three-dimensional/four-dimensional (3D/4D) volume transperineal ultrasound images using 4D View and Matlab® software and evaluate its intra- and interobserver reliability.

METHOD

The data of 23 women in their first trimester were included. 3D/4D volume datasets were obtained at rest. Two inexperienced observers were trained by an experienced observer to construct tomographic ultrasound images (TUI) from the original data and to delineate all structures. Puborectalis muscle area (PMA) and the mean echogenicity of the puborectalis muscle (MEP) were calculated offline. Intra- and interobserver reliability were determined by intraclass correlation coefficients (ICC) and their 95% CIs.

RESULTS

The development of a semi-automated method to calculate puborectalis area and echogenicity is described in detail. PMA and MEP measurements in pregnant women demonstrated almost perfect intraobserver reliability for both inexperienced observers, with ICC values ranging from 0.88 to 0.99. The interobserver reliability showed ICCs of 0.63 for PMA and almost perfect ICC values, of 0.96–0.98, for echogenicity. The majority of intraobserver mismatch between two delineations of PMA occurred near the borders.

CONCLUSIONS

Matlab® software can be used to provide reliable measurements of the area and echogenicity of the puborectalis muscle. As the latter can be used to assess structural changes in the puborectalis muscle, it appears a promising new tool for studying pelvic floor structural anatomy.



Introduction

In recent years, three-dimensional (3D) and four-dimensional (4D) volume transperineal ultrasound imaging have become increasingly popular for studying pelvic floor anatomy, allowing visualization of the pelvic floor in the axial plane¹. The axial plane is used to measure dimensions of the pelvic floor anatomy, such as the area of the puborectalis muscle, area of levator hiatus and minimal hiatal distances². Although the measurement of dimensions of the pelvic floor is well developed, identifying structural changes in the puborectalis muscle, apart from levator avulsions, is still in its infancy²⁻⁵. One option to assess muscular structure in a more quantitative way is to measure its echogenicity^{6,7}. Echogenicity measurements are already being used in the diagnosis and evaluation of neuromuscular disorders, as well as in orthopedics^{8,9}. This study was designed to develop and test the reliability of a semi-automated method to measure mean echogenicity of the puborectalis muscle (MEP).

Method

Development of our method to measure echogenicity was carried out as part of a subanalysis of a large study in our University Hospital. Over a period of 2 years, 280 nulliparous pregnant women were seen for 3D/4D transperineal ultrasound assessment of their pelvic floor anatomy during and after pregnancy. For our research question we used the 4D ultrasound data subsets of 23 randomly chosen women with a singleton pregnancy at approximately 12 weeks' gestation. Women were excluded if they had a medical history of urinary and/or fecal incontinence, previous prolapse or anti-incontinence surgery, connective tissue disease or neurological disorders. The Institutional Human Research Ethics Committee approved the study and all women gave informed consent.

Sonographic assessment consisted of 4D transperineal ultrasound imaging using a GE Voluson 730 Expert system (GE Medical Systems Zipf, Austria) with a RAB 8–4 MHz curved array volume transducer. The angles of the acquired volume were set 85° longitudinal and 70° transverse to the probe, and the depth of the volume varied per measurement. A temporal resolution of 3 Hz was used to acquire the data, and settings that could influence the intensity values were kept constant for each measurement. These settings were: gain, 15; power, 100; Harmonics, mid; contrast, 8; gray map, 4; persistence, 8; and enhance, 3. All pelvic floor ultrasound examinations were performed with the participants supine and with an empty bladder². The ultrasound probe was placed on the perineum in the sagittal plane and measurements made with musculature at rest were used for our analysis. The datasets were stored on a hard disk for analysis offline.

Offline analysis of the data was performed using 4D View 7.0 (GE Medical Systems) and Matlab® R2010a (MathWorks, Natick, MA, USA) by two inexperienced observers (A.G. and A.V.) and one experienced observer (K.S.). The inexperienced observers were trained in two sessions, covering 20 cases, by the experienced observer. Image analysis was performed by first determining and fixating the point of time of total muscle relaxation (4D data turned to 3D data). The plane of minimal hiatal dimensions was selected as previously described^{2,10,11}. This plane

was used to obtain tomographic ultrasound images (TUI) in the axial direction. The first slice in which the symphysis seemed closed was used for analysis^{5,10}. This two-dimensional (2D) ultrasound image contained 1304×662 pixels and was exported as a .bmp file to Matlab® R2010a (Image Processing Toolbox 7.0).

Figure 1 shows the semi-automated method used to select the puborectalis muscle and levator hiatus in three steps. The Matlab® function *imfreehand* was used for delineation. First, an outer border around the area of interest, consisting of the puborectalis muscle and levator hiatus, was drawn (Figure 1, Step 1a). All data outside this area of interest were eliminated and turned black (Figure 1, Step 1b). The second step was to draw a line to select the levator hiatus. This line followed the inner border of the puborectalis muscle, pubic symphysis and inferior pubic ramus (Figure 1, Steps 2a and 3a). The third step was to select the puborectalis muscle by drawing two lines at the attachment of this muscle to the symphysis (Figure 1, Step 3b). The resulting image represents the area of the puborectalis muscle that is automatically calculated in cm^2 . Differences in measurements may occur if markers are positioned in areas with less well-defined demarcation. In order to analyze this mismatch area we obtained an overlay of two delineation attempts on the same puborectalis muscle image. When a pixel was included in a delineation on both attempts, the pixel turned black, whereas if the pixel was delineated in only one of two attempts, the pixel retained its original (gray) color. The qualitative analysis is based on identifying the largest areas of mismatch

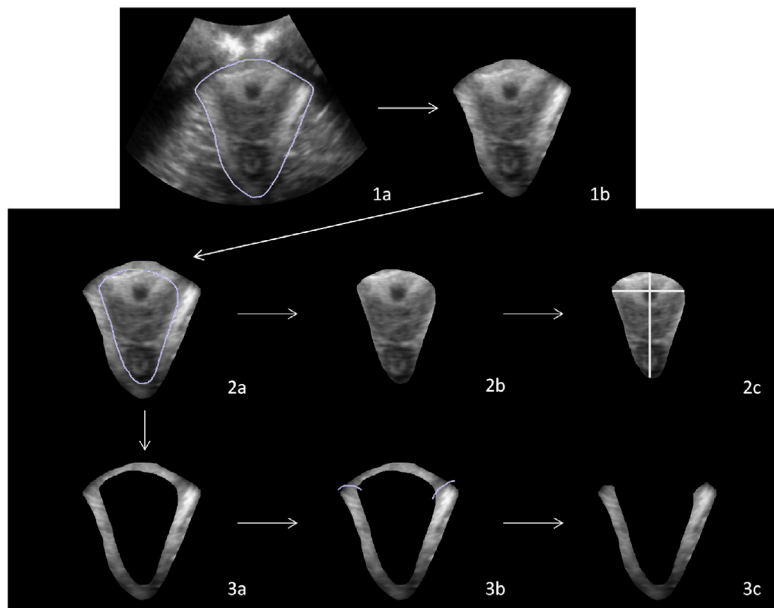


FIGURE 1 Semi-automated method using three steps (Steps 1–3) to select the puborectalis muscle and levator hiatus for assessment of area and echogenicity. Steps 1a and 1b: the area of interest is delineated. Steps 2a and 2b: the levator hiatus is delineated. Step 2c: minimal hiatal dimensions are visualized. Steps 3a–3c: the puborectalis muscle is delineated

between two delineation attempts. Quantitative analysis was performed by dividing the area of the mismatch (in pixels) by the total puborectalis muscle area (PMA) (in pixels).

Determination of echogenicity was based on the gray-scale image, in which the value for each pixel could range from 0 (black) to 255 (white). Normal muscle cells are rather echolucent and appear dark on the image. The connective and fatty tissues of the muscle have a higher echogenicity and appear brighter¹². The mean of all the pixel echogenicity values of the puborectalis muscle was calculated automatically, and is referred to subsequently as MEP.

The reliability of measuring PMA and MEP was tested in both intra- and interobserver series, with three independent examiners analyzing the patient's randomly ordered datasets. There was a 4-day time window between the repeat measurements and analysis of the offline dataset. At the time of the second delineation, the observers were blinded to the outcome of the first delineation and the datasets were again ordered randomly.

STATISTICAL ANALYSIS

Statistical analysis was performed using SPSS v. 20 (SPSS Inc., Chicago, IL, USA) and Excel 2010 (Microsoft Office, Microsoft Corp, Redmond, WA, USA). Means, SD and intraclass correlation coefficients (ICCs) with their 95% CIs were used to compare the datasets and validate the delineation. The ICC results were classified

TABLE 1 Intra- and interobserver reliability of delineation of pelvic floor musculature at rest

Intra-observer reliability				
Parameter	Observer	Mean (±SD)	ICC (95% CI)	
PMA (cm²)	A.G.	8.0 (1.6)	0.91 (0.80–0.96)	
	A.V.	9.2 (2.3)	0.88 (0.72–0.95)	
	K.S.	8.1 (2.0)	0.94 (0.85–0.97)	
Mean echogenicity	A.G.	128 (19)	0.99 (0.96–0.99)	
	A.V.	126 (19)	0.98 (0.96–0.99)	
	K.S.	126 (19)	0.99 (0.97–0.99)	
Inter-observer reliability				
Parameter	Observer	Mean Difference (SD)	ICC (95% CI)	LOA (lower to upper)
PMA (cm²)	A.G./A.V.	−1.17 (1.56)	0.69 (0.39–0.85)	−4.29 to 1.96
	A.G./K.S.	−0.08 (0.94)	0.87 (0.71–0.94)	−1.96 to 1.80
	A.V./K.S.	1.08 (1.86)	0.63 (0.31–0.84)	−2.63 to 4.80
Mean echogenicity	A.G./A.V.	2.53 (5.0)	0.96 (0.88–0.98)	−7.49 to 12.55
	A.G./K.S.	1.76 (4.0)	0.97 (0.93–0.99)	−6.27 to 9.78
	A.V./K.S.	−0.77 (4.2)	0.98 (0.94–0.99)	−9.12 to 12.55
Observers: A.G. and A.V., inexperienced; K.S., experienced				
ICC, intraclass correlation coefficient; LOA, limits of agreement; PMA, puborectalis muscle area				

Observers: A.G. and A.V., inexperienced; K.S., experienced
ICC, intraclass correlation coefficient; LOA, limits of agreement; PMA, puborectalis muscle area.

according to the subgroups defined by Landis and Koch, in which ICC values below 0.00 were considered poor, 0.00–0.20 slight, 0.21–0.40 fair, 0.41–0.60 moderate, 0.61–0.80 substantial and 0.81–1.00 almost perfect¹³. To evaluate the mean difference and limits of agreement (LOA) between observers, the Bland–Altman method was used¹⁴. A 95% CI for the bias was used to test for significance, to verify that the bias did not differ from zero.

Results

The mean age of the women was 30.9 (SD=3.8) years and their mean body mass index (BMI) was 23.3 (SD=4.3). Means with SD, ICC values with 95% CIs and mean differences with limits of agreement for PMA and MEP are shown in Table 1. Additionally, in Figure 2 the Bland–Altman LOAs for PMA measurements are shown.

PMA AND ECHOGENICITY

The PMA had an almost perfect intraobserver (ICC: 0.88–0.94) and a moderate to almost perfect interobserver (ICC: 0.63–0.87) reliability. The MEP measurement showed almost perfect intra- and interobserver ICCs (range, 0.96–0.99).

AREA OF MISMATCH

Figure 3 shows the differences in puborectalis muscle delineation caused by a mismatch in marker positioning. The left and center images show the two separate measurements and the right shows the mismatch. The mismatch tended to occur at the outer border of the puborectalis muscle. The total area of the mismatch ranged from 2 to 12%.

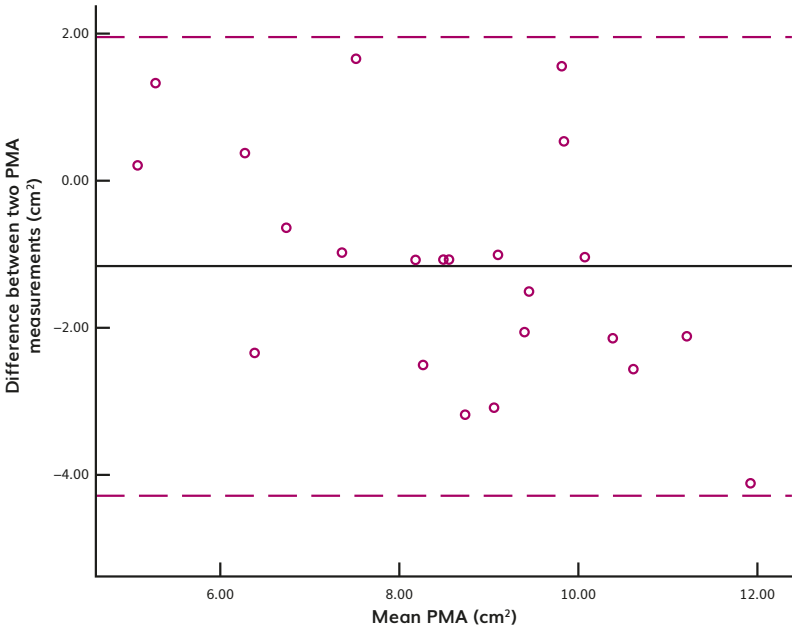


FIGURE 2 Bland–Altman analysis of puborectalis muscle area (PMA) measurements by Observer A.G., showing mean bias and limits of agreement

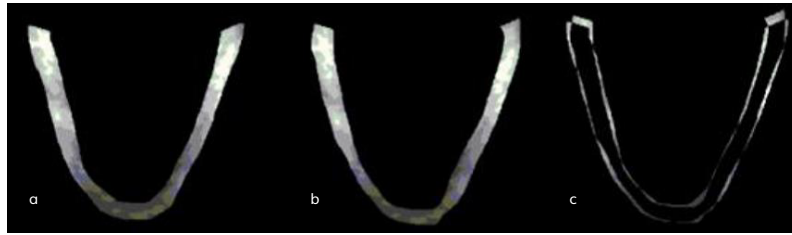


FIGURE 3 Qualitative analysis of the largest area of mismatch (c) between two delineation attempts (a and b). The mismatch occurs at the outer border of the puborectalis muscle and the total area of mismatch ranged from 2% to 12%.

Discussion

Our study shows that the area of the puborectal muscle and its echogenicity can be measured reliably using 3D/4D pelvic floor ultrasound combined with Matlab® software. Inexperienced observers were able to perform these measurements adequately after 20 training sessions.

To appreciate our findings, some possible limitations need to be discussed. First of all, we included healthy pregnant nulliparous women without previous delivery trauma to their pelvic floor. This provided us with high-quality images of intact pelvic floor musculature, which might have improved the accuracy of measurements. Another limitation is that two inexperienced observers (A.G. and A.V.) were trained by the same experienced observer (K.S.), which might have introduced instructor bias. However, training was given according to universally accepted pelvic floor ultrasound image-analysis guidelines (TUI reconstruction). Studying gray-scale images introduces another possible drawback. Different settings, within or between the ultrasound system(s), may produce different gray-scale images. This issue has been recognized and conversion equations can be applied to address this problem¹⁵. In our study we used deliberately the same specific settings in all measurements to ensure that the changes we observed in MEP were not affected by this potential bias. Finally, although we blinded the observers to the first set of measurements, the limited sample size introduces the risk of recall bias. We tried to limit this by using a 4-day time window between the time of image delineation and a separate random analysis of the two image sets.

One of the major strengths of our study is that we introduced a new parameter, MEP, into the research area of studying pelvic floor anatomy. We showed that measurements of MEP can be performed reliably.

The interobserver reliability of measuring PMA had the lowest ICC value. As shown in Figure 3, this is most probably caused by mismatching the outer border of the puborectalis muscle. Clear landmarks, such as dark edges indicating the border, were often not found, forcing the observers to delineate the structure more arbitrarily. This resulted in an average difference of 300–2000 pixels between measurements. Relative to the total number of pixels in the PMA, this accounts for 2–12% of the total area. However, the almost perfect MEP ICC values show that this mismatch did not affect the mean echogenicity measured.

Adding computer software to the delineation process of structures in the pelvic floor decreases the likelihood of human error and might also result in a reduction in the time taken for the procedure as all parameters can be calculated from one cycle instead of from multiple separate drawings produced by 4D View software. Further (quantitative) research should indicate how much time can be saved by using our method. Applying the software requires limited experience with pelvic floor image interpretation and delineation. However, currently we still need to use two separate software systems (ultrasound and Matlab®).

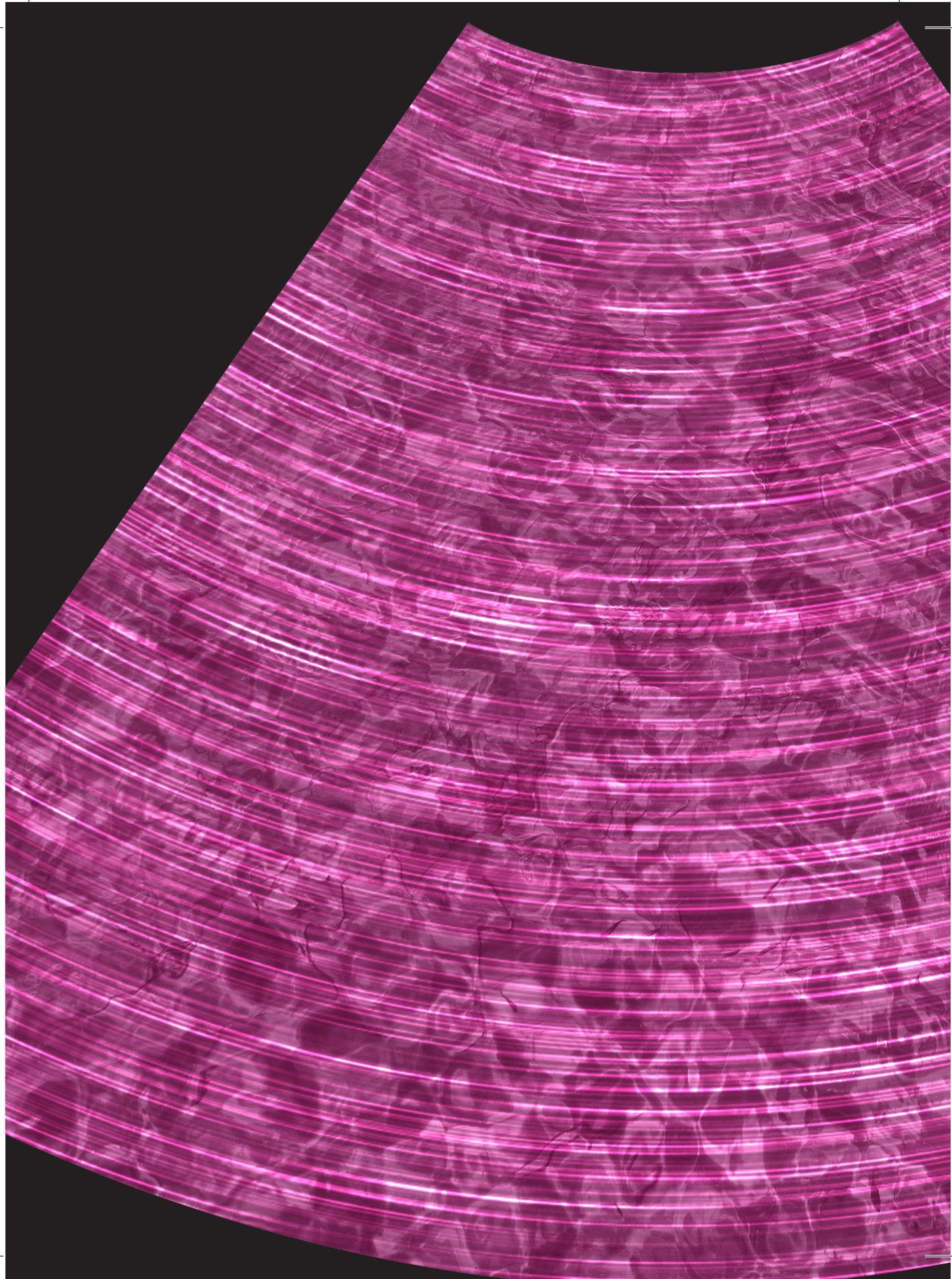
The semi-automatic detection method offers the possibility to study new parameters, such as echogenicity. Three recent studies examining the link between echogenicity and clinical parameters were performed by Tsai and coworkers⁸, Maurits and coworkers^{16,17} and Pillen and coworkers¹⁸. Tsai and coworkers demonstrated that a decrease in the mean gray-level (echogenicity) may be used as a sonographic indicator of rotator cuff partial-thickness tear or tendinopathy of the shoulder⁸. Maurits and coworkers demonstrated that they could separate, almost completely, two types of disorders (myopathies and neuropathies) based on abnormality of ultrasound muscle density and homogeneity^{16,17}. In the study by Pillen and coworkers¹⁸, a comparison was made between the sensitivity and specificity of visual vs quantitative evaluation of skeletal muscle ultrasound in children suspected of having a neuromuscular disorder (NMD). The quantitative analysis resulted in a higher interobserver agreement ($\kappa=0.86$) compared with visual evaluation ($\kappa=0.53$). This indicates that quantification of echo intensity is a more objective and accurate method compared with visual analysis and thus is better suited for the screening task in the diagnostic phase of children with a NMD.

These associations between muscle echogenicity and clinical outcome parameters, as demonstrated in other areas of medicine, may also prove to be useful in pelvic floor research. Measuring echogenicity may add to our understanding of what happens in (sub)total levator ani avulsions or in the recovery process after trauma.

In conclusion, this study showed that 3D/4D ultrasound imaging combined with Matlab® software is a reliable method to delineate structures of the pelvic floor in nulliparous women and measure puborectalis muscle echogenicity. Future studies using this parameter may add to our understanding of pelvic floor structural anatomy and function.

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CHAPTER 4

The effect of probe pressure, body mass index, age and sport activity on the puborectalis muscle echogenicity and area

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Abstract

INTRODUCTION To determine the effect of body mass index (BMI), age, physical activity and ultrasound probe pressure on the Mean Echogenicity of the Puborectalis muscle (MEP) and Puborectalis Muscle Area (PMA).

METHOD Pelvic floor ultrasound volume datasets of 108 women during first pregnancy and postpartum, retrieved from a larger cohort, were studied on the relation between physical conditions (BMI, age, physical) and MEP and PMA. Statistical testing was done by Pearsons' correlation coefficients and an independent T-test. Additionally two experienced examiners were asked to obtain the three-dimensional (3D) and four-dimensional (4D) volume datasets of 15 primiparous women at 6 months postpartum at different probe pressures (soft, normal and hard). Mean differences in MEP and PMA caused by the amount of applied probe pressure were studied by ANOVA (Tukey's post-hoc testing, 0.05 significance level).

RESULTS BMI and age showed poor correlation coefficients with MEP and PMA and there are no significant mean differences in MEP between women who do and do not sport. The PMA was not significantly affected by the amount of applied probe pressure during examination, however the MEP is significantly lower when soft pressure is applied (mean 90, SD=15.3), compared to normal (mean 117, SD=16.0; $p<0.003$) and hard pressure (mean 124, SD=22.3; $p<0.001$).

CONCLUSION BMI, age and physical activity do not affect the MEP and PMA in our study population. Additionally we found that the echogenicity of the pelvic floor musculature can be reliably studied when soft pressure on the ultrasound probe during examination is avoided.

Introduction

Ultrasound imaging is a safe, easy and relatively cheap medical diagnostic tool. The introduction of three-dimensional (3D) and four-dimensional (4D) perineal ultrasonography offers the possibility of observing the axial plane of the female pelvic floor^{1,2}. In this axial plane the levator ani, in particular the puborectalis muscle sling, can be visualised and hiatal distances and area can be reliably measured²⁻⁴. In ultrasound studies there is a growing interest in the structural composition of muscle tissue by measuring its echogenicity. Especially in neuromuscular disease in children, but also in orthopaedics, this echogenicity proved to be a valuable tool to measure muscle damage and disease progression⁵⁻⁸. We recently demonstrated that it is feasible to measure echogenicity of the puborectalis muscle by means of a semi-automatic delineation method in a reliable (both intra and inter-observer) fashion⁹. One of the concerns regarding measuring echogenicity is the effect of probe pressure on the tissue that lies between the probe and the area of interest. Odegaard and co-workers studied the effect of endoscopic ultrasound probe pressure on the appearance of the gastrointestinal wall¹⁰. Other factors that may be associated with muscle echogenicity are Body Mass Index (fat), age (muscle degeneration) and physical activity (muscle composition)^{6,11,12}. The aim of our study was twofold. First, we assessed the association between Body Mass Index (BMI; kg/m²), age and physical activity and the Mean Echogenicity of the Puborectalis muscle (MEP) and Puborectalis Muscle Area (PMA). Second, we set out to determine if probe pressure during image acquisition affects the MEP and PMA.

Materials and Method

STUDY DESIGN

Between April 2009 and February 2011, 280 nulliparous women with a singleton pregnancy were recruited for 3D transperineal ultrasound imaging of their pelvic floor during and after pregnancy. Exclusion criteria were a medical history of urinary or fecal incontinence, previous prolapse or anti-incontinence surgery, connective tissue diseases, neurological disorders and an inability to perform a maximum Valsalva maneuver, because of cardiac or pulmonary disease. The primary objective of the original study was to study possible associations between stress urinary incontinence symptoms and changes in levator ani anatomy and hiatal distances¹³. The current study is a secondary analysis of the data after introducing the concept of echogenicity in a thesis project in 2012, which was online published in 2014⁹. The study was approved by the Institutional Human Research Ethics Committee of our centre and all women gave informed consent.

At the time of the conduction of our present study complete ultrasound datasets from 108 women were available offline and analysed for MEP and PMA. Ultrasound measurements were obtained at 12 weeks of gestation, 36 weeks of gestation and 6 months postpartum. This database was used to assess possible associations between BMI, age and physical activity (this was only recorded at 12 weeks of gestation), and MEP and PMA. In addition, the mean MEP and standard deviation values were used to perform a sample size calculation for the prospective probe pressure study.



Information about physical activity was retrieved from the questionnaire that the women filled in directly after their ultrasound examination at 12 weeks of gestation. The question 'do you exercise' was used to divide the women into active or non-active in sports. Data on age and BMI were collected from the questionnaires and medical files at all three moments of examination.

ULTRASOUND EXAMINATION

Ultrasound images were obtained, with women in supine position and empty bladder, using a GE Kretz Voluson 730 Expert system (GE Medical Systems GmbH, Zipf, Austria) with RAB 8–4 MHz curved array volume transducer. Image settings were set as: gain 15; power 100; Harmonics mid; contrast 8; grey map 4; persistence 8; enhance 3^{1,4,9}. Imaging was performed with pelvic floor musculature at three different maneuvers, rest (R), pelvic floor muscle contraction (C) and Valsalva (V).

To determine the effect of ultrasound probe pressure during examination one trained examiner (GAvV) was asked to perform a pelvic floor ultrasound examination with normal, hard and soft applied probe pressure (respectively NP1, HP1, SP1) to the perineum. The amount of pressure was determined subjectively, where normal was defined as the pressure normally used in practice to obtain high quality images, high pressure as forceful compression of the probe against the skin, and soft as keeping the probe in loose contact with the skin, without applying pressure. A second examiner (KJS) repeated the examination, as an interobserver study, but only with normal pressure (NP2). The probe pressure measurements were all performed at 6 months postpartum because this part of the study was still running at the time of the probe pressure data acquisition.

IMAGE RECONSTRUCTION AND ANALYSIS

Offline image analysis was performed using program software 4D View 7.0 (GE Medical Systems Kretztechnik, Zipf, Austria) and Matlab® R2013a (MathWorks, Natick, MA). Tomographic ultrasound images were constructed from the axial plane^{2,3}. The musculature was delineated with the Image Processing Toolbox 7.0 – *imfreehand* of Matlab®. The MEP and PMA were calculated as previously described⁹. The MEP represents the mean brightness or echogenicity of all pixels in the delineated area of the puborectalis muscle.

SCALE ADJUSTMENT

In 4D View the acquired ultrasound volumes were visualised in a square that always contained the same amount of pixels and pixel size. As the pixel size on the screen remained equal, but the volumes slightly varied in size, the size of the area covered by the pixel varied per volume. Therefore, since we want to use this measurement for calculating dimensions, a scale had to be calibrated per volume to know which distance in centimetres is covered by the length of a pixel. This was done by measuring an arbitrary distance in centimetres in the used volume using 4D View. This image was loaded in Matlab® and the same distance was measured in pixels by calculating the distance between the pixel location at the beginning of the line and the pixel location at the end of the line. The scale was calculated by dividing the distance in centimetres by the distance in pixels. Scale adjustment was not required for calculating echogenicity. To calculate the Puborectalis Muscle Area, the adjusted area of one pixel (cm²) was multiplied by the automatically calculated number of pixels within the region of interest.

SAMPLE SIZE CALCULATION

From the 108 completed datasets we calculated the mean MEP and standard deviation (SD) of the postpartum women (124; SD=21). We arbitrarily considered a difference of 15% between two different probe pressure MEP assessments as clinically relevant. At an alpha of 5% and power of 0.8 we needed to measure 11 women. To account for possible difficulties in data acquisition or analyses we decided to include 15 women in the prospective probe pressure measurement group. We used BMI and age to check for comparability to the main group.

STATISTICAL ANALYSIS

SPSS v. 20 (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. Descriptive statistics were used to describe the population and its variables. We assumed normal distribution of MEP and PMA in the dataset of 108 women, but tested this assumption with Levene statistics on homogeneity in the students T-test and ANOVA models. Pearson's correlation coefficients were calculated between BMI and age versus MEP and PMA. An independent T-test was used to assess the mean differences in MEP and PMA between women who performed physical activity and those who did not. Finally, mean differences in MEP and PMA between different probe pressures were calculated using ANOVA statistics (Tukey's post-hoc testing, alpha set at 5%).

Results

The characteristics of the population are shown in Table 1. There were no significant differences between the main study group and the women included for the pressure probe study.

ASSOCIATIONS BETWEEN MEP AND PMA, AND AGE, BMI AND PHYSICAL ACTIVITY

There are no significant correlations between women's age and MEP or PMA during pregnancy and postpartum (Pearson's correlation coefficient -0.02 to 0.20). The Pearson's correlation coefficient between BMI at 12 weeks, 36 weeks gestation and 6 months postpartum, and the MEP and PMA at the same points in time varied between 0.06 and 0.12 (non-significant).

For women who performed physical activities at 12 weeks gestation, the MEP was 145 (SD=18) and the PMA was 5.2 cm^2 (SD=1.5 cm^2). For women not active in sports these values were MEP 145 (SD=21) and PMA 5.1 cm^2 (SD=1.6 cm^2). The

TABLE 1 Characteristics of the study population

Parameter	Main group – Mean (SD) (n=108)	Pressure group – Mean (SD) (n=15)
Age (years)	31.5 (4.3)	30.9 (4.4)
BMI 12 weeks gestation	23.9 (5.7)	24.2 (3.2)
BMI 36 weeks gestation	29.2 (8.6)	27.6 (3.2)
BMI 6 months postpartum	25.1 (7.3)	24.3 (3.4)

BMI in kg/m^2

mean differences between these two groups were not statistically significant ($p=0.92$ and $p=0.66$ respectively)

PROBE PRESSURE

The means \pm SD of MEP and PMA at different pressures are presented in Table 2. In Table 3 the mean differences and p-values between the four probe pressures, in relationship to MEP and PMA are shown. The only statistically significant finding was the lower MEP associated with soft probe pressure as compared to either one of the other probe pressures ($p<0.003$).

Discussion

We set out to study the potential effect of age, BMI, physical activity and ultrasound probe pressure to the perineum in the assessment of MEP and PMA in pregnant and early postpartum women. We demonstrated that soft pressure produced significantly lower MEP in perineal ultrasonography as compared to normal or hard pressure. The addition of extra pressure to a normal pressure scanning technique does not affect the MEP or PMA. In addition we showed that the MEP and PMA are independent of age, BMI and physical activity in this population.

In order to appreciate our findings some potential limitations have to be addressed. First, the amount of probe pressure (soft, normal or hard) was determined by the examiner's subjective judgement, and this may have introduced examiners' bias. However, in daily practice soft pressure of the ultrasound probe to the skin is usually avoided because it produces low quality images. Since the difference between normal and hard pressure in one examiner is non-significant, and the normal pressure MEP and PMA values between the two examiners are equal, we do not feel that the subjective probe pressure produces a clinical relevant difference. In addition, the subject of interest, the puborectalis muscle, will not be directly compressed by the probe.

A second limitation is that we assessed the association between age and MEP and PMA in a relatively narrow age range. Other studies have shown that muscle degeneration is associated with increasing age and differences in echogenicity^{7,14,15}. So our finding that there is no association between age and MEP and PMA is only valid for the population under study. Another remark and possible limitation of our study is the progress of BMI during pregnancy. Normally an increase in BMI

TABLE 2 MEP and PMA at different probe pressures

Probe pressure*	MEP (mean \pm SD) (n=15)	PMA (mean \pm SD)** (n=15)
NP1	117 \pm 16.0	5.1 \pm 0.8
HP1	124 \pm 22.3	5.5 \pm 0.8
SP1	90 \pm 15.3	4.9 \pm 0.9
NP2	118 \pm 22.3	5.3 \pm 1.2

*NP1=normal pressure applied by examiner 1; HP1=hard pressure applied by examiner 1; SP1=soft pressure applied by examiner 1; NP2=normal pressure applied by examiner 2.

**SD=Standard Deviation; PMA in cm²

represents an increase in body fat, while in our population the majority of BMI increase will be caused by fetal growth and amniotic fluid¹⁶.

Finally we did not assess physical activity into detail, for instance type, intensity and duration. Others have shown that muscle composition differs between athletes and untrained men¹⁷. Proper analysis of the effect of physical activity on MEP in women should be monitored in a quantified setting, e.g. including type and frequency of physical activity. Our study only refers to a population without professional athletes. A major strength of our study is the continuity of examination and image settings. Using the pelvic floor settings we described and keeping these parameters such as gain identical, enabled us to gather a reliable and comparable dataset.

There is little known on the effect of ultrasound probe pressure on tissue characteristics and image parameters. The study by Odegaard and coworkers showed that as transducer pressure against the gastrointestinal wall was increased from 0 to 10 KPa, changes were seen on the images in wall thickness, tissue echogenicity, and the number of layers¹⁰. These results were found using endoscopic ultrasound data, in which direct compression of the probe against the area of interest was examined. The difference in results between the study by Odegaard and coworkers and our study is presumably caused by the distance between the probe and the area of interest. Compression of the puborectalis muscle due to an increased probe pressure is unlikely to occur.

In conclusion, this study showed that BMI, age and physical activity do not affect MEP and PMA in pregnant and post-partum women. Additionally, normal or high pressure of the probe to the perineum does not significantly affect MEP and PMA. Normal scanning practices, with pre-fixed ultrasound equipment parameters, will produce reliable measurements of the MEP and PMA. Using these pre-set settings, the clinical value of these new parameters can be further assessed by different research groups.

TABLE 3 Difference in MEP and PMA at different probe pressures

Probe pressure*		MEP		PMA**	
		Difference (SD)	p-value	Difference (SD)	p-value
NP1	HP1	7.4 (14.0)	0.721	0.4 (0.9)	0.636
	SP1	-26.8 (16.4)	0.002	-0.2 (1.1)	0.915
	NP2	1.5 (21.6)	0.996	0.2 (1.1)	0.971
HP1	SP1	-34.2 (17.3)	0.000	-0.6 (1.2)	0.265
	NP2	-5.8 (24.5)	0.841	-0.2 (1.3)	0.876
SP1	NP2	28.4 (25.1)	0.001	0.4 (1.1)	0.697

*NP1=normal pressure applied by examiner 1; HP1=hard pressure applied by examiner 1; SP1=soft pressure applied by examiner 1; NP2=normal pressure applied by examiner 2.

**SD=Standard Deviation; PMA in cm²

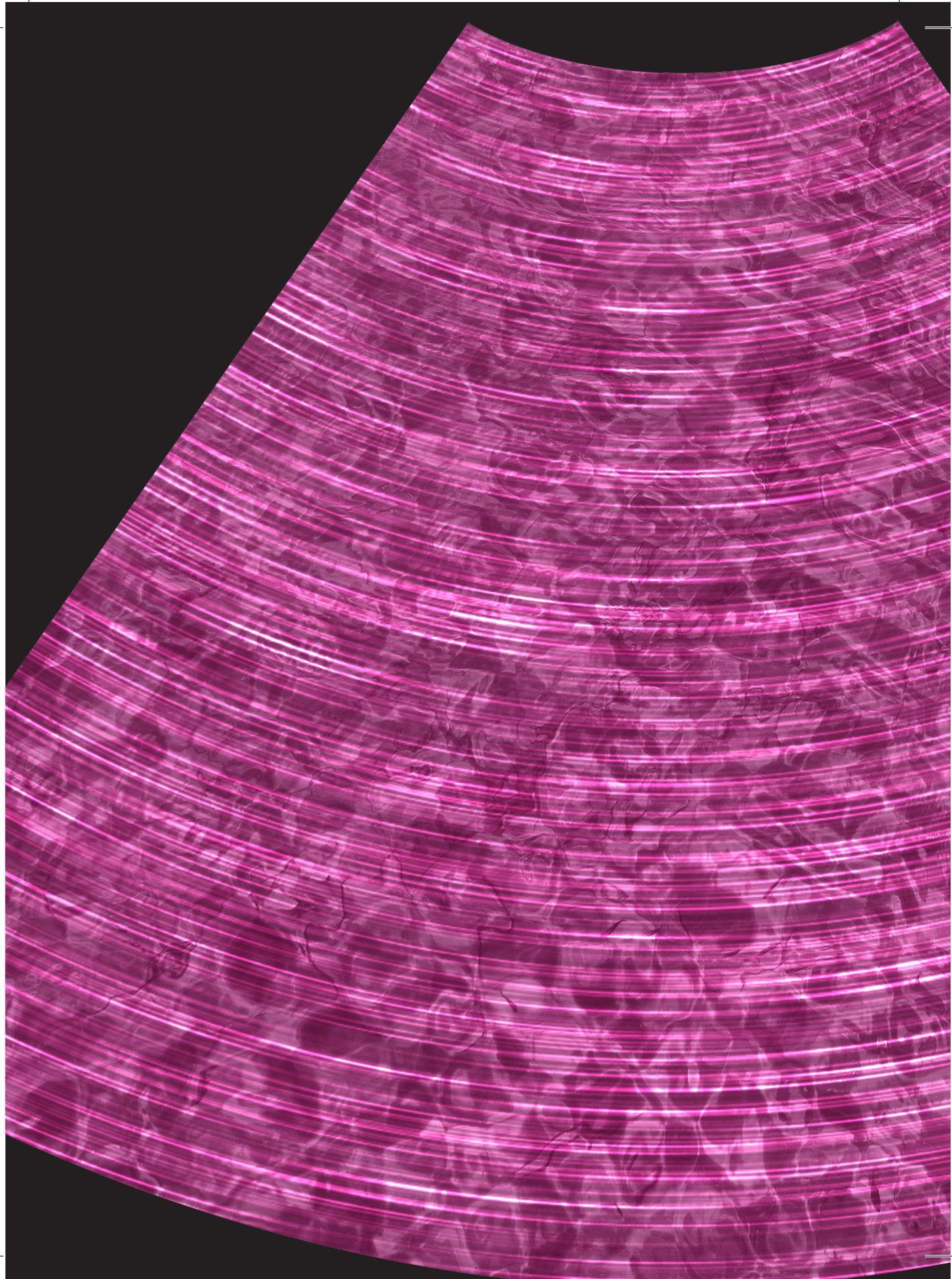


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The effect of probe pressure, body mass index, age and sport activity on the MEP and PMA







CHAPTER 5

Changes in the mean echogenicity and area of the puborectalis muscle during pregnancy and postpartum

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Abstract

INTRODUCTION Three-dimensional (3D) and four-dimensional (4D) volume transperineal ultrasound imaging is increasingly used to assess changes in the dimensions of the pelvic floor during pregnancy and after delivery. Little is known with regard to the area of the puborectalis muscle and its structural changes. Echogenicity measurement, a parameter that provides information on the structure of muscles, is increasingly used in orthopaedics and neuromuscular disease evaluation. This study is aimed at assessing the changes in the mean echogenicity of the puborectalis muscle (MEP) and the puborectalis muscle area (PMA) during first pregnancy and after childbirth.

METHODS The MEP and PMA of 254 women during first pregnancy were measured at 12 and 36 weeks' gestation and 6 months postpartum. To determine the effect of childbirth on MEP and PMA, the results at 6 months postpartum were separately analysed for vaginal deliveries, operative vaginal deliveries (vacuum) and cesarean section deliveries. Mean differences in MEP and PMA were analysed using ANOVA statistics.

RESULTS The MEP at 6 months postpartum was, independent of maneuver, significantly ($p < 0.001$) lower than MEP values during pregnancy. After cesarean delivery, the PMA was significantly smaller at maximum pelvic floor contraction than PMA after vaginal delivery ($p = 0.003$) or operative vaginal delivery ($p = 0.002$).

CONCLUSION Our study indicates that structural changes in the puborectalis muscle during and after pregnancy, as measured by MEP, occur and can be analysed. In addition, the mode of delivery affects the area of the puborectalis during contraction after delivery. For true volume analysis, as part of an assessment of contractility of the puborectalis muscle we will need 3D volume analysis.

Introduction

Pregnancy and childbirth are known risk factors for the development of pelvic floor disorders such as pelvic organ prolapse (POP) and urinary incontinence^{1,2}. Ultrasound imaging, and especially three-/four-dimensional (3D/4D) transperineal ultrasound, has contributed to our understanding of the anatomical changes that are involved in the pathophysiology of these symptoms^{3,4}. At present, data collected with ultrasound are either objective measurements such as hiatal dimension, bladder neck position, and levator urethral gap distances, or subjective observations such as levator ani avulsions or muscular haematomas^{5–9}. Recently, we described a method of reliably measuring the puborectalis muscle area (PMA) and mean echogenicity of the puborectalis muscle (MEP)¹⁰. Echogenicity is used to diagnose neuromuscular diseases in children without the need for tissue biopsy to discriminate between myopathies and neuropathies, in orthopaedics to analyse supraspinatus tendon tears, and in animal studies as an indicator of muscle healing processes^{11–14}.

The levator ani muscle, and especially the puborectalis part, is important in closing the genital hiatus and thereby offering support to the pelvic organs and ligaments¹⁵. Information about the composition of the puborectalis muscle by measuring its echogenicity during and after pregnancy may add to our understanding of the effect of pregnancy and delivery on pelvic floor function.

The aim of our study was to measure changes in the PMA and MEP using 3D/4D transperineal ultrasound in women during and after their first pregnancy.

Materials and methods

STUDY DESIGN AND POPULATION

Over a period of 2 years, 280 nulliparous pregnant women were seen for 3D/4D transperineal ultrasound assessment of their pelvic floor anatomy during and after pregnancy. The current study is part of this larger prospective observational study on the association between pelvic floor symptoms and changes in pelvic floor anatomy during and after first pregnancy^{16,17}. Women were excluded when they had a medical history of urinary and/or faecal incontinence, previous prolapse or anti-incontinence surgery, connective tissue disease or neurological disorders. The Institutional Human Research Ethics Committee approved the study and all women gave informed consent.

ULTRASOUND EXAMINATION

The assessment consisted of 4D transperineal ultrasound imaging using a GE Voluson 730 Expert system (GE Healthcare, Zipf, Austria) with an RAB 8–4 MHz curved array volume transducer. The angles of the acquired volume are set 85° longitudinal and 70° transverse to the probe, a temporal resolution of 3 Hz was used to acquire the data, and settings that might influence the intensity values were kept constant for each measurement (e.g. gain 15, power 100, Harmonics mid, contrast 8, grey map 4, persistence 8, enhance 3, depth 6 cm, 1 focus point, at a fixed height according to preset, time gain compensation (TGC) in a straight line in the centre). All pelvic floor ultrasound examinations were performed with the participants in the supine position and with an empty bladder⁵. The ultrasound

probe was placed on the perineum in the sagittal plane. Measurements were taken with the musculature at rest, during contraction and during Valsalva at approximately 12 weeks' gestation, 36 weeks' gestation and 6 months after delivery. The data sets were stored on a hard disk for offline processing.

IMAGE RECONSTRUCTION AND ANALYSIS

Offline analysis of the data was performed using 4D View 7.0 (GE Medical Systems Kretztechnik, Zipf, Austria) and Matlab® R2010a (MathWorks, Natick, MA, USA) by two of the authors (ATMG and MKW). Observers were blinded to the delivery mode during postprocessing of the data. Image analysis was carried out by first determining and fixing the point of time of the muscle at rest, at maximal muscle contraction and during Valsalva (4D data turned into 3D data). The plane of minimal hiatal dimensions is selected following the guidelines^{5,18,19}. This plane is used to obtain tomographic ultrasound images (TUI) in the axial direction. The first slice in which the pubic bones are closed is used for analysis. This 2D ultrasound image contains 1,304×662 pixels and is exported as a .bmp file to Matlab® R2010a (Image Processing Toolbox 7.0).

DELINEATION OF STRUCTURES

The region of interest (ROI), the puborectalis muscle, was delineated semi-automatically using the software Matlab® (function *imfreehand*) as described previously and visualised in Figure 1¹⁰. The PMA is calculated by multiplying the number of pixels within the ROI with the size of one pixel (cm²).

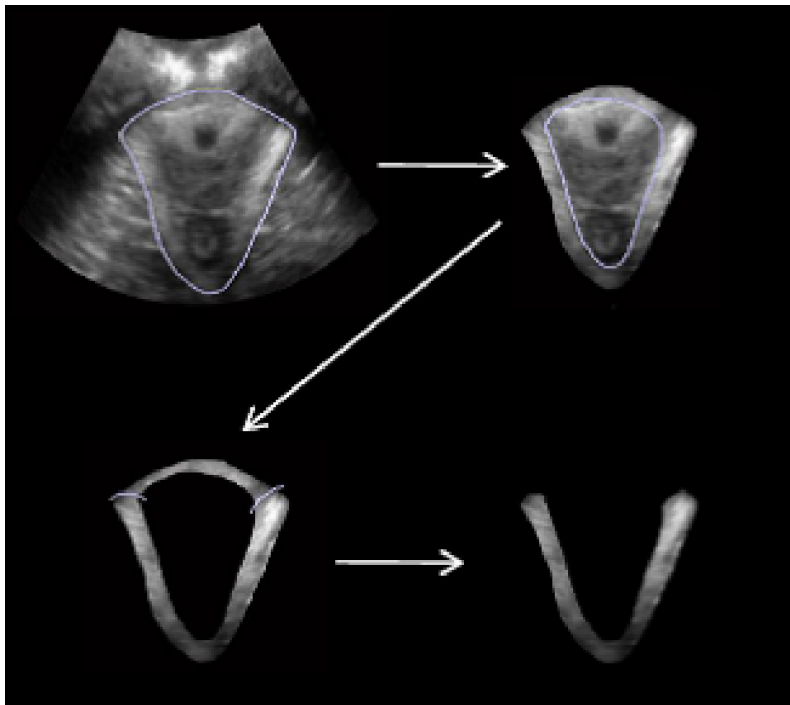


FIGURE 1 Delineation of the puborectalis muscle by hand

Echogenicity is based on the greyscale image in which the value for each pixel can vary between 0 (black) and 255 (white). Normal muscle cells are echolucent and appear dark on the image. The connective and fatty tissues around and within the muscle have a higher echogenicity and appear brighter²⁰. The MEP was determined automatically by calculating the sum of the echogenicity of each pixel and dividing that number by the number of pixels. We recently demonstrated that the interobserver reliability is moderate for measuring PMA and almost perfect for the MEP¹⁰. For both PMA and MEP the intraobserver reliability is almost perfect.

STATISTICAL ANALYSIS

Data collected at 12 and 36 weeks' gestation and 6 months postpartum were compared. The data at 6 months postpartum were separately analysed for women who delivered vaginally, women who had an operative vaginal delivery (vacuum) and those who delivered by cesarean section. The PMA and MEP of the groups were compared using ANOVA statistics. To correct for the number of comparisons made, the level of statistical significance level was adjusted using the Bonferroni method. (ANOVA $p=0.05/9$ comparisons, Bonferroni-adjusted $p=0.006$).

Results

Of the 280 women recruited from the clinic, 26 were excluded. Two women were incorrectly included (1 had a twin pregnancy and the other had a neurological disorder), 1 woman had an immature delivery at 19.9 weeks' gestation, 17 women were excluded based on loss to follow-up and/or missing ultrasound volume datasets because of technical errors during file saving (at least 2 out of 3 datasets were missing), and 6 datasets were excluded because the symphysis was located outside the view of the ultrasound images.

The mean age of the women was 31.1 years (SD: 4.1) and their mean body mass index (BMI) at 12 weeks', 36 weeks' gestation and 6 months postpartum was 23.4 (SD=3.9), 27.6 (SD=3.8) and 24.0 (SD=3.9) kg/m² respectively. Mean gestational age at first visit (weeks) was 13.3 (SD=1.9), at second visit 36.0 (SD=0.9) weeks and 40.2 (SD=1.6) weeks at delivery.

Of the 254 women included, optimal data analysis at 12 weeks' gestation was possible for 247 cases at rest, 240 during contraction and 223 during Valsalva. At

TABLE 1 MEP values during pregnancy and postpartum

MEP	At 12 weeks gestation		At 36 weeks gestation		At 6 months postpartum					
					Vaginal Delivery		Vaccum Delivery		Cesarean section	
	n	mean (SD)	n	mean (SD)	n	mean (SD)	n	mean (SD)	n	mean (SD)
Rest	247	141 (20)	219	148 (20)	144	128 (21)	40	130 (17)	43	127 (20)
Contraction	240	133 (21)	206	138 (21)	121	122 (23)	33	122 (23)	41	116 (23)
Valsalva	223	135 (21)	194	134 (23)	114	115 (22)	31	113 (20)	41	123 (21)

MEP=Mean Echogenicity of the Puborectalis muscle; SD=standard deviation

36 weeks' gestation these numbers were 219, 206 and 194, and postpartum 226, 195, 186 respectively.

Of the 254 women included, 47 (18.5%) underwent a cesarean section, 157 (61.8%) had a spontaneous vaginal delivery, 45 (17.7%) had an operative vaginal delivery (15 based on fetal distress, 15 based on failure to progress, 9 based on a combination of failure to progress and fetal distress and 6 with an unknown reason for vacuum extraction) and in 5 patient files the mode of delivery was not recorded.

In the cesarean group, 11 women had an elective cesarean section, 14 had an emergency cesarean section owing to foetal distress and 17 had an emergency cesarean section because of failure to progress. Additionally, in 5 women the indication for the cesarean section was fetal distress combined with failure to progress.

MEP

The MEP values at 6 months postpartum were all, independent of maneuver, significantly lower ($p < 0.001$) than MEP values during gestation (Table 1; Figure 2). No differences were found between the different modes of delivery. In addition, MEP values were significantly higher at 36 weeks' gestation than at 12 weeks, with the pelvic floor muscles at rest and during contraction.

PMA

Tables 2 and 3 show that women at 6 months postpartum who had a cesarean section have a significantly smaller puborectalis muscle area during contraction compared with vaginal delivery ($p = 0.003$) and operative vaginal deliveries ($p = 0.002$; Figure 3). There was no significant difference in PMA between the vaginal delivery group and women who had an operative vaginal delivery. The

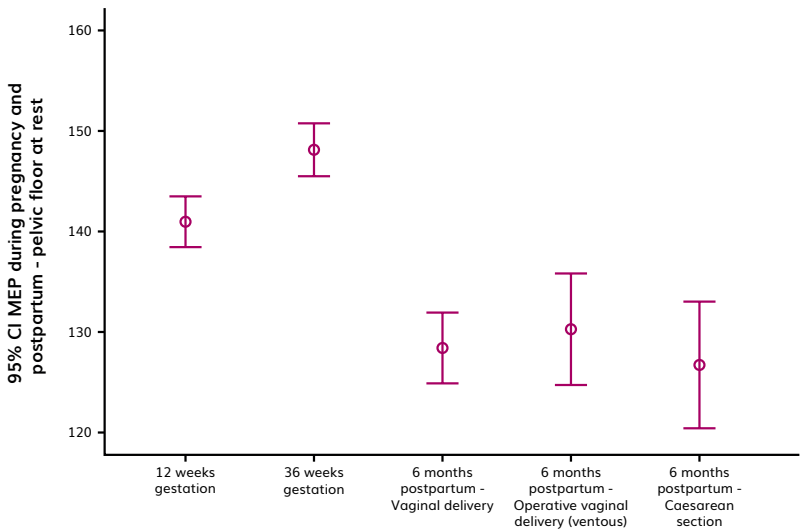


FIGURE 2 Mean echogenicity of the puborectalis muscle (MEP) during pregnancy and postpartum: pelvic floor at rest. *CI: confidence interval*

PMA at 6 months postpartum is significantly larger at rest ($p=0.004$), during contraction ($p=0.004$) and during Valsalva ($p=0.001$) compared with PMA at 36 weeks' gestation. Additionally, the PMA at 12 weeks' gestation during Valsalva is significantly larger ($p<0.001$) than PMA at 36 weeks' gestation.

Discussion

We studied the effect of pregnancy and delivery on the MEP and PMA using 3D/4D transperineal ultrasound. During pregnancy MEP significantly increased from 12 to 36 weeks' gestation when measured with the pelvic floor muscle at rest and during contraction. After delivery MEP significantly decreased compared with pregnancy values. We observed a significantly smaller PMA during contraction at 6 months after cesarean delivery than in women who had a vaginal delivery.

To appreciate our findings several issues need to be discussed. The observation that there is a significantly higher MEP during pregnancy than postpartum may be explained in several ways. The first, and most suggestive, explanation is that during pregnancy MEP increases as a result of changes in the intramuscular balance between muscle cells and connective or fatty tissue, in favour of the more echogenic connective or fatty tissue. This theory is supported by the observation that as early as the first stage of gestation, when fetal growth is very limited; the body aims to store nutrients for future demands²¹. The increased pregnancy levels of progesterone act as an insulin antagonist, which causes, together with the increased intake of nutrients, an increase in intracellular and intramuscular fat storage²². This observation is supported by the work of Herrera, who reported accumulation of fat during pregnancy in humans and in rats²¹. As Reimers and co-workers described that fat replacement is the main cause of increased muscle echogenicity, this increased lipogenesis may well explain our findings of the significant increase in echogenicity during pregnancy and the decrease at 6 months after delivery²³. It would have been instructive if baseline values for nulliparous non-pregnant women had been available; however, these were not obtained in this study. The second possible explanation for the changes in MEP lies in the distance and angle between the ultrasound probe and the target organ, the puborectalis muscle. When ultrasound waves travel through a deeper or different tissue composition, the returning waves have different characteristics. These differences could result in a change in echogenicity²⁴. However, the effect

TABLE 2 PMA values during pregnancy and postpartum

PMA	At 12 weeks gestation		At 36 weeks gestation		At 6 months postpartum					
					Vaginal Delivery		Vacuum Delivery		Cesarean section	
	n	mean (SD) (cm ²)	n	mean (SD) (cm ²)	n	mean (SD) (cm ²)	n	mean (SD) (cm ²)	n	mean (SD) (cm ²)
Rest	247	5.62(1.35)	219	5.85(1.33)	144	5.48(1.35)	40	5.88(1.55)	43	5.17(1.20)
Contraction	240	5.07(1.21)	206	5.33(1.25)	121	5.10(1.35)	33	5.43(1.66)	41	4.38(1.10)
Valsalva	223	5.84(1.35)	194	6.36(1.42)	115	5.83(1.43)	31	6.28(1.63)	41	5.71(1.41)

PMA puborectalis muscle area; SD standard deviation

of increasing probe pressure to the women's perineum, shortening the distance between the probe and the puborectalis muscle, produced no significant changes in MEP when at least normal pressure to the perineum was applied²⁵. The effect of the transducer angle to the muscle should also be considered. Given that the reflection of an acoustic ultrasound wave is strongest when the propagation direction of the wave is perpendicular to muscle fibres²⁶, this would suggest a different orientation of the ultrasound beam to the fibres of the puborectalis muscle during pregnancy. We found no supporting literature to indicate a major difference in muscle angle during pregnancy; therefore, the expected difference in angulation of the puborectalis muscle in comparison to the ultrasound beam is minor.

The final possible explanation for the difference between MEP during gestation and at 6 months after delivery is that delivery itself changes MEP. In this scenario we assume that the pre-pregnancy MEP is equal to the MEP during pregnancy, and that delivery trauma itself results in a decrease in MEP. As we did not observe a difference in MEP between women who had a vaginal delivery and those who had a cesarean delivery, we do not find this hypothesis plausible.

We found a significantly smaller PMA during contraction, but not at rest, at 6 months after cesarean delivery compared with vaginal delivery. This outcome should be interpreted within the perspective of the physical law of consolidation of mass. This law indicates that a decrease or increase in volume, in our case the puborectalis muscle, in two directions, will always affect the volume in the third dimension in the opposite way. The smaller muscle area during contraction indicates that in the plane we analysed the puborectalis muscle could be contracting better after a cesarean delivery than after a vaginal delivery. However, for true volume

TABLE 3 Mean difference in PMA and MEP values during pregnancy and postpartum; Mean difference in MEP and PMA value between different types of delivery (Vaginal–Vacuum and Vaginal–Cesarean section)

	12 – 36 weeks gestation	36 weeks – 6 months postpartum (general)	12 weeks – 6 months postpartum (general)	6 months postpartum		
				Vaginal versus Vacuum	Vaginal versus Cesarean	Vacuum versus Cesarean
	Mean difference (p-value)	Mean difference (p-value)	Mean difference (p-value)	Mean difference (p-value)	Mean difference (p-value)	Mean difference (p-value)
Rest						
MEP	6.88(<0.001)	–19.6(<0.001)	–12.7(<0.001)	–1.86(0.61)	2.2(0.53)	3.55(0.40)
PMA (cm ²)	–0.23(0.07)	0.37(0.004)	0.14(0.25)	–0.40(0.11)	0.39(0.09)	0.71(0.02)
Contraction						
MEP	5.2(0.005)	–17.1(<0.001)	–11.9(<0.001)	0.09(0.98)	6.8(0.10)	6.55(0.22)
PMA (cm ²)	–0.26(0.02)	0.37(0.004)	0.11(0.37)	–0.32(0.18)	0.71(0.003)	1.04(0.002)
Valsalva						
MEP	–0.5(0.821)	–17.8(<0.001)	–18.3(<0.001)	1.56(0.72)	–8.1(0.03)	–9.50(0.05)
PMA (cm ²)	–0.52(<0.001)	0.49(0.001)	–0.03(0.84)	–0.45(0.14)	0.21(0.39)	0.57(0.11)

MFP mean echogenicity of the puborectalis muscle; PMA puborectalis muscle area

MEP mean echogenicity of the puborectalis muscle; PMA puborectalis muscle area

analysis, as part of an assessment of contractility of the puborectalis muscle we will need 3D volume analysis. The same issue of consolidation of mass holds true for explaining the larger PMA at 12 weeks' gestation during Valsalva compared with 36 weeks' gestation.

We did not find a significant difference in MEP or PMA between the vaginal delivery and operative vaginal delivery. This indicates that the passage of the child's head itself causes the changes in PMA, and that this effect is not exaggerated by the use of instrumental delivery. In the literature there is some controversy about the longterm consequences of a vacuum delivery. In multiparous women the vacuum delivery was reported to be associated with pelvic floor symptoms²⁷. In contrast, a study performed in primiparous women showed no additional effect of a vacuum delivery on symptoms²⁸. This would be in line with our results, showing no difference in PMA and MEP between vacuum and normal vaginal delivery.

A limitation of this study was that we could not collect complete datasets for all women included. Some women withdraw from the study (because of pregnancy related problems or non-attenders), others had an early delivery (before the second visit) or were lost to follow-up without reason. The second reason for incomplete datasets was the inability of some women to perform a maximum contraction or Valsalva. As described by Orejuela and co-workers, a maximum Valsalva should at least have a duration of >6s²⁹. We did not specify this duration in our study protocol. Together with our clinical observation that thirdtrimester pregnant women are less willing or are unable to perform a maximum Valsalva, we believe that our data on Valsalva have to be interpreted with caution.

One of the major strengths of our study is its prospective design, including measurements at 12 and 36 weeks' gestation, but also postpartum at 6 months after delivery. Additionally, the large sample size and fixed ultrasound device

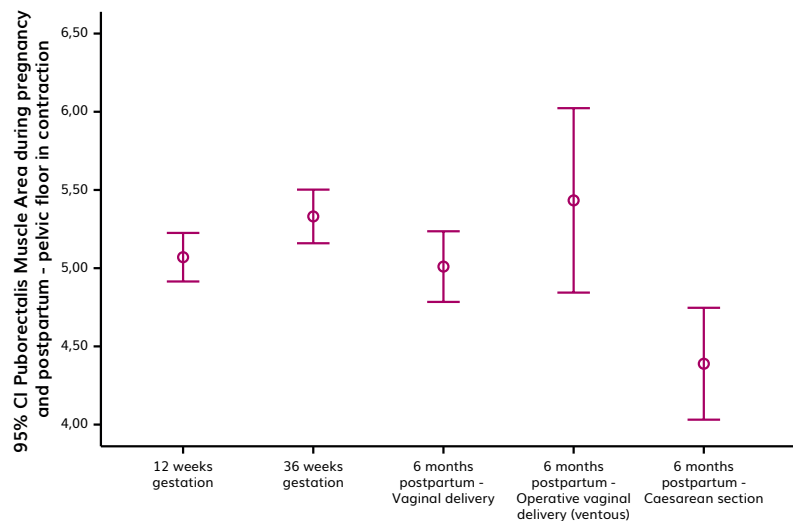


FIGURE 3 Puborectalis muscle area (PMA) during pregnancy and postpartum: pelvic floor during contraction. *CI: confidence interval*

settings are the key elements of this study. The key element in reproducing our echogenicity measurements is the use of identical system settings. Most segmentation of the TUIs and analyses of the echogenicity is done by the computer, decreasing the observer variability.

When comparing our study results with those of the current literature we found that measuring echogenicity of the puborectal muscle is new in urogynaecology, but has been shown to be clinically relevant in other fields of medicine, for instance, as an indicator of rotator cuff partial-thickness tear or tendinopathy of the shoulder and discrimination between myopathies and neuropathies in children^{12,14}. In the current literature Weinstein and co-workers reported puborectalis muscle areas of $4.8 \text{ cm}^2 \pm 2.4$ at rest and $5.3 \text{ cm}^2 \pm 2.1$ during contraction³⁰. In both maneuvers our results are slightly higher. One explanation could be that we studied nulliparous pregnant women, whereas Weinstein and coworkers studied only non-pregnant women. Another explanation could be that they measured the PMA by subtracting the inner from the outer hiatal area, instead of using the numbers of pixels in the delineated muscle area for the calculation. In our previous paper we described that the mismatch between two measurements occurred along the border of the delineated area, and was between 8 and 15%.

In conclusion, this study shows that puborectalis muscle echogenicity, as an indicator of muscle composition, significantly changes during pregnancy and after delivery. The next step is to investigate whether these changes might be associated with urogenital symptoms or pregnancy outcome. As measuring echogenicity has been shown to be of practical use in other fields of medicine, further exploring its potential value seems warranted. The most critical issue remains the use of identical settings of the software to be able to compare future research.

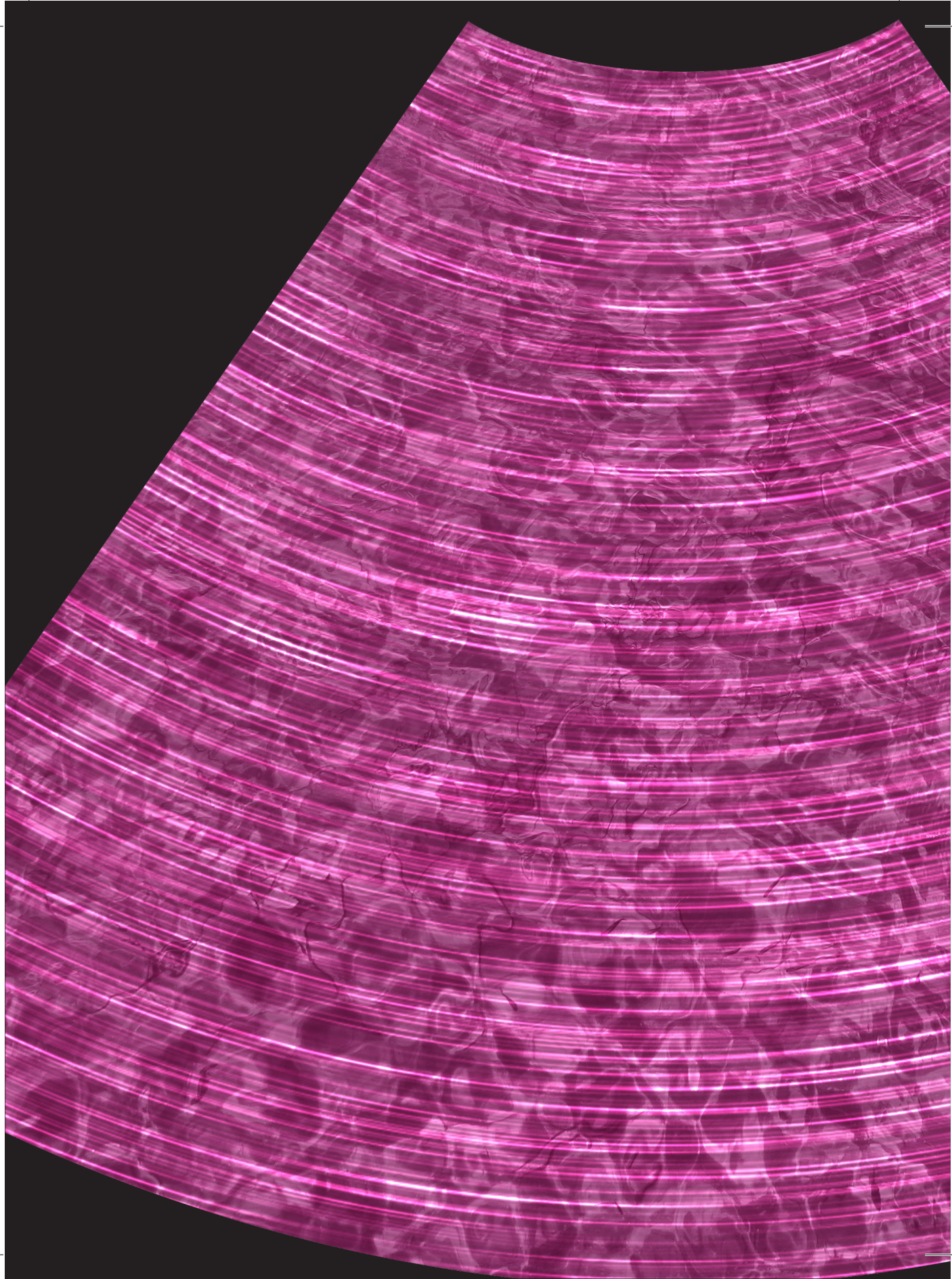
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Changes in the MEP and PMA during pregnancy and postpartum







CHAPTER 6

Association of first-trimester echogenicity of the puborectalis muscle with mode of delivery

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Abstract

OBJECTIVE

To evaluate the association between mean echogenicity of the puborectalis muscle, measured using transperineal ultrasonography, in women during their first pregnancy and the subsequent mode of delivery.

METHODS

This is a secondary analysis of a prospective observational study on the association between stress urinary incontinence and levator muscle avulsion after delivery of a first pregnancy. In this study, 280 nulliparous women with singleton pregnancies were examined with transperineal ultrasound examination at 12 and 36 weeks of gestation. Patients were recruited from an obstetrics practice associated with the university medical center in Utrecht, the Netherlands. Mean echogenicity of the puborectalis muscle values were measured at rest, in pelvic floor muscle contraction, and during the Valsalva maneuver. The subsequent mode of delivery was classified into five categories: spontaneous vaginal delivery, instrumental vaginal delivery, elective cesarean delivery, cesarean delivery resulting from nonreassuring fetal status, and cesarean delivery resulting from failure to progress. Mean echogenicity of the puborectalis muscle values according to mode of delivery were compared by analysis of variance and Tukey's post hoc test.

RESULTS

Of the 254 women included, 157 had spontaneous vaginal delivery, 47 underwent cesarean delivery (11 elective, 36 emergency), and 45 had operative vaginal delivery (vacuum); in five patient files, the mode of delivery was not recorded. Of the analyzed women, those who delivered by cesarean because of failure to progress had a significantly lower mean echogenicity of the puborectalis muscle in pelvic floor contraction at 12 weeks of gestation (mean echogenicity of 116 ± 14) than women who had spontaneous vaginal delivery (132 ± 21 ; Tukey's post hoc test, $p=0.03$), instrumental vaginal delivery (138 ± 21 ; $p=0.004$), and cesarean delivery resulting from nonreassuring fetal status (139 ± 20 ; $p=0.02$).

CONCLUSION

Lower mean echogenicity of the puborectalis muscle values in pelvic floor contraction during the first pregnancy at 12 weeks of gestation is associated with subsequent cesarean delivery as a result of failure to progress.

Introduction

Obstructed or dysfunctional labor, a failure to progress, is a common obstetric problem, with an estimated incidence of 3–6 per 100 live births worldwide¹. The cause can be mechanical, such as a cephalopelvic disproportion, but failure to progress also can be the result of functional factors such as inadequate uterine contractions or failure of the cervix to dilate^{2–4}. A three-dimensional computer model based on magnetic resonance imaging demonstrated that the pelvic floor muscles must undergo extensive stretching during vaginal delivery⁵. However, the potential role of pelvic floor muscles in the progression of labor and mode of delivery has not been widely studied. Van Veelen and coworkers⁶, investigated the association between levator hiatal dimensions, measured with transperineal ultrasonography, during pregnancy and subsequent mode of delivery in nulliparous women. They reported a significantly smaller levator anteroposterior dimension in contraction at 12 weeks of gestation in women who subsequently had cesarean delivery as a result of failure to progress as compared with women who had normal or vaginal-assisted delivery or emergency cesarean delivery as a result of nonreassuring fetal status. This finding raises the question of whether the puborectalis muscle is different structurally, functionally, or both in the women who undergo cesarean delivery as a result of failure to progress. Another way to look at the structure of muscles is to study its echogenicity. Echogenicity is clinically used in children with neuromuscular disease as a diagnostic tool and monitoring tool for disease progression^{7–9} so may well provide information of the puborectalis muscle.

The objective of the present study was to study the association between the mean echogenicity of the puborectalis muscle in pregnant nulliparous women and the subsequent mode of delivery. If changes in echogenicity reflect functional changes, as in neuromuscular diseases in children, we hypothesized that differences in the echogenicity could be linked to the mode of delivery.

Materials and Methods

This study is a secondary analysis of a prospective observational study on the association between stress urinary incontinence and levator muscle avulsion after delivery of a first pregnancy. The Medical Ethics Committee of the University Medical Center Utrecht approved this study (reference 08-299) and all women gave written informed consent. Patients were recruited from an obstetrics practice associated with the university medical center in Utrecht, the Netherlands. A total of 280 nulliparous pregnant women were examined with four-dimensional transperineal ultrasound assessment of their pelvic floor anatomy during pregnancy at 12 and 36 weeks of gestation and 6 months postpartum. Pregnancy was confirmed and dated based on the crown–rump length measured with early ultrasonography in regular care. The ultrasound data sets at 12 and 36 weeks of gestation were used for the purpose of this study. Women were excluded when they had a medical history of incontinence (urinary, fecal, or both), previous pelvic organ prolapse or anti-incontinence surgery, connective tissue disease, or neurologic disorders¹⁰. Clinicians who managed the patients' labor were blinded to the ultrasound data.

Delivery data were retrieved by one of the authors (M.K.W.) from the medical reports and the institutional database.

The mode of delivery was classified according to the Dutch nationwide perinatal register into five categories: spontaneous vaginal delivery, instrumental vaginal delivery, elective cesarean delivery, emergency cesarean delivery resulting from nonreassuring fetal status, and emergency cesarean delivery resulting from failure to progress¹¹.

The assessment consisted of four-dimensional transperineal ultrasound imaging using a GE Voluson 730 Expert system with a RAB 8–4 MHz curved-array volume transducer placed on the perineum in the sagittal plane. Two experienced ultrasonographers performed all the ultrasound examinations¹². All ultrasound system settings were kept identical during all examinations¹³. The pelvic floor ultrasound examinations were performed with the participants in a supine position and with an empty bladder. Echogenicity was measured with the puborectalis muscle at rest, during maximal pelvic floor muscle contraction, and during Valsalva¹³.

Offline analysis of the data were performed using 4D View 7.0 and Matlab® R2010a. The plane of minimal hiatal dimensions, resulting in the axial plane, is selected following the worldwide consensus¹⁴. The puborectalis muscle was delineated using the software Matlab® (function *imfreehand*) as described previously and shown in Figure 1¹⁵. The mean echogenicity of the puborectalis

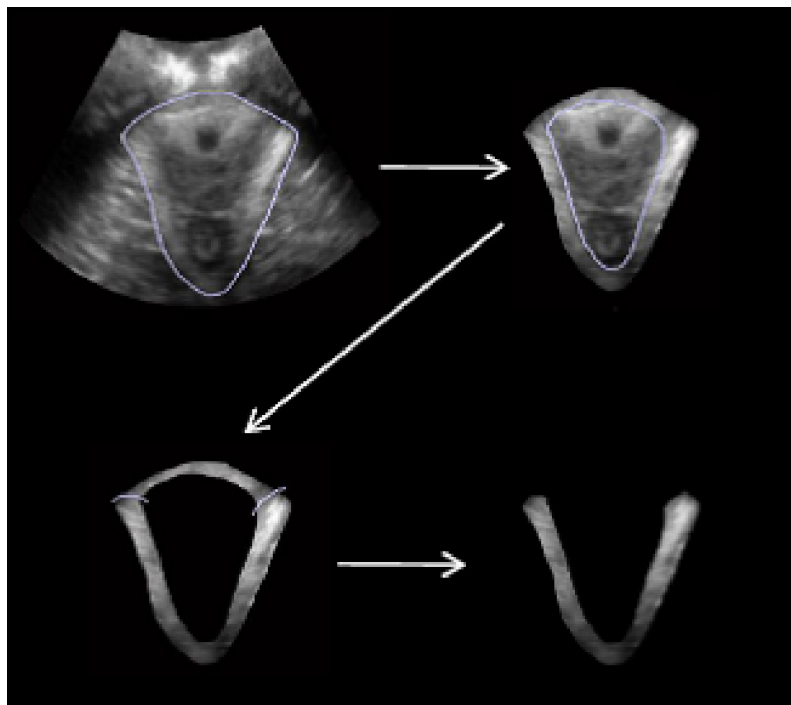


FIGURE 1 Delineation of the puborectalis muscle by hand

muscle was determined automatically by calculating the sum of the echogenicity of all pixels and dividing that sum by the number of pixels. Observers were blinded to delivery mode during postprocessing of the data.

Mean echogenicity of the puborectalis muscle values at 12 weeks and 36 weeks of gestation were compared between the different modes of delivery by analysis of variance followed by a Tukey's post hoc test when appropriate. To determine the magnitude of the effect, we calculated the effect size of the statistically significant findings by Cohen's *d*. A post hoc receiver operating characteristic curve was derived to evaluate the ability of mean echogenicity of the puborectalis muscle to correctly classify the need for an emergency cesarean delivery as a result of failure to progress. Statistical analysis was carried out using SPSS 20.0 for Windows.

Results

Of the 280 women recruited from the clinic, 26 were excluded. Two women were incorrectly included (based on a twin pregnancy and a neurologic disorder), one woman had a premature delivery at 19.9 weeks of gestation, 17 women were excluded based on loss to follow-up or missing ultrasound volume data sets because of technical errors during file saving (at least two out of three data sets were missing), and six data sets were excluded because the symphysis were located outside the view of the ultrasound images.

The mean age of the women was 31.1 years (standard deviation SD=4.1) and their mean body mass index—calculated as weight [kg] / (height [m])²—at 12 weeks and 36 weeks of gestation was 23.4 (SD=3.9) and 27.6 (SD=3.8), respectively. Mean gestational age at the first visit was 13.3 weeks (SD=1.9) and 36.0 weeks (SD=0.9) at the second visit.

Of the 254 women included, data analysis at 12 weeks of gestation was possible for 247 patients at rest, 240 in contraction, and 223 during Valsalva. At 36 weeks of gestation these numbers were 219, 206, and 194, respectively. Of the included women, 157 (61.8%) had spontaneous vaginal delivery, 47 (18.5%) underwent cesarean delivery (11 elective, 36 emergency), and 45 (17.7%) had operative vaginal delivery (vacuum); in five (2.0%) patient files, the mode of delivery was not recorded¹⁴. In the operative vaginal delivery group, 15 deliveries were based on nonreassuring fetal status, 15 on failure to progress, nine on a combination of failure to progress and nonreassuring fetal status, and six had unknown reasons for vacuum extraction. In the emergency cesarean delivery group resulting from nonreassuring fetal status, 15 women were in the first stage of labor, one in the second stage of labor, whereas in three patients, the stage of labor was missing from the records. In the emergency cesarean delivery group resulting from failure to progress, 13 were in the first stage of labor (median cervical dilation 5 cm, range 1–10 cm) and four in the second¹⁴.

In Table 1, the mean echogenicity of the puborectalis muscle values at 12 weeks and 36 weeks of gestation is shown at rest, in contraction, and during Valsalva. At 12 weeks of gestation, at rest and during Valsalva, no statistical significant differences in mean echogenicity of the puborectalis muscle between groups were found. However, during contraction, there was a statistically significant lower

TABLE 1 Mean echogenicity of the puborectalis muscle at 12 and 36 weeks of gestation by delivery type and maneuver

Delivery type	Maneuver					
	at rest		at contraction		during Valsalva	
	12 wk of gestation	36 wk of gestation	12 wk of gestation	36 wk of gestation	12 wk of gestation	36 wk of gestation
Vaginal (n=157)	139±20	148±20	132±21	139±21	133±20	135±22
Assisted vaginal (vacuum) (n=45)	146±18	148±20	138±21	136±21	141±25	128±23
Elective cesarean (n=11)	150±15	155±10	137±18	142±13	139±24	148±15
Cesarean based on nonreassuring fetal status (n=19)	144±28	147±19	139±20	140±20	142±20	138±19
Cesarean based on failure to progress (n=17)	134±15	145±26	116±14	133±14	128±20	138±31

Data are mean±standard deviation unless otherwise specified

mean echogenicity of the puborectalis muscle for the women who underwent cesarean delivery based on failure to progress as compared with vaginal deliveries ($p=0.03$), assisted vaginal deliveries ($p=0.004$), and cesarean delivery based on nonreassuring fetal status ($p=0.02$) with effect sizes of 0.90, 1.23, and 1.33, respectively. The results of the post hoc tests are shown in Table 2 and Figure 2. At 36 weeks of gestation no statistically significant differences in mean echogenicity of the puborectalis muscle between groups were observed for all maneuvers.

The area under the curve (Figure 3), representing the performance of mean echogenicity of the puborectalis muscle in distinguishing between vaginal deliveries and the need for an emergency cesarean delivery based on failure to progress, is 0.75 (SD=0.049, 95% confidence interval bounds 0.656–0.848).

Discussion

Women who delivered by cesarean as a result of failure to progress had a statistically significantly lower mean echogenicity of the puborectalis muscle on pelvic floor contraction at 12 weeks of gestation than women who had spontaneous or instrumental vaginal delivery or women who underwent cesarean delivery as a result of nonreassuring fetal status. No differences in mean echogenicity of the puborectalis muscle between modes of delivery groups at 36 weeks of gestation were found.

Echogenicity, or grayscale value analysis, is a parameter derived from imaging tools such as ultrasonography and varies between 0 (black) and 255 (white). In case of muscle tissue it represents the ratio between muscle cells that appear

dark in grayscale imaging and the brighter extracellular matrix. The two major components of this extracellular matrix are collagen and fat. Higher echogenicity values are associated with increased amounts of either fat in muscle tissue or connective tissue^{16,17}. The echogenicity provides us with structural information and in case of neuromuscular disease in children, it is also used as a diagnostic tool and monitoring tool for disease progression⁷⁻⁹.

During the course of pregnancy, the extracellular matrix of the puborectalis muscle changes. One of the greatest challenges lies within a pathophysiologic explanation of the echogenicity changes, because it cannot be based on human histology data. In a recent study by Alperin and coworkers¹⁸, it was demonstrated that the collagen content of the intramuscular extracellular matrix increases during pregnancy. This adaptation already started in early pregnancy and returned to nonpregnant virgin rat levels after delivery¹⁸. This is in line with our clinical observation that the mean echogenicity of the puborectalis muscle increases over time during pregnancy and decreases after delivery¹⁵. The significantly lower mean echogenicity of the puborectalis muscle in women who underwent emergency cesarean delivery as a result of failure to progress may be an indication of a disturbed early adaptation of this collagen metabolism with less collagen being formed. One of the key factors in the preparation for childbirth is the weakening of collagen in the pelvic tissues¹⁹. Less intramuscular collagen during pregnancy may

TABLE 2 Analysis of Variance (Tukey post-hoc) – Mean difference in mean echogenicity of the puborectalis muscle at 12 weeks of gestation among different types of delivery

Cesarean delivery for failure to progress vs	Echogenicity of the puborectalis muscle	p
Puborectalis muscle in rest		
Vaginal delivery	-5.1±5.1	0.917
Operative vaginal delivery (vacuum)	-12.7±5.7	0.228
Elective cesarean delivery	-16.6±7.9	0.299
Cesarean delivery for nonreassuring fetal status	-10.6±7.1	0.677
Puborectalis muscle in contraction		
Vaginal delivery	-15.9±5.5	0.032
Operative vaginal delivery (vacuum)	-21.7±6.1	0.004
Elective cesarean delivery	-20.8±8.4	0.098
Cesarean delivery for nonreassuring fetal status	-22.2±7.6	0.023
Puborectalis muscle in Valsalva		
Vaginal delivery	-4.7±5.5	0.955
Operative vaginal delivery (vacuum)	-12.9±6.3	0.310
Elective cesarean delivery	-10.8±8.4	0.803
Cesarean delivery for nonreassuring fetal status	-13.7±7.8	0.506

Data are mean difference±standard deviation unless otherwise specified.

Vaginal delivery n=157; operative vaginal delivery n=45; elective cesarean delivery n=11; cesarean delivery based on nonreassuring fetal status n=19; cesarean delivery based on failure to progress n=17.

be associated with the inability of the pelvic floor to stretch during delivery. With respect to failure to progress at the second stage of labor, this is an intriguing explanation. However, it is unlikely that the puborectalis muscle itself limits first stage of labor progression, which was shown to be the cause of failure in the majority of women. To explain this, the low echogenicity of the puborectalis needs to be considered as a constitutional difference in muscle cell to extracellular matrix balance. Although hypothetical, a diminished amount of connective tissue in the cervix may be associated with poor weakening and dilatation. This possible association is currently under investigation at our institute.

The association we found at 12 weeks of gestation was only significant during muscle contraction, although there was a same, nonsignificant trend with the puborectalis muscle at rest and during Valsalva. Our explanation for this finding is that, during contractions, the number of muscle cells per square centimeter in the contracting part increases, increasing the ratio between muscle cells and extracellular matrix in favor of the first²⁰. The net effect is that the ultrasound image becomes darker. Therefore, the effect of the limited collagen amount on mean echogenicity of the puborectalis muscle may be more expressed during contraction as compared with at rest and during Valsalva.

The significant differences between groups in mean echogenicity of the puborectalis muscle we found at 12 weeks of gestation was not present at 36 weeks of gestation. We hypothesize that this is the effect of intramuscular fat storage. With respect to fat metabolism, it has been shown in both humans and rats that the increased pregnancy levels of progesterone and increased intake

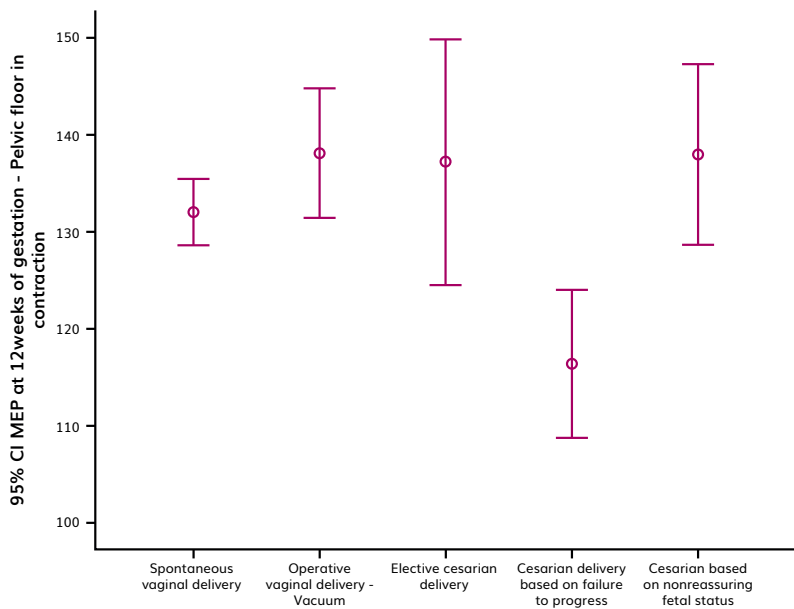


FIGURE 2 Distribution of mean echogenicity of the puborectalis muscle at 12 weeks of gestation, pelvic floor in contraction. *CI: confidence interval*

of nutrients increase intracellular and intramuscular fat storage^{21,22}. It also has been demonstrated that fat replacement causes a substantial increase in muscle echogenicity¹⁷. An increasing mean echogenicity of the puborectalis muscle as a result of intramuscular fat storage during pregnancy may well obscure a limited increase in intramuscular collagen.

If we test the diagnostic characteristics of the mean echogenicity of the puborectalis muscle between women who had vaginal delivery and those who had cesarean delivery as a result of failure to progress, we found an area under the curve in the receiver operating characteristics curve of 0.75. Stated otherwise, in our population, 75% of participants who had cesarean delivery as a result of failure to progress had a lower mean echogenicity of the puborectalis muscle than their normal vaginal delivery counterpart. In its current form the mean echogenicity of the puborectalis muscle is not suitable as a clinical prognostic test and does not provide a single optimal cutoff value. Additionally, there are promising new techniques to analyze ultrasound images, such as strain and elasticity, becoming available for pelvic floor ultrasonography. These may, in combination with clinical

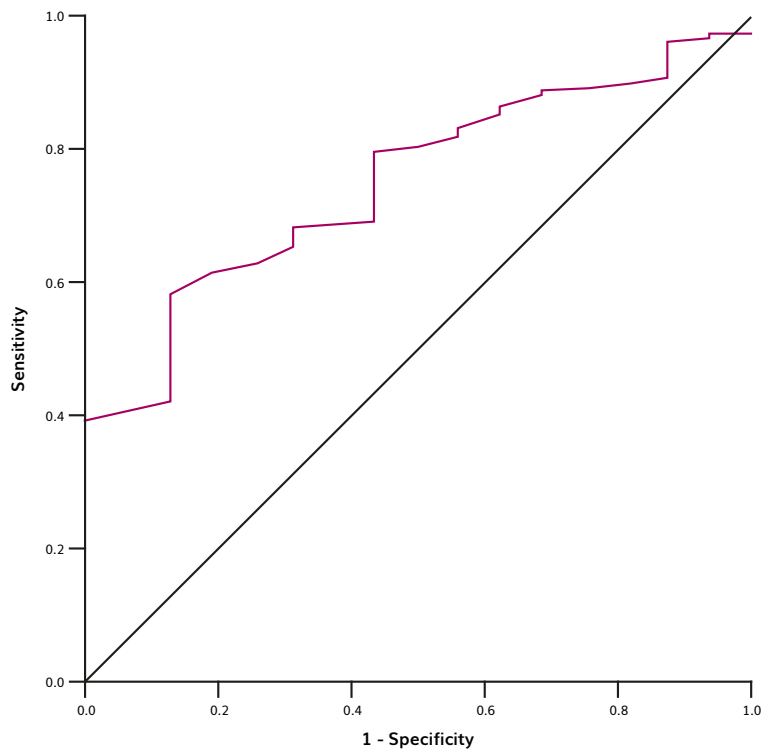


FIGURE 3 Receiver operating curve of mean echogenicity of the puborectalis muscle. Vaginal delivery (n=192) compared with cesarean delivery for failure to progress (n=16) at 12 weeks of gestation, pelvic floor in contraction. Area under curve=0.752; standard error=0.049, 95% confidence interval 0.656–0.848

characteristics and mean echogenicity of the puborectalis muscle measurements, provide a better predictive clinical tool in the future.

A possible limitation of this study is the discrimination among different types of delivery. Worldwide there is a large variation in cesarean delivery rates¹. Varying incidence in obstructed labor per country is likely to be the result of a number of factors including variations in case definition and case ascertainment^{1,23,24}. Because we undertook a single-center study, this may have resulted in bias based on delivery type indication. The second limitation of this study is that it is a secondary analysis and therefore it was not powered for the outcome under study. However, the differences in mean echogenicity of the puborectalis muscle among delivery type groups were found to have an effect sizes of greater than 0.8, indicating a very strong effect. However, we would like to emphasize the preliminary nature of this novel study. It needs corroboration with larger, preferably multi-center series.

One of the major strengths of our study is its prospective design, including measurements at 12 and 36 weeks of gestation. Furthermore, we used four-dimensional transperineal ultrasonography, which is a reliable technique for measuring echogenicity in pregnant women¹³. Additionally, the fixed ultrasound device settings are key elements of this study.

In conclusion, this study shows that mean echogenicity of the puborectalis muscle at 12 weeks of gestation in women who need an emergency cesarean delivery as a result of failure to progress in nulliparous women differs from mean echogenicity of the puborectalis muscle in women with a different mode of delivery.

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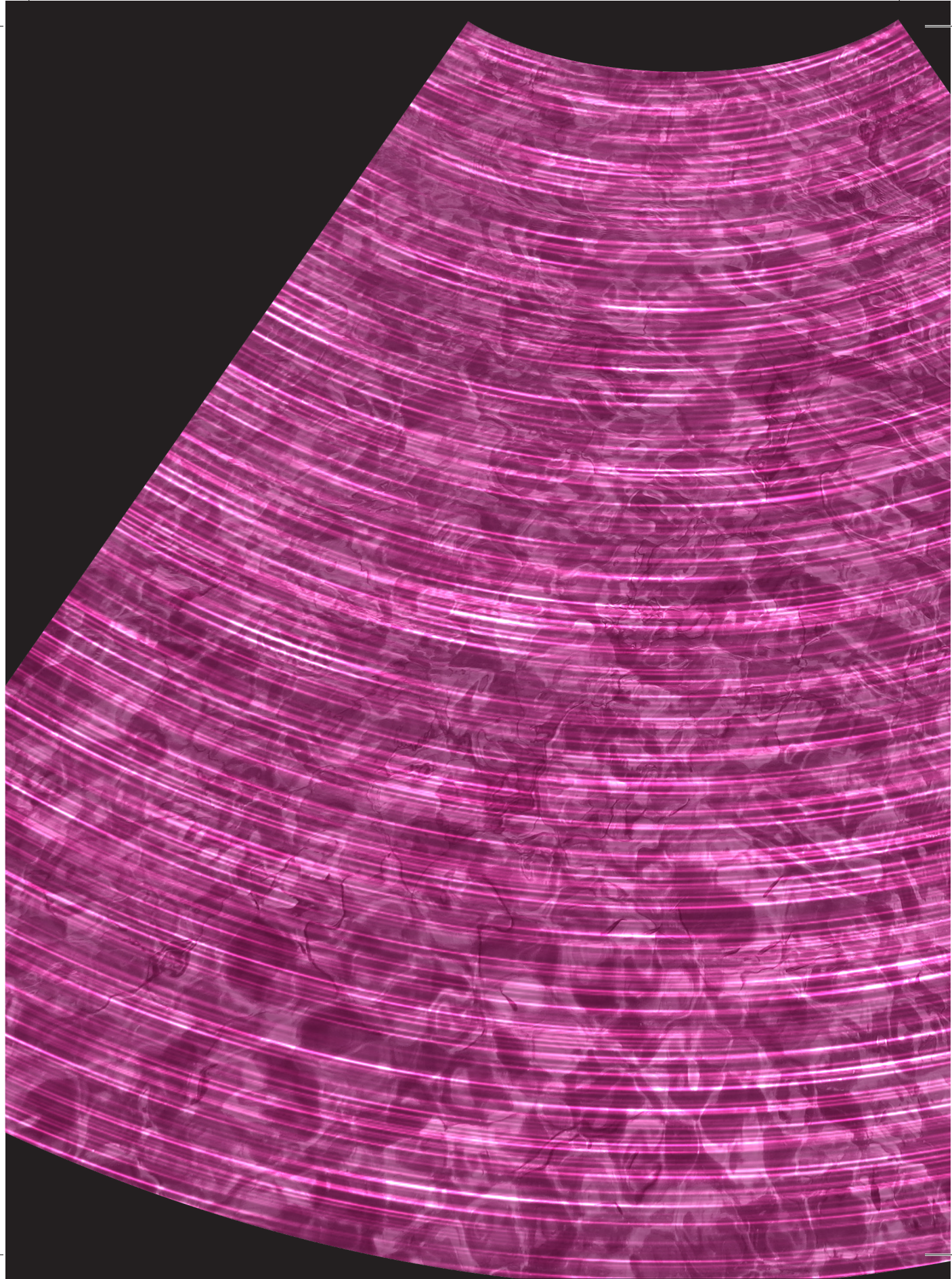
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Chapter 6

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Association of first-trimester echogenicity of the puborectalis muscle with mode of delivery







CHAPTER 7

Changes in the global strain of the puborectalis muscle during pregnancy and postpartum

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Submitted for publication

Abstract

INTRODUCTION One of the functional parameters that can be assessed by ultrasound is muscle strain. Strain represents the amount of deformation from its original shape when forces are applied to the tissue under study. We set out to assess the global strain of the puborectalis muscle during and after pregnancy.

OBJECTIVE To evaluate the effect of pregnancy and child-delivery on global strain of the puborectalis muscle.

METHOD This is a secondary analysis of a prospective observational study on the association between stress urinary incontinence and levator muscle avulsion after delivery. Two-hundred and eighty nulliparous pregnant women had 4D transperineal ultrasound assessments at 12 and 36 weeks of gestation, and 6 months postpartum. Tomographic Ultrasound Images were constructed and the puborectalis muscle was delineated by hand using programming software. After delineation, the length of the midline of the puborectalis muscle was measured in the rest and contraction and global strain was expressed as percentile difference. The postpartum results were analysed separately for vaginal, operative (vacuum) vaginal and cesarean deliveries. To compare the global strain at the three measurement moments per type of delivery, paired sample t-testing was used. The project was approved by the Institutional Human Research Ethics Committee and all women gave informed consent.

RESULTS A total of 254 datasets could be analysed. During pregnancy the global strain did not change. After spontaneous and operative vaginal delivery the global strain significantly diminished as compared to pregnancy values. This did not occur in women who had a cesarean delivery.

CONCLUSION Vaginal childbirth negatively influences the strain of the puborectalis muscle.

Introduction

The orientation of the puborectalis muscle, from its attachment at the pubic symphysis, with a sling shape surrounding the urethra, vagina and rectum, to the other attachment at the pubic symphysis, makes it possible to provide active support (by contraction) to the pelvic organs. During vaginal delivery the puborectalis muscle is exposed to stress by the passage of the fetal head and prone to direct trauma¹. Muscle trauma during delivery is described as possible cause of postpartum pelvic floor muscle dysfunction (e.g. stress urinary incontinence and pelvic organ prolapse)^{2,3,4}. Assessing muscular function, like contractility, in a quantitative way is possible by measuring its strain^{5,6}. Strain, in case of muscle tissue, represents the amount of deformation during contraction or relaxation. Strain during contraction is therefore defined as $(\text{Length}_{\text{contraction}} - \text{Length}_{\text{rest}})$ divided by the $\text{Length}_{\text{rest}}$. In cardiology, strain measurements provide important clinical functional information. For instance, longitudinal strain of the left ventricle has proven to be an adequate parameter to diagnose ventricular function⁷. Measuring strain in the puborectalis muscle during and after pregnancy may add to our understanding of the physiological and functional effect of pregnancy and delivery on the pelvic floor.

The introduction of 4D transperineal ultrasound allowed us to visualize the puborectalis muscle in the axial plane, and to study movement and contractility⁸. The shortening of the puborectalis muscle during contraction, e.g. its strain, can be measured by means of new software segmentation and data analyses techniques. As stated, during vaginal childbirth the puborectalis muscle is susceptible to traumatic injury^{2,3,4}. We set out to study changes in strain of the puborectalis muscle during pregnancy and after spontaneous vaginal, operative vaginal (vacuum) or cesarean delivery.

Materials and Method

STUDY DESIGN AND POPULATION

The development of our method to measure strain was a secondary analysis of a large prospective cohort study in our University Hospital⁹⁻¹⁷. Perineal ultrasound volume imaging data sets of the pelvic floor of 280 nulliparous women, who were measured at 12 and 36 weeks of gestation and 6 months after delivery, were used⁹. Patients were included from an obstetrics practice, associated with the university medical center in Utrecht, the Netherlands.

Women were excluded when they had a medical history of urinary and, or fecal incontinence, previous pelvic organ prolapse or anti-incontinence surgery, connective tissue disease or neurological disorders⁹. The original study (reference 08-299) was approved by the Institutional Human Research Ethics Committee of the University Medical Center Utrecht and all women gave informed consent.

ULTRASOUND EXAMINATION

The assessment consisted of 4D transperineal ultrasound imaging using a GE Voluson 730 Expert system (GE Healthcare, Zipf, Austria) with RAB 8–4 MHz curved array volume transducer. All pelvic floor ultrasound examinations were performed with the participants in supine position and with an empty bladder⁹.



The ultrasound probe was placed on the perineum in the sagittal plane, after which the investigators who performed the ultrasound examinations verbally instructed each woman and at least three attempts with visual feedback on the ultrasound monitor during the maneuver were acquired for each woman. The examinations were performed by two experienced sonographers, who gave similar instructions during the trial and final examination to ensure that the women performed maximal pelvic floor muscle contraction.

IMAGE RECONSTRUCTION AND ANALYSIS

Offline analysis of the data was performed using 4D View 7.0 (GE Medical Systems Kretztechnik, Zipf, Austria) and Matlab® R2010a (MathWorks, Natick, MA). Observers were blinded to delivery mode during post processing of the data. Image analysis was done by first selection of the Tomographic Ultrasound Images (TUI) in rest and contraction following the worldwide consensus^{8,18}. The slice on which we performed our strain measurement is the slice of minimal hiatal dimensions, e.g. the TUI slice in which the pubic bones are closed.

DELINEATION OF STRUCTURES AND MIDLINE DRAWING

The region of interest, the puborectalis muscle, was delineated semi-automatic using the software Matlab® (function *imfreehand*) as described previously¹⁴. Additionally software was developed to automatically load, process and calculate the length of the puborectalis muscle (Figure 1). Based on the previously delineated regions of interest, a centerline was generated. The length of this centerline was then defined as the length of the puborectalis muscle and used for the calculation of the strain. The results from this automated software were validated by manual spline measurements using the Matlab® tool "Image Measurement Utility".

$$\text{Strain} = \frac{\text{Length}_{\text{contracted}} - \text{Length}_{\text{relaxed}}}{\text{Length}_{\text{relaxed}}} \cdot 100\%$$

For every dataset, an overlaid image of the original gray value image, the contour of the segmentation, the approximating spline and the intersection points and is saved (Figure 1d) and visually inspected by an expert. Strain calculation is done based on centerlines that are generated from the previous delineation. The reliability for this delineation is described earlier, with an interobserver ranging from 0.63–0.87 and intra-observer 0.88–0.94¹⁴. Since the generation of the centerline is done fully automatically and the centerlines were afterwards visually inspected by an expert, the strain calculation uses the same ground truth as the described calculation.

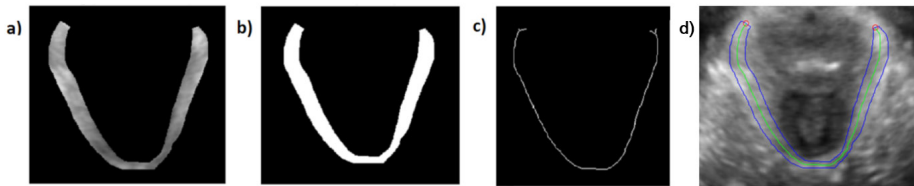


FIGURE 1 Drawing a midline in the segmented puborectalis to obtain the global strain

DATA AND STATISTICAL ANALYSIS

In our study, strain is defined as the shortening of the muscle from its resting length. If no movement of the muscle, e.g. no strain, is found this may be explained in two ways. The first explanation is that the woman does not know how to perform a proper contraction. The second explanation is that the muscle is severely damaged. A strain value of zero (no movement) at 6 months postpartum was considered to be the result of puborectalis muscle damage only if the woman had shown to be able to contract her puborectalis muscle at 12 and 36 weeks pregnancy measurements. If not, we classified the woman as not being able to perform a voluntary contraction and she was excluded from analysis.

To check the correlation between strain and the parameters birth weight, duration of second stage labour and the moment that the ultrasound scans were made. Since vaginal delivery, both spontaneous and assisted, has been reported as one of the main factors associated with pelvic floor disorders, we decided to analyse our postpartum results separately for women who had a cesarean delivery, vaginal delivery or operative vaginal delivery (vacuum)^{3,19}. Data collected at 12 and 36 weeks' of gestation were compared with paired samples t-test to assess possible changes in strain during pregnancy. In addition, differences in absolute strain values at 6 months postpartum between the delivery groups were compared with ANOVA statistics and Tukey post hoc testing.

Results

Of the 280 women recruited from the clinic, we were able to analyze 254 datasets, based on exclusion criteria as presented in the flowchart (Figure 2).

Within the final dataset of 254 images there were some additional missing data based on no-shows on one of the three occasions. A total of six women were not able to perform an adequate contraction after delivery as well as during pregnancy and were excluded as previously indicated. Twenty women who had a vaginal delivery and 5 women who had an operative vaginal delivery had a

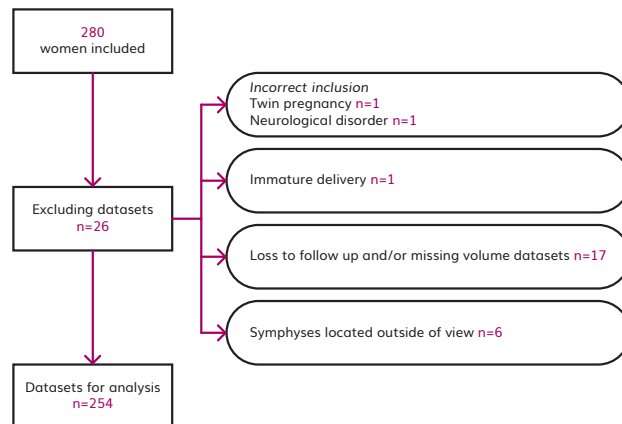


FIGURE 2 Exclusion flow chart

TABLE 1 Weeks of gestation and months postpartum at different examination times

	Ultrasound 1 weeks of gestation (SD)	Ultrasound 2 weeks of gestation (SD)	Ultrasound 3 months postpartum (SD)
Vaginal delivery	12.8 (1.86)	35.8 (0.79)	6.3 (0.91)
Operative vaginal delivery	12.7 (1.55)	35.7 (0.84)	6.1 (0.54)
Cesarean delivery	12.7 (1.77)	35.5 (0.82)	6.0 (0.46)

true zero strain value. After cesarean delivery all women were able to perform a contraction.

The mean age of the women was 31.1 years (SD=4.1) and their mean body mass index (BMI) at 12 weeks, 36 weeks of gestation and 6 months postpartum was 23.4 (SD=3.9); 27.6 (SD=3.8); 24.0 (SD=3.9) kg per m², respectively. The number of women who had a first and second stage cesarean delivery was described before¹⁶. The mean and SD values of the examination time points for the different delivery groups show that the women were examined at the same time points in pregnancy and postpartum (Table 1).

The only statistical significant correlation on strain and birth weight and duration of second stage labour was between the global strain and second stage delivery. However, after correction for multiple testing (Bonferroni correction 0.05/6) this was not significant anymore and the correlation coefficient of 0.173 is considered as poor.

GLOBAL STRAIN

In Table 2 the absolute strain values, expressed as percentages, for the different time points for each type of delivery group are shown in columns 1 to 3. Figure 3 shows the graphical expression of these values and their standard deviations. In addition, we studied the difference in absolute strain values between pregnancy and after delivery. In the vaginal delivery and operative vaginal delivery group there was a statistical significant reduction in absolute strain value after delivery. In Table 1 this difference is expressed as relative percentage change between 12 or 36 weeks gestation and after delivery. No statistical significant reduction in absolute strain values after delivery were seen in the cesarean delivery group.

TABLE 2 Global strain values at 12 weeks' and 36 weeks' of gestation and 6 months postpartum (pp) and mean relative differences between time points per type of delivery

	12w		36w		6mpp		Difference 12w vs 6mpp (p-value)	Difference 36w vs 6mpp (p-value)
	n	GSV (SD)	n	GSV (SD)	n	GSV (SD)		
Vaginal delivery	149	-10.3 (8.2)	141	-11.2 (8.0)	141	-8.3 (8.3)	-17% (0.042)	-28% (<0.001)
Operative vaginal delivery	39	-11.2 (8.2)	40	-10.5 (8.8)	34	-6.5 (9.7)	-42% (0.017)	-34% (0.043)
Cesarean delivery	44	-12.0 (8.5)	34	-9.2 (9.5)	41	-10.8 (9.1)	-16% (0.273)	+29% (0.250)

GSV= Global strain value; SD=standard deviation; w=weeks; mpp=months post partum

Changes in the global strain of the puborectalis muscle during pregnancy and postpartum

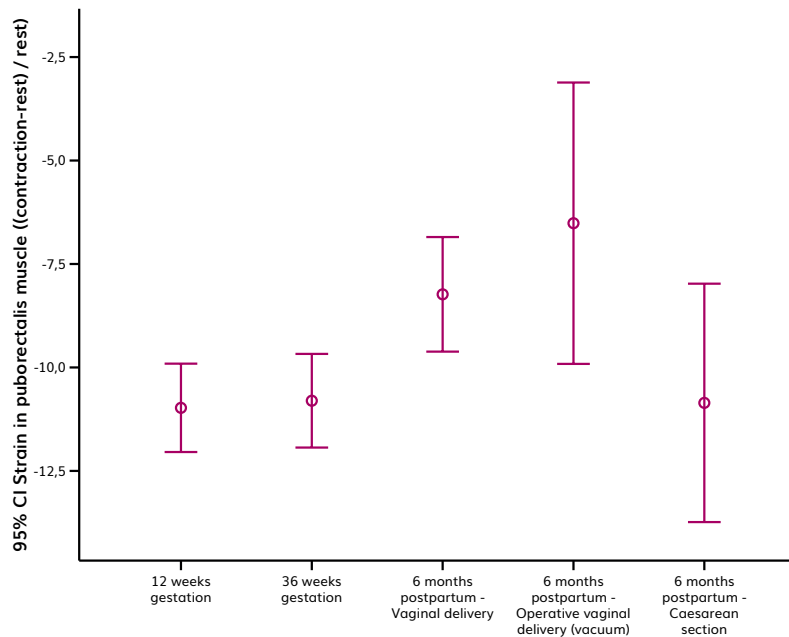


FIGURE 3 Strain during pregnancy and postpartum. *CI: confidence interval*

In addition, the ANOVA statistics and Tukey post hoc testing indicated no significant differences in absolute strain values at 6 months postpartum between the delivery groups, with p-values of respectively vaginal–operative vaginal 0.702; vaginal–cesarean 0.172 and operative vaginal–cesarean 0.144.

Discussion

Women who delivered by spontaneous or operative (vacuum) vaginal delivery had a statistically significant reduced global strain of the puborectalis muscle at 6 months postpartum compared to their pregnancy levels, ranging from –17% to –42%. This reduction is not found in women who had a cesarean delivery. We did not find a significant difference in absolute strain values at 6 months postpartum between the delivery groups.

Strain represents the amount of deformation between resting and contracting state of a muscle. In this perspective a reduction in strain in the puborectalis muscle is equal to a reduction in contraction or shortening of the muscle, e.g. diminished contractile function.

The ability of the puborectalis muscle to contract and relax is an essential functional feature, and obtaining functional information through ultrasound may be of diagnostic and therapeutic importance. We consider strain during contraction of clinical importance for pelvic floor function since contraction is the mechanism behind continence. Quantifying strain could be a helpful tool in diagnostics and therapy in pelvic floor muscle training. The majority of studies on



the functional aspects of the pelvic floor look at hiatal area and angles, which are secondary markers of puborectalis muscle function^{8,9,20–22}. If the anteroposterior and transversal distances of the genital hiatus equally change in opposite directions (shortening and lengthening), the net result of the change in area value will be zero. However, in such a situation the change in shape of the hiatal area will be due to muscle contraction or relaxation, which will represent itself as global strain. This concept was already shown in cardiology by Pedrizzetti and coworkers²³.

Thyer and co-workers were the first to introduce the concept of strain into the ultrasound assessment of pelvic floor muscle function²⁴. They measured strain by measuring the changes in hiatal circumference between rest and contraction, and rest and Valsalva. They showed that the correlation between the digital assessment of pelvic floor strength, using the modified Oxford scale²⁴, and strain was statistical significant but moderate for contraction, and weak for Valsalva. Accordingly, in 2009 two studies were published on strain in the pelvic floor in relation to clinical outcome. Abdool and co-workers assessed the effect of levator avulsions on strain measurements of the levator muscle and concluded that avulsion injury is associated with abnormal levator biometry and function²⁵. Svabik and co-workers defined the degree of stretch/strain of the levator hiatus in childbirth. They indicated that normative data for the distension was obtained, but no conclusion could be drawn yet²⁶. These reports on strain of the pelvic floor muscles underline the potential of this type of measurement in assessing pelvic floor behavior and changes.

Vaginal child delivery causes trauma to the pelvic floor muscles^{27,28}. We believe that the reduction in strain is primarily caused by the excessive stretch/overdistension, which the puborectalis muscle has to overcome during delivery. From this perspective our finding of significantly reduced strain of the puborectalis muscle after vaginal delivery, but not after cesarean delivery, is logical. A similar results was presented by the group of Staer-Jensen. They found that the estimated mean levator hiatus area during contraction was significantly larger postpartum (6 weeks, 6 months and 12 months) in the vaginal delivery group compared to the cesarean deliveries²⁹. One of the explanations for the decrease in absolute strain after vaginal delivery is the occurrence of major levator avulsion trauma. However, prior to starting the analysis for levator avulsions in our dataset we performed a multicenter reliability study on the accuracy of diagnosing these avulsions across centers and between observers. We found, in a subset of 40 women, that in this particular group of women 6 months after first vaginal delivery, trained observers highly disagreed on the presence or absence of levator avulsions¹¹. With this poor reproducibility result we decided not to analyze our remaining data for levator avulsions. However, levator avulsions after delivery have been consistently reported by other observers and it would be worthwhile to analyze those datasets with our strain analyses tool^{25,30}. The absolute strain values between the different groups at 6 months postpartum were lower after vaginal delivery, although not statistically significant. This could be due to an inadequate sample size, since the original study was not powered to detect differences in strain. Furthermore, since the ultrasound was made 6 months postpartum, regeneration of the muscle might already improve the function and diminish the difference between the different



groups^{31,32}. The effect of the muscle healing is not clear, since we did not scan at multiple moments postpartum.

One of the drawbacks of our study is that it is a secondary analysis of a prospective observational study on the association between stress urinary incontinence and levator muscle avulsion after delivery. Therefore, it was not powered to detect strain differences between different delivery groups, and sample size may have been inadequate to answer our questions. However, the differences in global strain between delivery type groups were found to be significant.

One of the major strengths of our study is its prospective design, including measurements at 12 and 36 weeks of gestation, but also postpartum at 6 months after child delivery. Furthermore, measuring strain is dependent on proper delineation of the puborectalis muscle from its attachment to the pubic symphysis. A slight variation in the visual identification of this place of attachment is possible, which may result in a difference in length of the puborectalis muscle as a consequence. The measurements on global strain are based on the method of semiautomatic delineation, with good reliability, which we described in an earlier study¹⁴. After manual segmentation the analyses of the strain is done automatically by the computer, decreasing the observer variability.

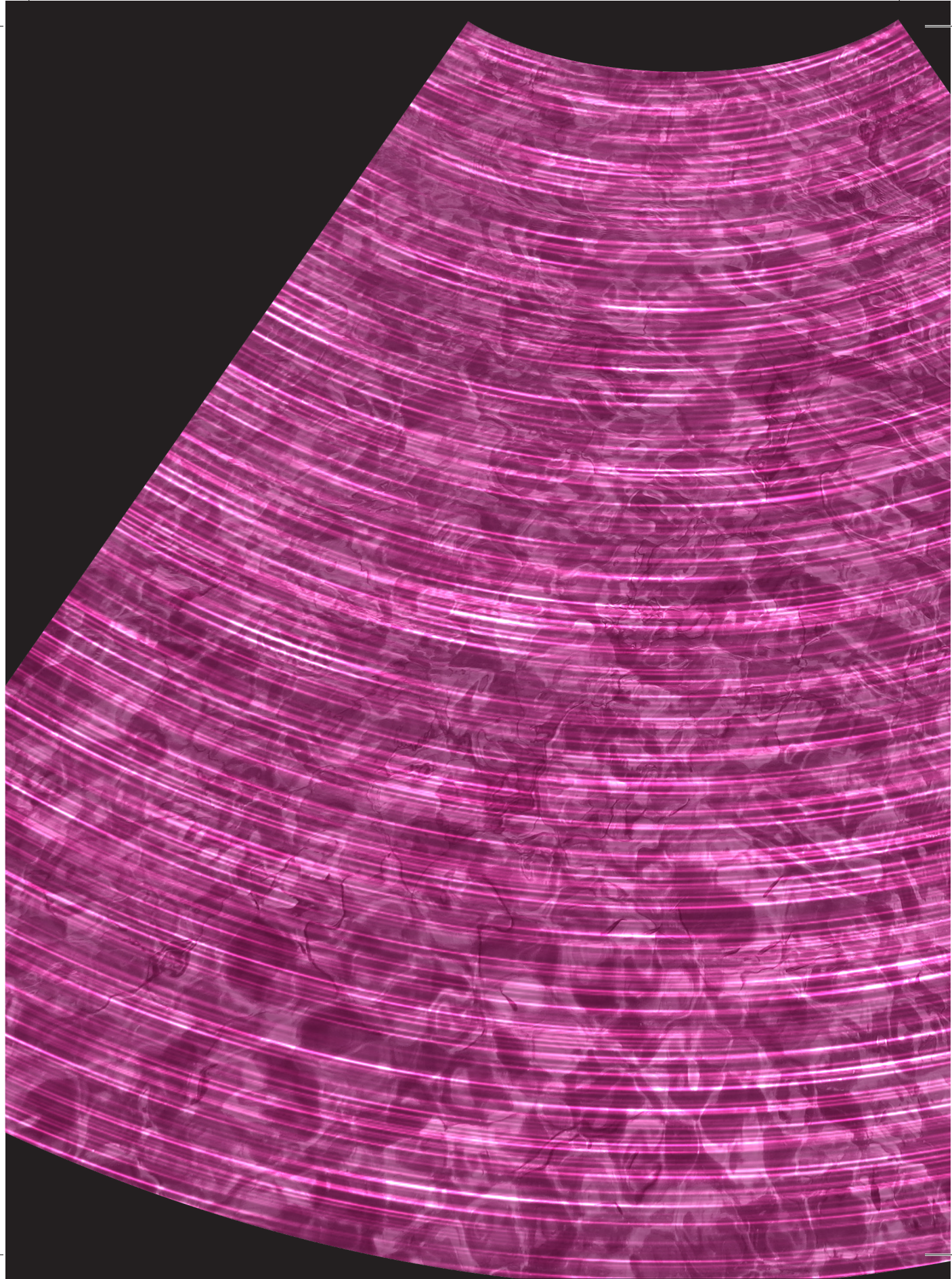
In conclusion, the diminished global strain of the puborectalis muscle after vaginal or operative vaginal delivery indicates that childbirth negatively influences the contractility of the puborectalis muscle.

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CHAPTER 8

General discussion

Chapter 8



General discussion

This thesis was performed on the research questions as outlined in the introduction. We studied two main topics in the field of ultrasound in urogynecology: echogenicity of the puborectalis muscle (method and clinical value), and the changes in pelvic floor muscle strain during pregnancy and postpartum.

Previous studies leading to this thesis.

Previous research by Van Veelen and co-workers between 2013 and 2015, indicated that changes in the area of the genital hiatus occur during and after first pregnancy¹⁻⁵. At 36 weeks of gestation, women who delivered vaginally showed a persistent significant increase in hiatal dimensions on Valsalva, whereas women who delivered by prelabor or first-stage cesarean section showed no significant changes in hiatal dimensions on Valsalva. These results are indirect indications that the composition or structure as well as function of the muscles surrounding the genital hiatus change.

The structure of muscles is studied by a parameter called "echogenicity". In previous studies echogenicity was already used in the diagnosis of neuromuscular diseases in children and in the analyses of supraspinatus tendon tears, and monitoring muscle healing processes⁶⁻⁹. Echogenicity describes the ratio between muscle fibers (echolucent) and extra cellular matrix consisting of connective and fatty tissue (echogenic) and is expressed on a greyscale in which the value for each pixel can vary between 0 (black) and 255 (white)¹⁰. The mean of all the ultrasound pixel values within the region of interest is defined as the mean echogenicity. In order to better study and understand the structure of the pelvic floor muscles, our studies focused on introducing echogenicity and defining its value.

Changes in hiatal area are a consequence of activity of the puborectalis muscle. Assessing muscular function, like contractility, in a quantitative way is possible by measuring its strain. In cardiology, strain measurements provide important clinical functional information. For instance, longitudinal strain of the left ventricle has proven to be an adequate parameter to diagnose ventricular function¹¹⁻¹³. This pelvic floor muscle function was studied by strain in this thesis.

Main findings of studies presented in the current thesis

The specific aims of this thesis were:

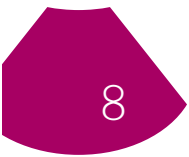
- To evaluate the compliance of prospective diagnostic accuracy studies investigating pelvic floor ultrasound, with Standards for Reporting of Diagnostic Accuracy (STARD) guidelines (Chapter 2).
- To develop a semi-automated method to assess puborectalis muscle echogenicity on 3D/4D volume transperineal ultrasound images using 4D View and Matlab® software and evaluating its intra- and interobserver reliability (Chapter 3).

- To determine the effect of body mass index (BMI), age, physical activity and ultrasound probe pressure on the Mean Echogenicity of the Puborectalis muscle (MEP) and Puborectalis Muscle Area (PMA) (Chapter 4).
- To assess the changes in the Mean Echogenicity of the Puborectalis muscle (MEP) and the Puborectalis Muscle Area (PMA) during first pregnancy and after childbirth (Chapter 5).
- To evaluate the association between mean echogenicity of the puborectalis muscle, measured using transperineal ultrasound, in women during their first pregnancy and the subsequent mode of delivery (Chapter 6)
- To evaluate the effect of pregnancy and child-delivery on global strain of the puborectalis muscle (Chapter 7)

Clinical relevance of measuring echogenicity.

The mean of all the pixel values of the puborectalis muscle was defined as the Mean Echogenicity of the Puborectalis muscle (MEP). In chapter 3 the method and reliability of assessing the MEP and puborectalis muscle area (PMA) were reported. The method we developed proved to be reliable and reproducible. The area in which the largest mismatch between observers occurred was near the attachment of the muscle to the pubic symphysis. An important remark in relation to these ICC values is that we included healthy pregnant nulliparous women without previous delivery trauma to their pelvic floor. This provided us with high quality images of intact pelvic floor musculature, which might have improved the accuracy of measurements. Since there are no other studies who report on the echogenicity of the puborectalis muscle we cannot compare our results. However, in relation to our PMA measurements, studies on dimensions of the pelvic floor also showed moderate to almost perfect intra and interobserver reliabilities^{1,14-17}. The advantage of measuring MEP is that it is can be calculated automatically, increasing the reliability to 100%.

The echogenicity values during pregnancy and postpartum are compared in chapter 5. The MEP at 6 months postpartum was, maneuver independent, significantly lower as compared to MEP values during pregnancy. A possible explanation could be that already during the first stage of gestation, when fetal growth is very limited; the body aims at storing nutrients for future demands¹⁸. The increased pregnancy levels of progesterone act as an insulin antagonist which causes, together with the increased intake of nutrients, an increase in intracellular and intramuscular fat storage^{19,20}. However, since pre-pregnancy values are lacking in our study results, we cannot proof this hypothesis with certainty. Another explanation can be that echogenicity decreases due to muscular trauma during vaginal delivery. Trauma to the muscle (like hematoma) will appear dark on ultrasound and reduce the echogenicity. However since we are measuring at 6 months postpartum, the majority of the hematoma are expected to have healed²¹. A follow-up period with more (frequent) ultrasound scans could have been an added value to the study, since it would have allowed us to study the effect of muscle regeneration on MEP. The detachment of the puborectalis muscle from the pubic bone (avulsion) will also affect muscle shape and echogenicity. Scoring



these avulsions and looking at the effect on MEP would be interesting. However the reliability of diagnosis of avulsions has been subject of debate^{3,22}.

Since pregnancy has a significant effect on the pelvic floor, information on muscle composition and changes during pregnancy and postpartum could be of value within two areas of clinical care: predefining mode of delivery and monitoring and treatment of trauma.

MODE OF DELIVERY

First, during the course of pregnancy the muscles of the pelvic floor have to adapt as preparation for childbirth. In a recent study by Alperin and co-workers it was demonstrated that the collagen content of the intramuscular extracellular matrix of the pelvic floor muscle increases during pregnancy²³. This adaptation already starts in early pregnancy and returns to non-pregnant virgin rat levels after delivery²³. Although not part of their research question, this change in collagen content would have reflected itself in the echogenicity of muscles. Maladaptation of the collagen changes in the extracellular matrix may be one of the reasons some women fail to deliver vaginally.

Since decades, researcher have searched for parameters that are associated with mode of delivery. Factors, such as parity, maternal age, body mass index (BMI), induction of labor, epidural analgesia and birth weight, have already been associated with the mode of delivery in pregnant women, however these factors do not cover all failures to deliver vaginally²⁴⁻²⁶. Measuring echogenicity, as an indirect marker of the collagen content of muscle tissue, may be of additional value. The association between the mean echogenicity of the puborectalis at 12 weeks gestation, with the pelvic floor in rest, contraction and during Valsalva maneuver, and mode of delivery was described in chapter 6. The significantly lower mean echogenicity of the puborectalis muscle in contraction in women who had an emergency cesarean section due to failure to progress, indicates a different muscle to extracellular matrix ratio, in favor of the muscle cell component. We hypothesize that a low MEP in early pregnancy is a result of a disturbed early adaptation of the collagen metabolism, with less collagen being formed or initially present. Since collagen weakening is necessary in preparation for childbirth, a limited amount of collagen may inhibit this process. In order for this explanation to hold ground we need to hypothesize that the low echogenicity is constitutional.

Two main points need to be mentioned in this perspective. To answer the question on collagen concentration a histological examination on the muscle tissue would be indicated. However, obtaining a muscle biopsy from pregnant women is ethically complex. A second point of discussion is the variation in case definition and case ascertainment. In the Netherlands we have other cut-off values when defining the need for a cesarean delivery compared to for example the United States of America or Germany. We have a relatively high rate of home deliveries and we are almost unfamiliar with elective cesarean deliveries, not based on a clinical need²⁷⁻²⁹. Including echogenicity measures in clinical decision making is therefore more likely to be accepted and of added value in countries with a higher number of vaginal deliveries. However the wide spread variation in clinical decision making should also acquire more attention to improve the effectiveness of study outcomes.

MONITORING AND TREATMENT OF TRAUMA

The second area of clinical relevance, in which knowledge on structure and function of the muscle could be of added value, is monitoring trauma and improving regeneration. Minor and major trauma of the puborectalis muscle are known to occur in many women as a consequence of vaginal delivery^{30,31}. The effectiveness of treatment of this type of injury, for instance tissue regeneration techniques, could be monitored with ultrasound.

Clinical value of measuring global strain

We consider strain during contraction of clinical importance for pelvic floor function since contraction is the mechanism behind continence. Quantifying strain could be a helpful tool in diagnostics and therapy of pelvic floor muscle training. The majority of studies on the functional aspects of the pelvic floor study the hiatal area and angles, which are indirect markers of puborectalis muscle function^{2,32,33}. Van Veelen and co-workers evaluated changes in the contractibility of the levator hiatus during and after first pregnancy by means of the in- or decrease of levator hiatal dimensions during pelvic floor contraction and Valsalva maneuver². However, when the anteroposterior and transversal distances of the genital hiatus equally change in opposite directions (shortening and lengthening) during contraction, the net result of the change in area value will be zero. In such a situation the change in shape of the hiatal area, does not represent the strain of the different parts of the muscle³⁴. In Chapter 7 the results of studying the strain of the pelvic floor with a new software tool, are described. The main outcome of this study was that after spontaneous and operative vaginal delivery the global strain significantly diminished as compared to pregnancy values. This did not occur in women who had a cesarean delivery. Three other studies described strain in the pelvic floor, but none of these studies define the changes pre- and postpartum³⁵⁻³⁷. The major pitfall in our study was the number of complete dataset inclusions. When either the "rest" or "contraction" data was missing, it was not possible to define strain. Adding the separation of data among the three groups of modes of delivery, vaginal delivery, operative vaginal delivery (vacuum) and cesarean section, resulted in a limited number of inclusions per group. The strain values per patient between the time points were significantly different, but to define absolute mean difference in strain postpartum, larger groups need to be analyzed. In addition, the same arguments accounts as in chapter 4, we did not exclude data with muscle trauma from our research results. Considering the effect of minor and major avulsions on the functionality of the puborectalis muscle³⁸, our decision to not define avulsions in our dataset might be considered debateable. Especially, since both intact and damaged muscles are analyzed as if they are one group. A re-exam of our data on strain, defining those datasets with avulsions of the puborectalis muscle could be interesting and could have an effect on our conclusions on the outcome of strain

Issues in study design

In chapter 4 the results of our study on the effect of several demographic characteristics on MEP were described. Differences in echogenicity caused by

probe pressure were described by Odegaard in a different clinical setting before³⁹. The results of our low, medium and hard pressure of the probe on the perineum showed that soft pressure needs to be avoided since the MEP is significantly lower when soft pressure is applied compared to normal and hard pressure.. However in clinical practice soft pressure will produce poorer quality images and is avoided anyway, extra instructions on probe pressure during examination is not necessary.

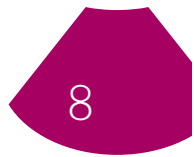
In relation to the study design it is important to realize that the study was primarily conducted as an observational study to determine the association between stress urinary incontinence and levator avulsions (approval number 08/299). Studying the functionality and structural components of the puborectalis by means of echogenicity and strain was only designed later. All examinations were done using the same pre-sets and without changes to any ultrasound setting, enabling us to use the dataset for a secondary goal. This pre-set is accurate for echogenicity measurements, however, the pre-set did not fully fulfill the needs for our strain study. One of the upmost important parameters to consider when studying strain is the framerate, representing the number of images captured per second. A pelvic floor muscle contraction occurs within a second, in order to visualize and analyze this contraction step by step (from rest tot maximum contraction) it is required to have multiple ultrasound frames within this one second. To put things in perspective, we currently analyzed our strain ultrasound images with a framerate of 3 frames per second (fps), while strain research in cardiology is done at 30 fps or more⁴⁰.

One of the limitations in reproducing our findings is the fact that different settings, within or between the ultrasound system(s), will produce different echogenicity values. Fortunately, within our dataset all parameters were kept identical (by means of a pre-set). Nevertheless, there is a clear need to develop algorithms or calibration devices (phantoms) for ultrasound equipment in order to obtain reproducible echogenicity values. For strain measurements, described in chapter 7, differences between ultrasound systems setups is not a problem.

Recommendations and future research

In this thesis we showed that new parameters in the field of ultrasound in urogynecology adds to the current understanding of the structure and function of the pelvic floor. The next step is to translate these findings into clinical practice. For instance, patients with a poor score on strain postpartum may benefit from early pelvic floor exercises. Measuring strain can also be useful for monitoring progression of muscle function under pelvic floor muscle training exercises. On the other hand, if large muscle trauma is detected with MEP, by means of areas of scar tissue formation, this may be associated with a poor outcome of pelvic floor muscle training and can help in early decision making (surgery). Finally, primigravid women with a very low MEP at 12 weeks gestations can be counseled to deliver at the hospital (not at home), or even consider elective cesarean section. Cut-off values need to be determined and our results need to be externally validated.

- One of the limitations of our study is that all measurements were obtained in a two dimensional (2D) plane. The pelvic floor, including all its muscles, ligaments and bones is a complex three dimensional (3D) system. A next step in studying



the pelvic floor is defining it in 3D. This would limit the effect of so called out of plane movement during contraction and Valsalva. Out of plane movement occurs when the muscle, which is a volume, reshapes and moves during contracting. When the muscle is visualized by 2D ultrasound, this reshaping might occur outside the view of the 2D plane. The main recommendation therefore is to develop a volume (3D) segmentation, preferably automatic, and additionally expand to 4D (time) segmentation. In that way strain of the muscle can be visualized in a way that more accurately represents the movement.

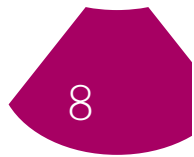
- A multicenter follow-up study on echogenicity, the PURE-study, is designed to control our results on the association between echogenicity and mode of delivery and draw conclusions based on these outcomes. Additionally women are scanned with a transvaginal probe to visualize the cervix and with a linear probe to visualize the vastus lateralis muscle (upper leg). Since transvaginal ultrasound imaging is common practice in obstetrics and 2D scanning of the vastus lateralis is easier compared to transperineal ultrasound, these two methods of defining echogenicity could improve simplicity of echogenicity measurements.

General conclusion

With the results presented in this thesis we provided an indication to consider new structural and functional parameters, retrieved from ultrasound, to diagnose patients during pregnancy and consider as a tool during treatment after levator ani muscle injury.

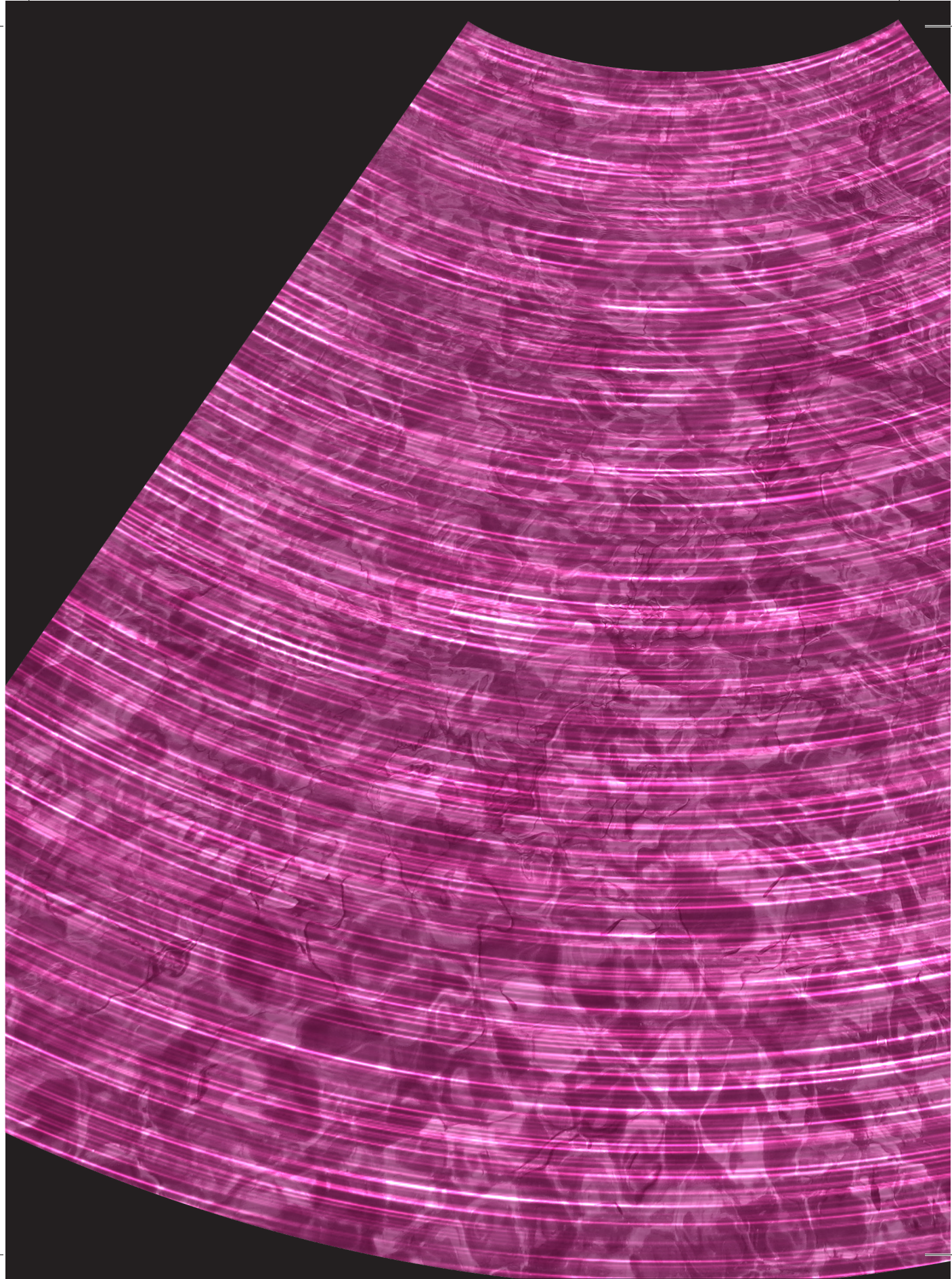
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CHAPTER 9

Summary

Samenvatting

Chapter 9



Summary

This thesis focussed on the composition and functionality of the puborectalis muscle based on calculating echogenicity and strain from transperineal ultrasound recordings. As described in **chapter 1**, it is its specific orientation that allows the levator ani muscle to lift (vertically in the standing posture) the pelvic organs and close the levator hiatus (horizontally in standing posture). Dysfunction of the pelvic floor muscles can be caused by instant trauma or long term deterioration. The most frequently reported risk factors for developing incontinence and pelvic organ prolapse are: pregnancy, mode of delivery, parity and Body-Mass-Index. The fact that the puborectalis muscle can be visualized on ultrasound images allows clinicians and researchers to study composition and function.

In recent years, a large number of studies have been published on the clinical relevance of pelvic floor three-dimensional (3D) transperineal ultrasound. In **chapter 2** we reviewed literature to determine the compliance of diagnostic accuracy studies investigating pelvic floor 3D ultrasound, with Standards for Reporting of Diagnostic Accuracy (STARD) guidelines by means of a systematic review. The overall compliance with reporting guidelines of diagnostic accuracy studies of pelvic floor 3D transperineal ultrasound is relatively good, compared to other fields of medicine. However specific items, such as quality of reporting on handling of indeterminate results or missing responses, adverse events and time interval between tests, should require more attention when reported.

In recent years three-dimensional (3D) and four-dimensional (4D) volume transperineal ultrasound imaging has become increasingly popular to study pelvic floor anatomy. Although measuring dimensions of the pelvic floor is well developed, identifying structural changes in the puborectalis muscle, apart from levator avulsions, is still in its infancy. In **chapter 3** we described the development of a semi-automated method to assess puborectalis muscle echogenicity on 3D transperineal ultrasound images using 4D View and Matlab® software and evaluate its intra- and interobserver reliability. Echogenicity and area measurements of the puborectalis muscle can be performed reliably, and appears a promising new tool for studying pelvic floor anatomy.

In **chapter 4** we presented the effect of body mass index (BMI), age, physical activity and ultrasound probe pressure on the Mean Echogenicity of the Puborectalis muscle (MEP) and Puborectalis Muscle Area (PMA). Two experienced examiners were asked to obtain ultrasound volume datasets of 15 primiparous women at 6 months postpartum at different probe pressures (soft, normal and hard). BMI, age and physical activity do not affect the MEP and PMA in our study population. Additionally we found that the echogenicity of the pelvic floor musculature can be reliably studied when soft pressure on the ultrasound probe during examination is avoided.

In **chapter 5** we reported on changes in the mean echogenicity (MEP) and area (PMA) of the puborectalis muscle during first pregnancy and after childbirth. The MEP and PMA of 254 women during first pregnancy were measured at 12 and 36 weeks gestation and 6 months postpartum. To determine the effect of child-birth on MEP and PMA, the results at 6 months postpartum were separately analysed for vaginal deliveries, operative vaginal deliveries (ventous) and cesarean-

section deliveries. The MEP at 6 months postpartum was, maneuver independent, significantly ($p < 0.001$) lower as compared to MEP values during pregnancy. Our study indicates that structural changes in the puborectalis muscle during and after pregnancy, as measured by MEP, occur and can be analysed. In addition, after cesarean delivery, the PMA was significantly smaller at maximum pelvic floor contraction as compared to PMA after vaginal delivery ($p = 0.003$) or operative vaginal delivery ($p = 0.002$). Thus the mode of delivery affects PMA during contraction after delivery. For true volume analysis, as part of an assessment of contractility of the puborectalis muscle we will need 3D volume analysis.

In **chapter 6** we evaluated the association between mean echogenicity of the puborectalis (MEP) muscle, measured using transperineal ultrasonography, in women during their first pregnancy and the subsequent mode of delivery. The mode of delivery was classified into five categories: spontaneous vaginal delivery, instrumental vaginal delivery, elective cesarean delivery, cesarean delivery due to non-reassuring fetal status and cesarean delivery due to failure to progress. Of the 254 women included 157 had a spontaneous vaginal delivery, 47 underwent a cesarean delivery (11 elective, 36 emergency) and 45 had a vacuum operative vaginal delivery and in 5 patient files the mode of delivery was not recorded. Of the analyzed women, those who delivered by cesarean because of failure to progress had a significantly lower mean echogenicity of the puborectalis muscle on pelvic floor contraction at 12 weeks of gestation (116 ± 14) than women who had a spontaneous vaginal delivery (132 ± 21 ; Tukey's post-hoc test, $p = 0.03$), instrumental vaginal delivery (138 ± 21 ; $p = 0.004$) and cesarean delivery due to non-reassuring fetal status (139 ± 20 ; $p = 0.02$). The significantly lower mean echogenicity of the puborectalis muscle in women who had an emergency cesarean section due to failure to progress may be an indication of a disturbed early adaptation of this collagen metabolism, with less collagen being formed.

In **chapter 7** we described the global strain of the puborectalis muscle during and after pregnancy. After delineation of the muscle on ultrasound images, the length of the midline of the puborectalis muscle was measured in the rest and contraction and global strain was expressed as percentile difference. During pregnancy the global strain did not change. After spontaneous and operative vaginal delivery the global strain significantly diminished as compared to pregnancy values. This did not occur in women who had a cesarean delivery. Vaginal childbirth thus negatively influences the strain of the puborectalis muscle.

In **chapter 8** we discussed the results and conclusions that can be drawn from this thesis in a broader perspective. The information obtained in the research presented an indication to consider new structural and functional parameters, retrieved from ultrasound, to diagnose patients and consider during treatment after muscle injury.

Nederlandse Samenvatting

Dit proefschrift richt zicht op de samenstelling en functionaliteit van de puborectalis spier, gebaseerd op de parameters echogeniciteit en vervorming bepaald vanuit echo opnames. Zoals beschreven in **hoofdstuk 1**, is het de specifieke oriëntatie van de levator ani spier die deze in staat stelt om de bekkenorganen te tillen (verticaal in staande houding) en de levator hiatus te sluiten (horizontaal in staande houding). Disfunctie van de bekkenbodemspieren kan veroorzaakt zijn door direct trauma of langdurige achteruitgang. De meest voorkomende risico factoren voor het ontwikkelen van incontinentie en bekken orgaan prolaps zijn: zwangerschap, wijze van bevalling, pariteit en BMI. Aangezien de puborectalis spier goed gevisualiseerd kan worden met echo, stelt dit de artsen en onderzoekers in staat om de spier compositie en functie te onderzoeken.

De laatste jaren zijn er talrijke studies gepubliceerd over de klinische relevantie van driedimensionale (3D) transperineale echografie met betrekking tot de bekkenbodemspier. In **hoofdstuk 2** beschreven we een systematisch overzicht van literatuur en de kwaliteit en nauwkeurigheid van studies die bekkenbodemspier 3D echografie beschrijven, middels de richtlijn: Standards for Reporting of Diagnostic Accuracy (STARD). De algehele naleving van de richtlijn voor de rapportage van diagnostische nauwkeurigheid studies van bekkenbodemspier 3D transperineale echo is relatief goed, in vergelijking met andere gebieden van de geneeskunde. Echter specifieke onderwerpen, zoals de kwaliteit van de rapportage over de behandeling van onbepaalde resultaten of ontbrekende data, bijwerkingen en tijdsinterval tussen de tests, zou meer aandacht moeten krijgen wanneer gerapporteerd.

De laatste jaren is driedimensionale (3D) en vierdimensionale (4D) volume transperineale echografie steeds populairder geworden om de anatomie van de bekkenbodemspier te bestuderen. Hoewel het bepalen van afmetingen van de bekkenbodemspier goed ontwikkeld is, staat het identificeren van structurele veranderingen in de puborectalis spier, behalve betreffende het bepalen van levator avulsies, nog in de kinderschoenen. In **hoofdstuk 3** beschreven we de ontwikkeling van een semi-automatische methode om echogeniciteit van de puborectalis spier te beoordelen op 3D transperineale ultrasound beelden met behulp van software programma's 4D-view en Matlab®. Waarbij we intra- en interobserver betrouwbaarheid hebben geëvalueerd. Echogeniciteit en oppervlakte metingen van de puborectalis spier kunnen betrouwbaar worden uitgevoerd, en echogeniciteit lijkt een veelbelovend nieuw instrument voor het bestuderen van de anatomie van de bekkenbodemspier.

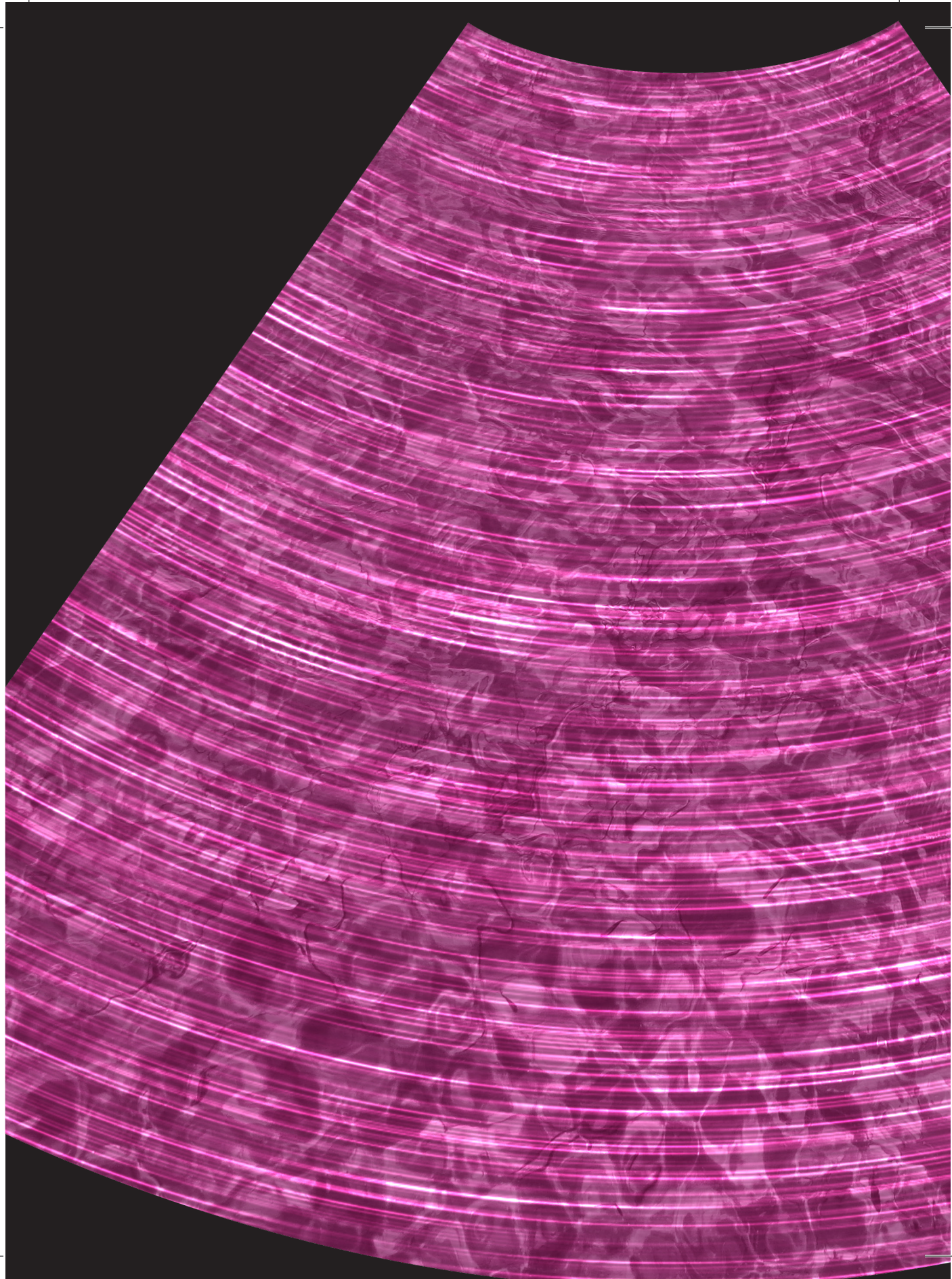
In **hoofdstuk 4** presenteerden we het effect van de body mass index (BMI), leeftijd, sporten en probe druk op de gemiddelde echogeniciteit (MEP) en oppervlak (PMA) van de puborectalis spier. Twee ervaren artsen werd gevraagd om bij 15 vrouwen (primiparae) echo volume datasets te maken op 6 maanden postpartum, waarbij de echo probe met verschillende druk op het perineum werd gepositioneerd (zacht, normaal en hard). De resultaten laten zien dat BMI, leeftijd en sporten geen invloed hebben op de MEP en PMA binnen onze studie populatie. Bovendien vonden we dat de echogeniciteit van de bekkenbodemspier betrouwbaar kan worden bestudeerd als zachte druk op de echo probe tijdens scannen wordt vermeden.

In **hoofdstuk 5** berichtten we over de veranderingen in de gemiddelde echogeniciteit (MEP) en oppervlakte (PMA) van de puborectalis spier tijdens de eerste zwangerschap en na de bevalling. De MEP en PMA van 254 vrouwen tijdens de eerste zwangerschap werd gemeten op 12 en 36 weken zwangerschap en 6 maanden na de bevalling. Om het effect van de bevalling op MEP en PMA te bepalen, werden de resultaten op 6 maanden na de bevalling afzonderlijk geanalyseerd voor vaginale bevallingen, operatieve vaginale bevallingen (vacuüm) en keizersnedes. De MEP op 6 maanden postpartum is, onafhankelijk van de positie (rust, contractie, persen), significant ($p < 0,001$) lager dan MEP waarden tijdens de zwangerschap. De studie geeft aan dat structurele veranderingen in de puborectalis spier tijdens en na de zwangerschap, optreden en gemeten kunnen worden met MEP. Bovendien, na een keizersnede, was de PMA aanzienlijk kleiner tijdens maximaal bekkenbodem contractie vergeleken met PMA na vaginale geboorte ($p = 0,003$) of operatieve vaginale bevalling ($p = 0,002$). Zo is de wijze van bevalling van invloed op de PMA tijdens contractie na de bevalling. Voor volume analyses, in het kader van een evaluatie van de contractiliteit van de puborectalis spier, zijn 3D-volume analyses nodig.

In **hoofdstuk 6** beschreven we de associatie tussen gemiddelde echogeniciteit van de puborectalis spier (MEP), gemeten met behulp van transperineale echografie, bij vrouwen tijdens hun eerste zwangerschap en de daarop volgende wijze van bevalling. De wijze van bevalling werd ingedeeld in vijf categorieën: spontane vaginale bevalling, instrumentale vaginale bevalling (vacuüm), electieve keizersnede, keizersnede als gevolg van foetale nood en keizersnede als gevolg van niet vorderen. Van de 254 geïnccludeerde vrouwen hadden er 157 een spontane vaginale bevalling, 47 vrouwen ondergingen een keizersnede (11 electief, 36 nood), 45 vrouwen had een vaginale bevalling met vacuüm extractie en in 5 patiëntendossiers was wijze van bevalling is niet opgenomen. De vrouwen die via een keizersnede, als gevolg van niet vorderen, bevallen waren hadden een significant lagere gemiddelde echogeniciteit van de puborectalis spier op echo (in contractie) bij 12 weken zwangerschap (116 ± 14) ten opzichte van vrouwen die een spontane vaginale bevalling (132 ± 21 ; post-hoc Tukey's test, $p = 0.03$), operatieve (vacuüm) vaginale bevalling (138 ± 21 ; $p = 0.004$) of een keizersnede als gevolg van foetale nood (139 ± 20 ; $p = 0.02$) hadden. De aanzienlijk lagere gemiddelde echogeniciteit van de puborectalis spier bij vrouwen met een keizersnede als gevolg van niet vorderen kan een indicatie zijn van een verstoorde vroegtijdige aanpassing van collageen metabolisme, waarbij er minder collageen gevormd is.

In **hoofdstuk 7** zijn de globale vervorming van de puborectalis spier tijdens en na de zwangerschap beschreven. Na het uitknippen van de spier aan op de echo afbeeldingen, werd de lengte van de middellijn van de puborectalis spier gemeten in rust en contractie. De globale vervorming werd uitgedrukt als verschil in percentage tussen de twee posities. Tijdens de zwangerschap veranderde de globale vervorming in de spier niet. Na spontane en operatieve (vacuüm) vaginale bevalling was de globale vervorming significant verminderd in vergelijking de vervorming tijdens met de zwangerschap. Deze afname deed zich niet voor bij vrouwen die een keizersnede hebben gehad. Vaginale bevalling beïnvloedt de vervorming in de puborectalis spier dus nadelig.

In **hoofdstuk 8** worden de resultaten en conclusies van dit proefschrift in een breder perspectief besproken. De resultaten van dit onderzoek kunnen worden gebruikt om nieuwe structurele en functionele parameters, bepaald uit echo afbeeldingen, toe te passen bij het diagnosticeren en behandelen van patiënten.





CHAPTER 10

Addendum

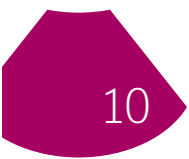
Review committee

Biography

List of publications and
presentations

List of abbreviations

Acknowledgement



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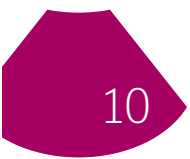
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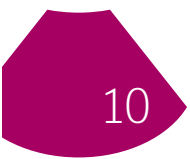
Biography

Anique Theodora Maria Grob was born on October 12th 1987 in Zevenaar, the Netherlands. She is the oldest of three, having a brother and sister. Anique is living with her husband, Jean-Michel Bellos, in Hengelo.



After graduating from the Liemers College (Zevenaar), she attended the University of Twente to study Technical Medicine and Health Sciences. During her master 2 and master 3 years of rotating internships her interest in (uro) gynecology was evoked. She graduated cum laude for her master Technical Medicine.

When she received her master degree in 2013, Anique continued to work in this field starting a PhD research track under supervision of prof. dr. ir. C.H. Slump (University of Twente) en prof. dr. C.H. van der Vaart (UMC Utrecht). She did her PhD part-time, while also working at the University of Twente as a teacher in Technical Medicine. She received her university teaching qualification in 2015.



List of publications and presentations

Grob ATM, Withagen MIJ, van de Waarsenburg MK, Schweitzer KJ, van der Vaart CH. Association of First-Trimester Echogenicity of the Puborectalis Muscle With Mode of Delivery. *Obstet Gynecol.* 2016; 127(6): 1021-6.

van de Waarsenburg MK, Withagen MIJ, **Grob ATM**, Schweitzer KJ, van Veelen GA, van der Vaart CH. Mean echogenicity and area of puborectalis muscle in women with stress urinary incontinence during pregnancy and after delivery. *Int Urogynecol J.* 2016. [Epub ahead of print]

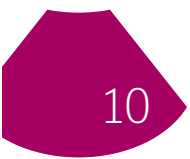
Grob ATM, Withagen MIJ, van de Waarsenburg MK, Schweitzer KJ, van der Vaart CH. Changes in the mean echogenicity and area of the puborectalis muscle during pregnancy and postpartum. *Int Urogynecol J.* 2016; 27(6): 895-901.

Grob ATM, Veen AAC, Schweitzer KJ, Withagen MIJ, van Veelen GA, van der Vaart CH. Measuring echogenicity and area of the puborectalis muscle: method and reliability. *Ultrasound Obstet Gynecol.* 2014; 44(4): 481-5.

TABLE 1 Presentations at conferences and meetings

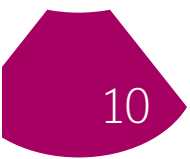
Date	What	Topic	Where
2014-07	Oral	Changes in the mean echogenicity of the puborectalis muscle during pregnancy and postpartum	IUGA*
2014-07	Poster	Changes in puborectalis muscle area during pregnancy and postpartum	IUGA*
2014-09	Oral	Assessment of puborectalis muscle echogenicity: effect of BMI, age, sport activity and ultrasound probe pressure	ISUOG*
2014-11	Oral	Measuring echogenicity and area of the puborectalis muscle: method and reliability.	ImagO*
2015-04	Oral	Echogeniciteit van de bekkenbodemspier gedurende zwangerschap en postpartum	Doelencongres*
2015-06	Poster	Appearance model of the puborectalis muscle in 3D ultrasound images	MIRA**
2015-06	Poster	Transperineal ultrasonographic echogenicity measurements in predicting caesarean delivery for failure to progress in nulliparous women	IUGA*
2015-06	Poster	Mean echogenicity of the puborectalis muscle and stress urinary incontinence during pregnancy and after delivery	IUGA***
2016-04	Poster	Automatic Segmentation of the Puborectalis Muscle in 3d Ultrasound Using Active Appearance Models	ISBI**
2016-08	Oral	Changes in the global strain of the puborectalis muscle during pregnancy and after child-birth.	IUGA*

* First author, ** Second author, *** Third author



List of abbreviations

2D	Two-dimensional
3D	Three-dimensional
4D	Four-dimensional
BMI	Body Mass Index
C	Contraction
CI	Confidence Interval
CT	Computed Tomography
HP	Hard Pressure
ICC	Intraclass Correlation Coefficient
LAM	Levator Ani Muscle
LOA	Limits Of Agreement
MEP	Mean Echogenicity of the Puborectalis muscle
MRI	Magnetic Resonance Imaging
NMD	NeuroMuscular Disorder
NP	Normal Pressure
PMA	Puborectalis Muscle Area
POP	Pelvic Organ Prolapse
POP-Q	Pelvic Organ Prolapse Quantification
R	Rest
SD	Standard Deviation
SP	Soft Pressure
STARD	Standards for Reporting of Diagnostic Accuracy
SUI	Stress Urinary Incontinence
TGC	Time Gain Compensation
TRIPOD	Transparent Reporting of a multivariable prediction model for Individual Prognosis Or Diagnosis
TUI	Tomographic Ultrasound Image
UI	Urinary Incontinence
V	Valsalva



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Het is het eindpunt van de trein,
bijna geen mens hoeft er te zijn,
bijna geen hond gaat zover mee:
Enschede.

uit *Textielstad*
door Willem Wilmink