ASSESSMENT OF ECHOGENICITY OF THE CERVIX AT 12 WEEKS PREGNANCY IN RELATION TO THE MODE OF DELIVERY

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Summary

The goal of this study was to associate the echogenicity of the cervix at 12 weeks pregnancy to the mode of delivery. This association may contribute to a prediction model, resulting in an improvement of emotional experience of childbirth and decrease of extra costs. Calibration of ultrasound images was necessary due to the use of data from different hospitals. There were differences between the images per device, despite the pre-set. The calibration is done based on phantom data and data of the vastus lateralis muscle, which functioned as control data. Three different corrections are applied in a semi-automatic calibration method. The ultrasound images have been corrected for the scaling of grey-values and density and for the strength of the signal over the depth. This method has an accuracy of approximately 4.5% a 5.5% and is suitable for the images obtained with the abdominal probe, with the Bergman device or the UMC Utrecht device.

A reliable and reproducible method developed is to determine the echogenicity of the cervix. The anterior lip showed the best results compared to the posterior lip, based on correlations, standard deviations and standard error.

Thereafter, 211 scans of the cervix from St. Antonius and UMC Utrecht have been analysed and associated to five different modes of delivery; vaginal, assisted delivery, primary caesarean section, secondary caesarean section because of fetal distress and secondary caesarean because of failure to progress. No significant differences in echogenicity between these groups are found. This also applies to ratios of different regions in the cervix. These regions were measured because literature shows that there are differences in histology per region of the cervix. In addition, the signal processing of the device plays a role between different regions. For all other associated variables, duration of pregnancy, induction, use of augmentation and the use of medication, no significant differences in echogenicity between groups are determined.

Abbreviations

CS - Caesarean section
LUT - look-up-table
FOC - fear of childbirth
ICC - interclass correlation
MOD - mode of delivery
ECM - extracellular matrix
NVOG - Nederlandse Vereniging voor Obstetrie en Gynaecologie

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Chapter 1. General introduction

1.1 Introduction

Pregnancy is associated with several adaptations in the pelvic connective tissue and the pelvic musculature in preparation for delivery.[1, 2] The pelvic connective tissue and musculature are commonly described as the pelvic floor. The pelvic floor plays an important role in the support of the uterus and pelvic organs like the bladder and rectum. The uterus, pelvic floor muscles and the cervix are important structures in the pelvis which, undergo changes to adapt the body for the ongoing pregnancy and which make a delivery possible.[3, 4] The function of the cervix during pregnancy is to prevent pre-term delivery and allow dilatation during actual delivery in order to let the baby pass.[2, 5-7] To accomplish this, the cervix undergoes various changes, called remodelling.[2, 8, 9] There are four phases of cervical adaptations during pregnancy. The first 32 weeks is the softening phase of the cervix. During these weeks there is collagen reorganization, growth edema and an increased vascularization. Between 32 weeks and the uterine contractions the ripening takes place. There is an increase in synthesis in collagen, hydrophilic glycosaminoglycans and proteoglycans. Therefore, the distensibility of the cervix increased. This phase is followed by the dilation phase. During this phase, a release of proteases and collagenases into the extracellular matrix (ECM), leukocytes infiltration and a decrease of collagen concentration occur. Resulting in softening and dilation of the cervix. The fourth phase is taking place after delivery and is characterized by repair of the tissue.[2, 8-10] Failure to undergo the adaptations will obstruct labour and vaginal delivery. An obstructed labour can be managed through interventions like an assisted-delivery or caesarean delivery. The composition of the cervix, with its balance between the ECM (mainly collagen) and muscle cells, may be important in the pathophysiology of obstructed labour and thus on the mode of delivery (MOD).

An indication of the composition of the cervix can be obtained with ultrasound. Brightness of muscles on ultrasound depends on the tissue composition in muscle cells and ECM.[11] The echogenicity is the grey-value of the pixel, varying from 0 (black) to 255 (white). Normal muscle cells are hypo-echoic and appear dark on ultrasound images. Connective and fatty tissue of muscles are more echoic compared to muscle cells, so brighter on the images. An increase of collagen is related to increase of echogenicity.[11] In other words, tissue with a higher concentration of collagen relative to muscle cells, has a higher echogenicity and appears brighter.[11, 12] This knowledge can be used to consider adaptations in the cervix during pregnancy; because the main component of the cervix is collagen. The brightness of the cervix is related to the histologic composition of the cervix.
Ultrasound contributes to the determination of the adaptations, since ultrasound grey-scale analysis has proven to be a useful technique.[11, 13-15] Studies have been done to assess adaptations in (neuro-) muscular disorders with the quantitative grey-scale analysis. These muscles undergo histological changes, which leads to loss of normal heterogeneity and to a more echogenic muscle on ultrasound images. This provides information about the progress of the disease.[13-15]

However, echogenicity or grey-scale analysis in gynecology is less common. Previous ultrasound research about the adaptions of the pelvic floor during pregnancy, in UMC Utrecht hospital has focused on the pelvic floor musculature.[16-18] There are significant differences in pelvic floor dimensions and echogenicity of the puborectalis muscle at 12 weeks pregnancy between women whom delivered with an (emergency) caesarean section (CS) and whom had a different MOD shown.[12] The differences in echogenicity reflect differences in composition of the pelvic floor muscle. The observation that at 12 weeks gestation the echogenicity of the puborectalis muscle is lower for women whom failed vaginal delivery indicates that the composition of the muscle favors muscle cell tissue over ECM. In the failure to deliver group there are women who failed to reach complete cervical dilatation. The levator ani is mainly important during the second stage of labour in which it has to adept to allow passage of the child.[3, 4] The first stage of labour, e.g. the dilatation of the cervix, is not influenced by the pelvic floor musculature, but important for obstructed labour. Therefore, the cervix is interesting.

1.2. Purpose of the study

Considering the role of the collagen during (obstructed) labour, it is interesting to investigate the echogenicity of the cervix in relation to the MOD. To determine the echogenicity there is data obtained from contributing hospitals (UMC Utrecht, St. Antonius Nieuwegein, UMC Radboud and Reinier de Graaf). Due to the use of different data sources, it must be investigated whether these data is consistent, despite pre-sets. For inconsistent data, a calibration is required to make the images comparable. This will be done with images of a phantom and of the vastus lateralis muscle post-partum (Chapter 2). Since the images are comparable, a reproducible and objective method to measure the echogenicity is required (Chapter 3). With the use of the developed method, the echogenicity of the cervix will be determined and will be brought into association with the MOD (Chapter 4).
So, the main goal of this research is to determine the cervical echogenicity from the ultrasound images at 12 weeks gestation with the use of an objective and reproducible method and to associate the echogenicity with the MOD.

Chapter 2. Ultrasound Calibration

2.1 Introduction

2.1.1. Problem

The database of the images is a multi-centre study in which the ultrasound images were made with three types of GE (General Electric Company, Boston, US) ultrasound devices in 5 different hospitals, see table 1. In this chapter, it is investigated whether there are differences between data obtained from contributing centres, what differences may be and how they can be calibrated. So, all data can be compared from contributing centres and used for the echogenicity analysis of the cervix.

Table 1, overview of the contributing hospitals with the used devices.

Hospital	Ultrasound device
UMC Utrecht	GE Voluson 730
St. Antonius	GE Voluson E8
Bergman Clinic	GE Voluson I Portable
Reinier de Graaf	GE Voluson E8
UMC Radboud	GE Voluson I Portable

2.1.2. Analysis

The database consist of images of the cervix and puborectalis of 211 pregnant women and images of the pubrectalis and vastus lateralis post-partum of 20 women. The internal scans of the cervix are made with the vaginal probe RIC 5-9H/GYN. The measurements of the muscle vastus lateral and the puborectalis muscle are made with a curved abdominal probe RAB 4-8L/GYN probe. A pre-set is used for obtaining comparable images per probe (see Appendix 4 for the settings).

There is post-partum data of 20 women, consisting several perineal images and images of the muscle vastus lateralis. At each measuring moment, one of two ultrasound devices is used, UMC Utrecht or Bergman device. The follow up is 1 day, 1 week, 2 weeks, 3 weeks, 4 weeks, 6 weeks, 12 weeks, 18 weeks and 24 weeks after delivery.
The images of the vastus lateralis are made as control compared to the puborectalis muscle images, because of research of Alperin and co-workers.[19] They showed that pregnancy leads to unique structural modifications in the pelvic floor muscles of rats. The fiber length increases by adding sarcomeres in series and there is increased intramuscular collagen synthesis in ECM of pregnant rats. Non-pregnant rats did not show these modifications. Furthermore, the changes were not visible in the tibialis anterior.[19] Based on these findings in rats, no differences in echogenicity of the muscle vastus lateralis are expected. Based on this assumption, the data is used to look at differences between the devices.

Analysing the echogenicity of the post-partum data of 5 women showed major differences in echogenicity of the muscle, both between the women and between different measurement moments per women. This can be caused by the effect over time, maybe women differ in collagen synthesis in the muscle vastus lateralis by adaptations related to pregnancy and recover during the 24 weeks post-partum. Another option is that it can be caused by the settings of the devices.

For both potential causes, the effect is visualized in the table and graph below. First, the data is classified per devices as is shown in Table 2. This does not take into account any possible effects over time. After that, there has been a distinction in every measurement moment, as is visualized in Figure 1. Due to the major differences in mean echogenicity per device in Table 2, the difference between devices has been taken into consideration and are therefore showed separately.

Table 2, echogenicity per device of the 5 women, with maximum 9 measurement moments post-partum.

	Bergman (n=23)	UMC Utrecht (n=17)
Mean	195	147
Std. deviation	14.0	12.2

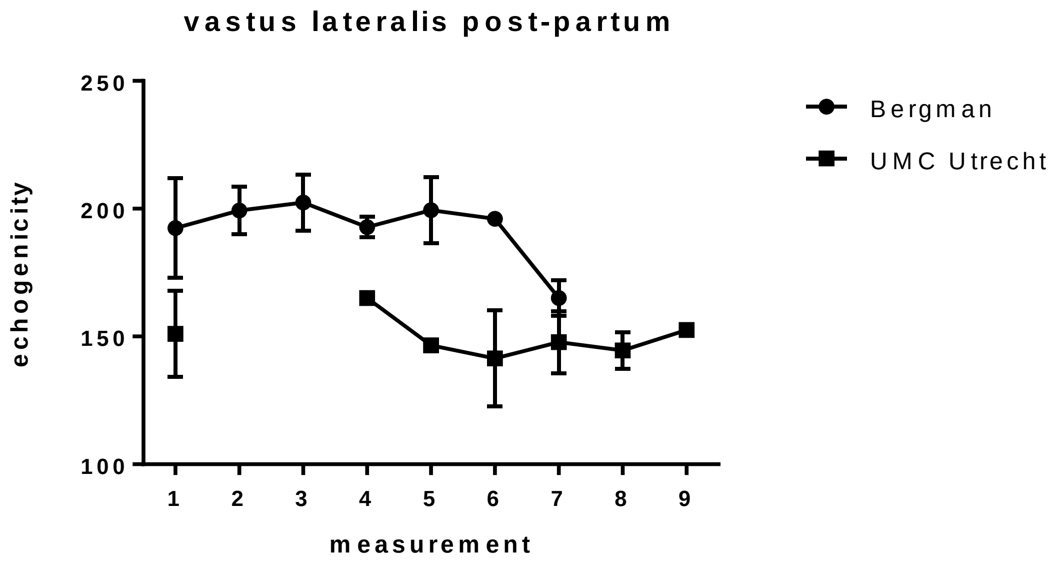


Figure 1, echogenicity of the vastus lateralis at 9 different measurement moments post-partum.

Both devices show a relatively straight line in time, relative to the standard deviation. Except measurement moment 7 of the Bergman device. The women are often scanned the first times with the Bergman device at home to be further scanned at the hospital as soon as they are recovered from the partus. Measuring 4 of UMC Utrecht is a result of one measurement while the rest of the measurements is an average of measurements. This woman may have higher echogenicity or it may be a measurement error. However, for the visualization over time, it does not matter. Given the relatively straight lines in the graph in relative to the stand deviation, where no effect over time is expected, the differences between settings are investigated.
Despite the pre-set, differences between the images are visible. The images made with the Bergman clinic device are generally much brighter compared to the images of the other devices, see images below.

Figure 2, image made with UMC Utrecht device. The settings are visible in the red box. The focus is visible in the blue box.

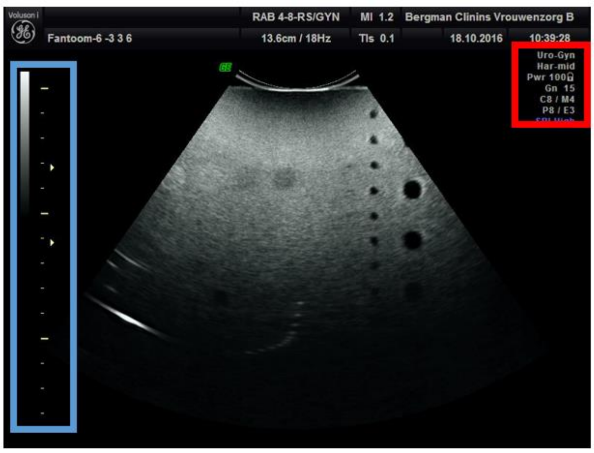


Figure 3, image made Bergman device. The settings are visible in the red box. The focus is visible in the blue box.

The depth and frequency is different for the Bergman device compared to the UMC Utrecht device. Thereby, the resolution is different in images of both devices. These differences makes the comparison of the echogenicity between different devices unreliable; therefore, calibration of the data is necessary.

2.1.3. Goal and requirements

To realize a reproducible assessment of echogenicity between devices, pertinent signal processing needs to be adapted to calibrate the different devices to eachother. After calibration, the echogenicity of each image can be determined, regardless of the used ultrasound device.
The calibration of the different devices should lead to a semi-automatic method to calibrate each image. A semi-automatic method is required to reduce the error sensitivity and reduce the required time. Furthermore, it is important that the method is validated, accurate and reliable, so it is reproducible. Therefore, an accuracy about 5% is desired in line with current clinical practice. To make the method user friendly, clear instruction and default settings are necessary.

2.2 Design

Given that a protocol is used, differences are caused by the processing of the signal by the devices or anatomically but not because of the settings. An ATS type 570 ultrasound phantom (ATS Labouratories, Bridgeport, USA) is used to create a constant, thereby eliminating the anatomically variation as a possible cause. Thus, only the effects of signal processing are made visible. The phantom is a multipurpose and endoscopic phantom with a frequency range of 2.25 to 7.5 MHz. It is useful for different measurements, varying from resolution to grey-scale. There are four grey-scale targets ranging in contrast from +6 dB to -6 dB, at 30 mm depth. The tissue mimicking material is made of urethane rubber and has a speed of sound of 1450 m/s at 23 ^{0}C. The attenuation coefficient is 0.5 dB/cm/MHz at 3.5 MHz.

The calibration is focused on the analysis of the puborectalis muscles and vastus lateralis, because the phantom is scanned with the abdominal probe. Calibration of the vaginal probe was not possible due to the absence of a suitable phantom. The penetration depth of the vaginal probe is not sufficient to image targets in a phantom, because of the high frequency. High frequencies are more attenuated than lower frequencies, due to absorption for a given distance. Therefore, high frequencies are useful for superficial tissues and lower frequencies for the deeper located tissues**.**

There is post-partum data of the vastus lateralis as control data obtained with UMC Utrecht and Bergman devices. Most data is obtained with the UMC Utrecht device. Therefore, this method is focused on the devices of UMC Utrecht and Bergman Clinic. Comparing the images of both devices leads to several differences, as mentioned earlier. First, images of the Bergman devices are much brighter. There are several parameters, that influence the brightness of an image. For example, the gain. The gain setting allows the adjustment of the overall brightness of the image or on different depth with the time-gain compensation (TGC bars). Compensation for attenuation on different areas (near, mid and far-field) is possible with TGC. Secondly, the dynamic range influences the brightness. This is the ratio between the largest and smallest signals, which an instrument can respond to, without distortion. The dynamic range controls the contrast on the ultrasound image making. With edge enhancement, the ultrasound attempts to make a sharper image by combining adjacent signals. This will show higher contrast and brighter edges of structures. Next to the gain, the grey-map influences the brightness. The grey-map is the distribution of the 256 different values. It is visualized as a grey-scale in the look-up-table (LUT). Ideally, the LUT is a linear line. The distribution will often differ from the linear line. The deviation is determined by the grey-map.
However, all these settings are equal because of the pre-set. Since the contrast plays a major role in these settings, the scale of the grey-values is considered. This scale, shown in the LUT, indicates the distribution of echogenicity values from the images. Ideally, linear from 255 to 0.

2.2.1. LUT correction

The values in the LUT are plotted against the length. An average of the three columns next to the column with the highest values in the LUT has been selected. Resulting in an array representing the distribution of the grey-scale, called the LUT line. Next, there is a linear line from 255 to 0 with numbers of steps equal as the length of the LUT line assessed. The ratio between those lines at each depth is the conversion factor. Eventually, each pixel in the original image is multiplied by the conversion factor which is associated with the most similar echogenicity value. As a result, all images should be linearly with equal step size in echogenicity. This rescaling makes it possible to compare different images, because, the distribution of the grey-values is almost the same for each image. The effect of applying this correction is showed in the Figures 4, 5 and 6, below.

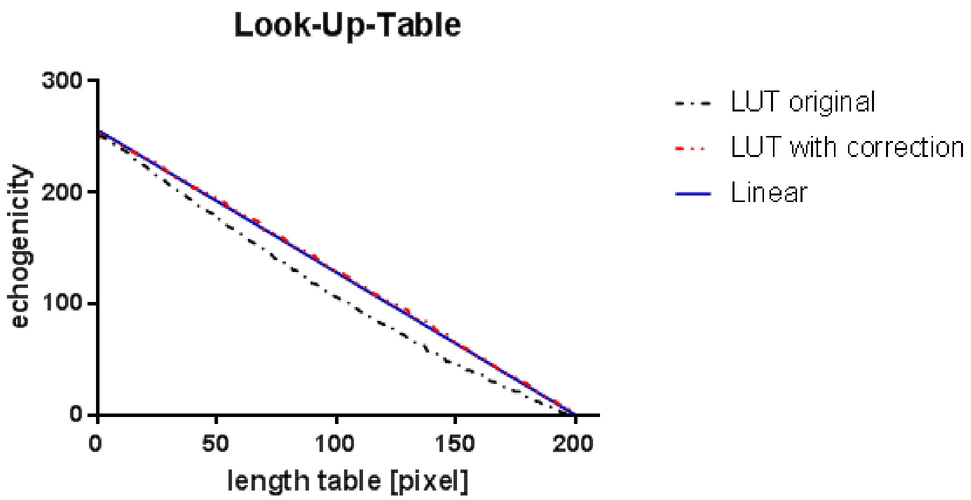


Figure 4, Look-up-table before and after correction, original LUT (black), corrected (red) and blue linear line

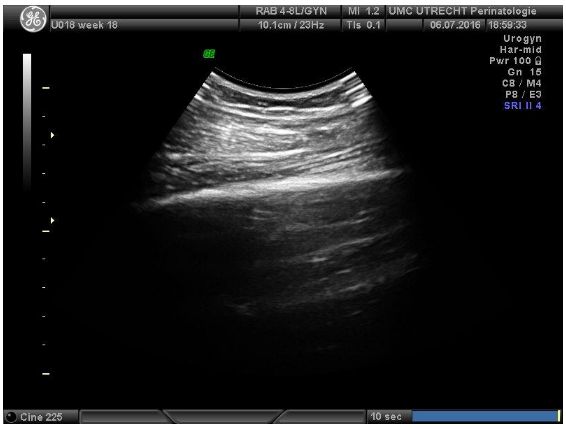


Figure 5, original image of the vastus lateralis muscle.

Figure 6, result of LUT correction on the image of the vastus lateralis muscle

Ideally, the corrected LUT (the red line) would be equal to the linear line (blue line). This is not the case at first, but it is better than the original table (black line). The values with an echogenicity of around 80 a 150 deviate most from the linear line. Therefore, these values are most amplified, see Figure 5 and 6. Despite this correction, still the images of different devices are not comparable. The images show that different focus, depth and frequency are used.

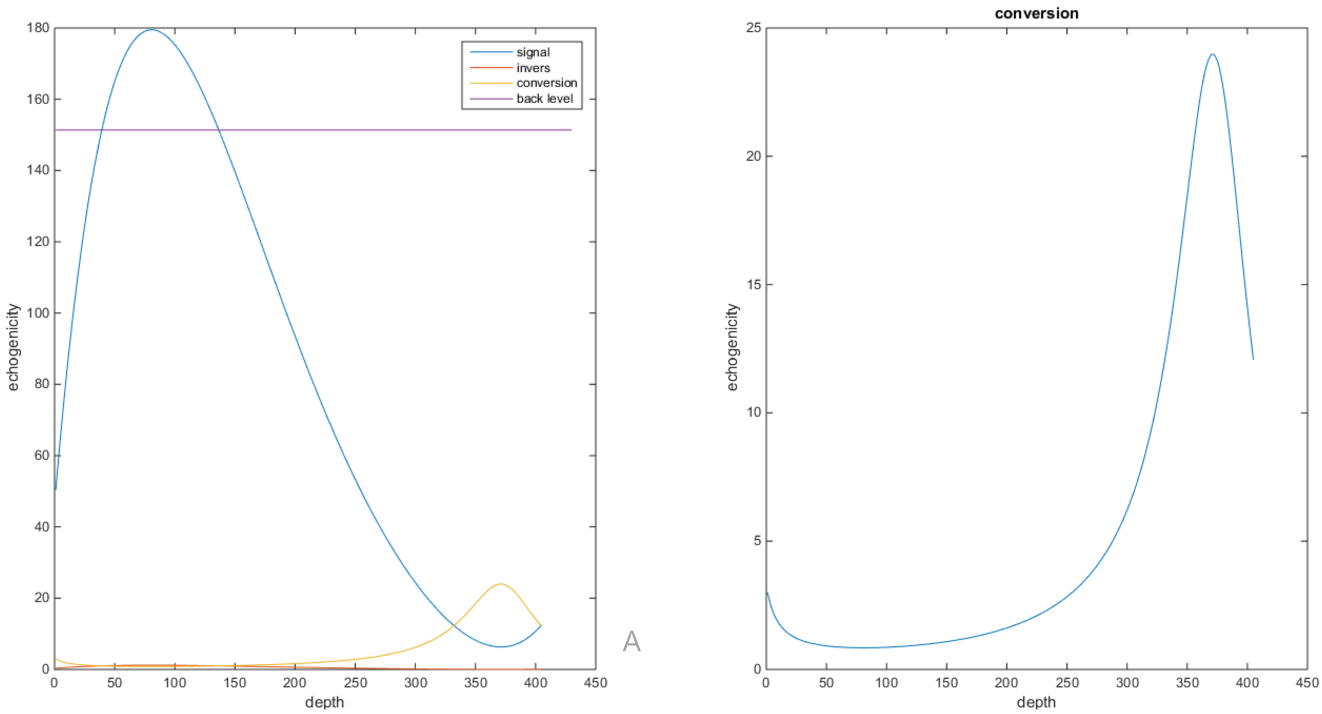
Table 3, settings per device for the contributing hospitals

Setting	Bergman	St. Antonius	UMC Utrecht
Depth	13.6 cm	10.1 cm	10.1 cm
Frequency	18 Hz	24	23
Focus	3 and 6 cm	3 and 6 cm	1.7 and 4.7 cm

These parameters influence the resolution, spatial and temporal. The spatial resolution consists of the lateral and axial resolution. Whereby the lateral resolution is the distinctive ability horizontally and axial resolution is the distinctive ability in the depth. The temporal resolution is the frame rate. The resolution has improved by adjusting the frequency at the expense of the penetration, or increase penetration at the expense of resolution. Imaging of superficial objects is possible with high frequencies; lower frequencies are more suitable for deeper objects. Because the high-frequent signals have high absorption in tissues. Persistence influences the temporal resolution used in grey-scale imaging. It is smoothing by average of different images, so reducing the variation in the images between different frames. High frame rate results in reduced spatial resolution. The depth influences the frame rate; an increase in depth results in reduced resolution. The resolution and frame rate will be worse at a higher depth because there is a longer distance to cover. Lateral resolution at a given depth can be adjusted by the focus. The frame rate will be lower at the depth of the focus, whereby the resolution improved. It is a specific area effect; the frame rate differs only at that depth. There is a gain of the signal at the depth of the focus. Given that the gain on a different height, per device, reinforce another structure does not make a reliable comparison possible. Therefore, it is important that the reinforcement will be assess equally over the depth.

2.2.2. Depth correction

To determine the reinforcement over the depth, QA4US software from Radboud UMC has been used for the calibration. The available software program MATLAB R2014b has been used for the grey-scale analysis. This software is used to analyse the signal in depth. Different images of the phantom background are required. The software determined an average image of several background images. Furthermore, different images are made of air, without gel on the probe. QA4US determine the signal in depth, corrected for the noise, by using the delivered images. The software program also gave the depth whereby the signal is not useful anymore. From that point of depth, the noise predominates the signal.
The signal represent the amount of reinforcement and weakening related to the background also called the back level. The back level is being obtained with the QA4US software. Hereby, the value of the phantom medium is been calculated at 30 mm depth.
The grey-scale course is determined, from the tip of the probe to the end of the image. The ratio between the signal and the back level (=0 dB) is the conversion factor, which is applied on each pixel value on a particular depth. Ultimately, you have an equal amount of signal throughout the entire depth of the images. The results are visualized in the Figures 7-12 below.



A B

Figures 7A and 7B, 7A: Signal per the depth visualized in blue, in yellow the conversion, the back level in purple and in orange the ratio between the back level and signal. 7B: The blue line is the conversion per depth.

Figure 8, original image of the vastus lateral muscle.

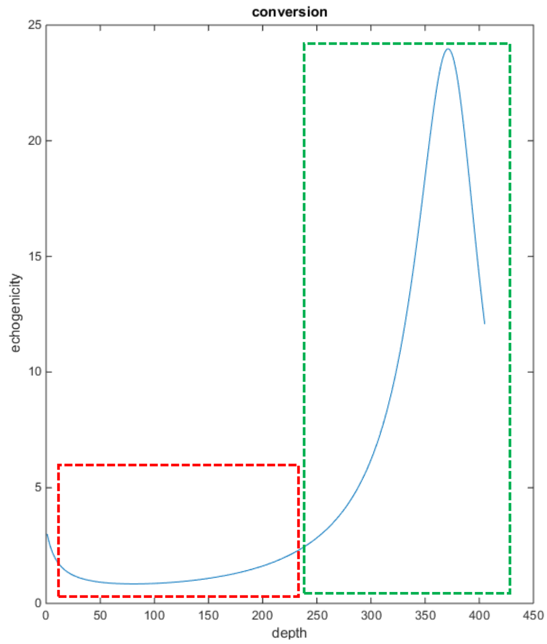


Figure 9, conversion, part of decreased intensity in red box, part of reinforcement in green box.

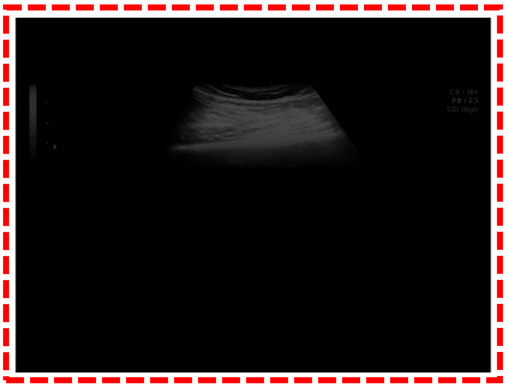


Figure 10, part of increased intensity

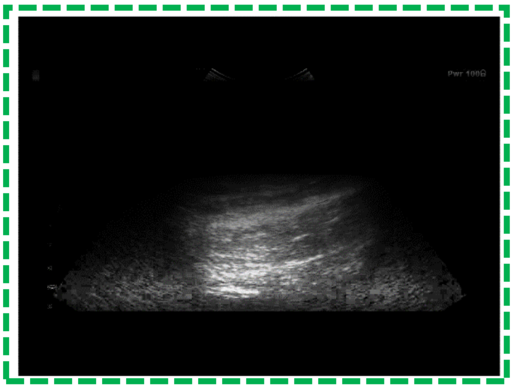


Figure 11, part of reinforcement

Figure 12, result after correction of the vastus lateralis muscle

Figure 8 showed the effect of the conversion per manipulation of the signal. Each pixel value in a region with a signal above the back level (see Figure 7A) is lower after the conversion, visualized in a red box (Figure 9). The pixel values in the regions with a signal below the back level are increased, visualized in a green box (Figure 9). Both regions are showed in Figure 10 and Figure 11, wherein Figure 10 shows the region of weakened and Figure 11 the region of reinforcement. The total effect of conversion is visualized in Figure 12, with the differences relative to the original image clearly visible. There is a reinforcement of the lower parts and weakening of the mid field region.
To validate this method, the echogenicity of the phantom over depth is determined and visualized, see Figure 13. After correction the echogenicity should remain the same over depth, see Figure 14.

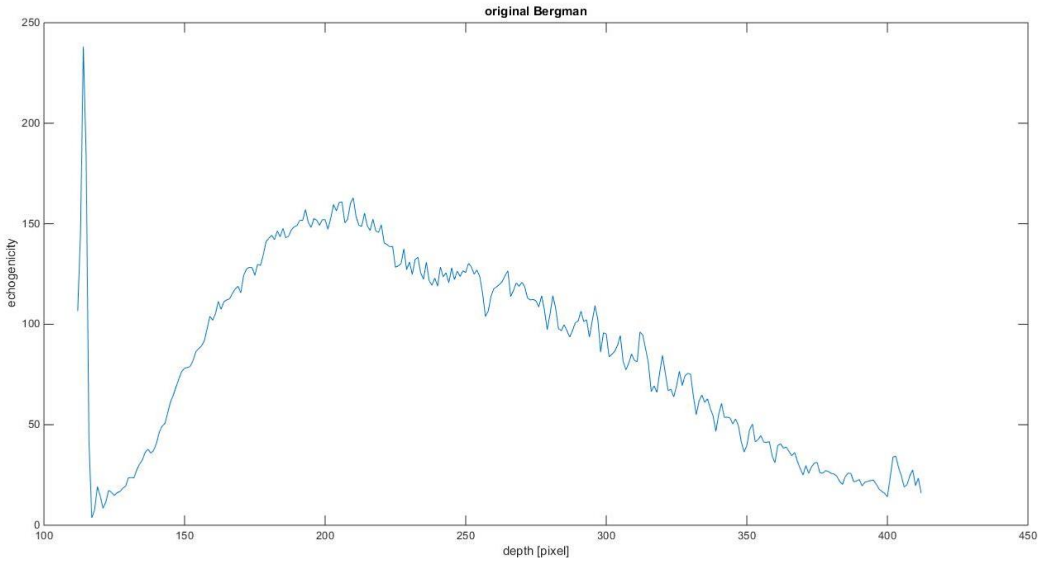


Figure 13, echogenicity per depth in phantom image for the Bergman device.

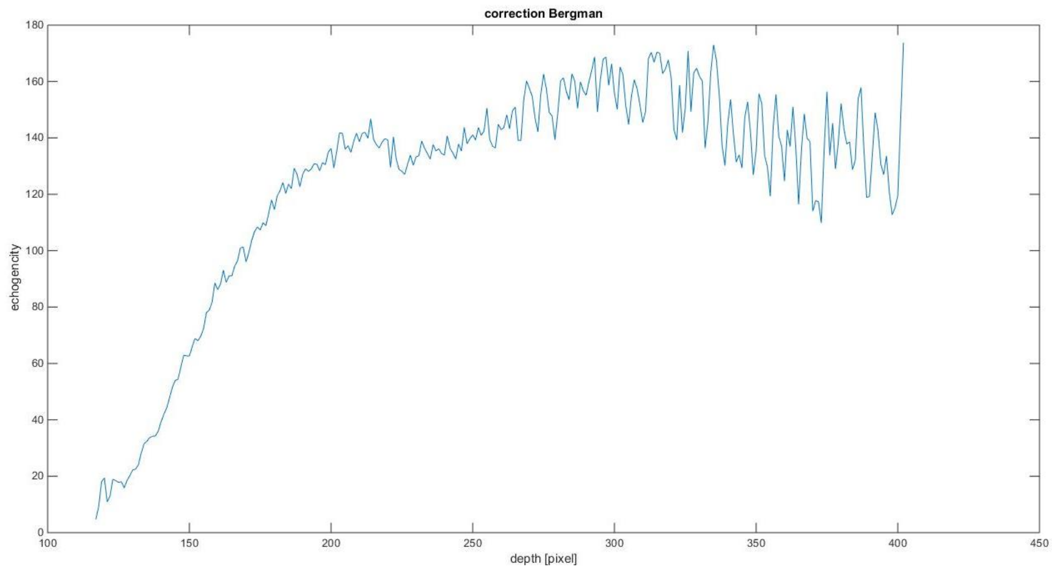


Figure 13, echogenicity per depth in corrected phantom image for the Bergman device.

Both graphs shows the mean echogenicity of a similar region over the depth of the phantom image. Graph 13 is without correction and therefore similar to the signal over depth (Figure 7A). Graph 14 shows the signal after correction for the penetration depth. Compared to the original image, it did not show the weakening of the signal in the lower region of the image. Since pixel depth is around 180, the signal remains relatively the same brightness over depth. It can be concluded that the correction of signal processing of the signal over depth has been filtered out.

However, after applying both corrections, there is still a difference in brightness between the images of the Bergman device and UMC Utrecht device. During the depth correction, is has noticed that there is a difference in back level value. The back level of the Bergman device is much higher than the back level of the UMC Utrecht device. Despite the rescaling of the distribution there seems to be a difference in the linearity of the density. Therefore, it is important to determine the echogenicity from different constant density areas.

2.2.3. Back level correction

In the phantom there are four different areas build to measure, varying from -6 dB to +6 dB compared to the 0 dB which is the back level. Of each region, the average of 3 measurements are used to measure the echogenicity per density. The trend lines are determined on the basis of the 5 regions. (-6,-3,0,+3,+6 dB). Next, the data of the Bergman is corrected, with the aim of the trend line formula, to the UMC Utrecht data. Because there is more data of the UMC Utrecht compared to Bergman data, the margin of the error is the smallest. Correcting both to eachother, the average of both data could thus lead to a smaller margin of error but a more frequent repetitive error.

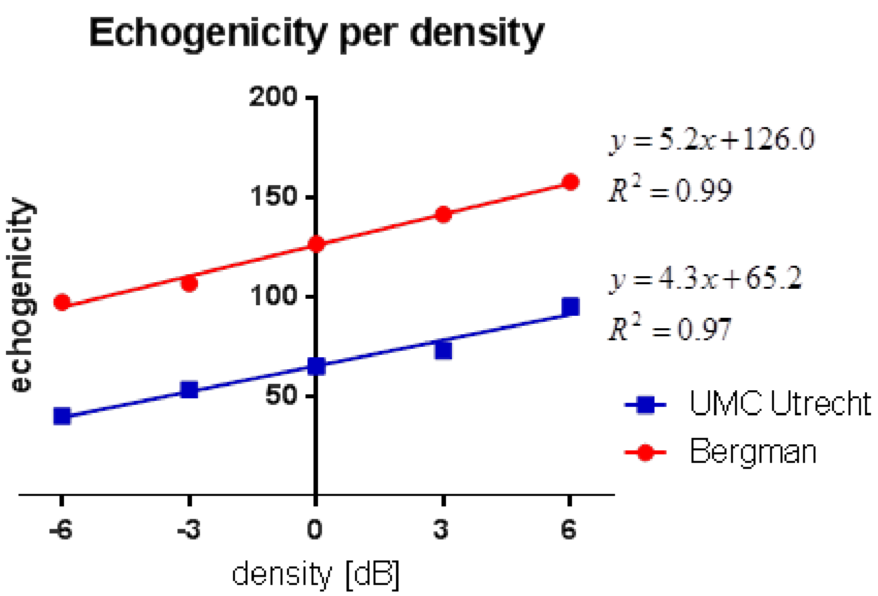


Figure 15, echogenicity per density region, with the formula of the linear trend line.

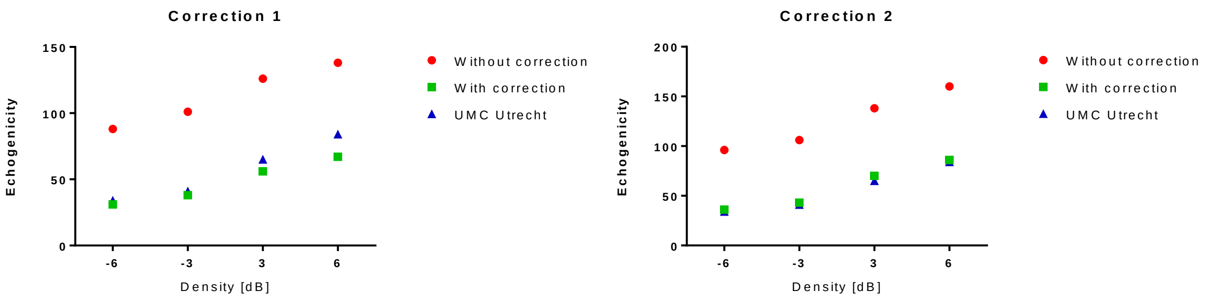


Figure 16, results of the correction on 2 different analysis. Red is result without correction, green is echogenicity with correction and blue is UMC Utrecht results.

Figure 15 clearly shows that all the values of the different densities are much higher at the Bergman compared to UMC Utrecht. In addition to a major difference in back level (+/- 60), there is also a small difference in the slope of the trend line. As in the LUT, there seems to be a difference in the scale, in this case of the densities. As validation, the formula of the trend line is applied on two analyses of the density lesions. The echogenicity of the different lesions are almost similar after the correction, see Figure 16. Within correction 1 is a greater deviation of the corrected values of 3 and 6 dB relative to the UMC Utrecht data compared to correction 2. This can be explained by a greater deviation of the echogenicity of these lesions relative to the trend line on which the correction formula is based.

2.2.4. Validation correction method

The images seem to be similar after applying all three correction methods after each other. As validation of the method, the different density regions are measured (-6 dB, -3 dB, 0 dB, +3 dB and +6 dB) 5 times.

Table 4, results with and without correction for both devices with the corresponding standard deviations

	UMC Utrecht		Bergman	
	Original	**Correction**	**Original**	**Correction**
-6 dB	39.9 (± 0.35)	46.1 (± 1.84)	97.9 (± 1.77)	50.3 (± 1.08)
-3 dB	52.4 (± 1.96)	55.8 (± 1.78)	106.6 (± 0.65)	58.0 (± 2.70)
0 dB	65.1 (± 0.48)	73.6 (± 1.04)	126.7 (± 1.01)	72.1 (± 2.75)
+3 dB	72.8 (± 0.38)	80.5 (± 0.75)	141.1 (± 0.87)	81.3 (± 1.93)
+6 dB	94.5 (± 1.92)	99.5 (± 1.18)	158.1 (± 1.33)	93.0 (± 0.71)

The method has an average echogenicity difference of 4.5%, varying from 9.1% (-6 dB) to 1% (+3 dB) compared to the echogenicity of the original images.

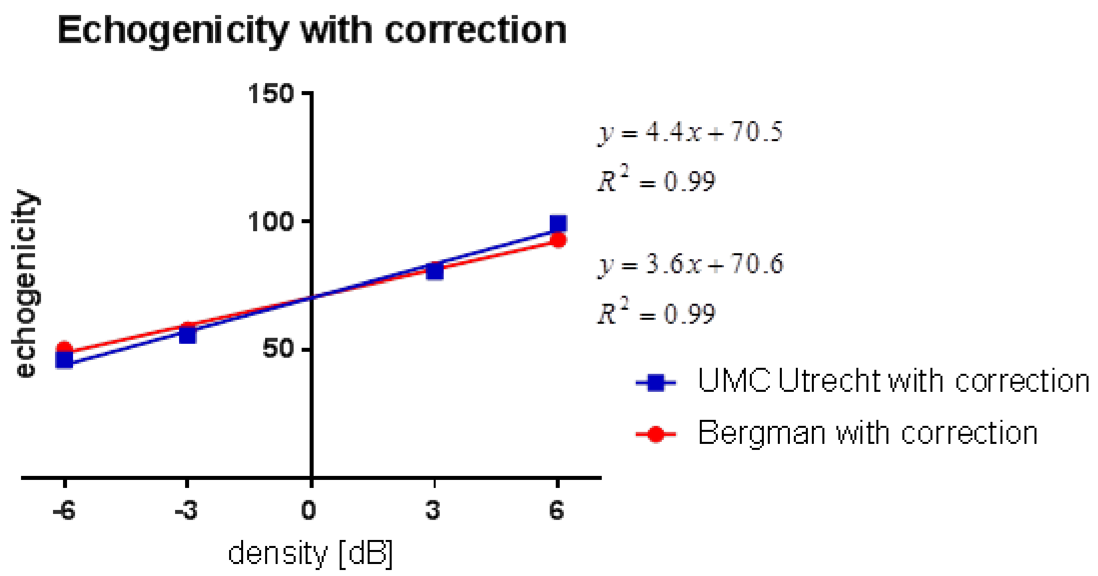


Figure 17, echogenicity of the different contrast lesions with correction.

The slope after correction differs with 0.8 echogenicity per density. There is a greater deviation of the correction in a greater difference of density compared to the background.

This method is validated also with the clinical data of the vastus lateralis of 5 women. Because of anatomical variations, the echogenicity before and after the correction per patient are shown.

Table 5, echogenicity per patient, per device without correction and with correction. With n is number of measurements and std. dev is standard deviation.

patient		UMC Utrecht	Bergman	With correction
1	mean	144.0	187.4	111.5
	Std. dev	7.0	17.4	8.3
	n	4	5	9
2	mean	152.0	186.2	108.1
	Std. dev		12.8	9.8
	n	1	6	7
3	mean	139.3	208.3	117.0
	Std. dev	5.0	8.7	10.8
	n	3	5	9
4	mean	146.9	201.0	114.4
	Std. dev	17.1	1.7	9.9
	n	6	3	9
5	mean	159.3	195.8	120.5
	Std. dev	3.2	8.3	8.7
	n	3	4	7

Table 6, echogenicity per patient for both devices, with and without correction. With n is number of measurements and std. dev is standard deviation.

	Without correction		With correction	
	UMCU (n=23)	Bergman (n=17)	UMCU (n=23)	Bergman (n=17)
mean	195	147	117	111
Std. dev	14.0	12.2	10.5	7.9

As before, the data per device on each measurement moment is shown in Table 7 and Figure 18 and 19.

Table 7, echogenicity of the vastus lateralis per meausurement moment, both Bergman and UMC Utrecht without correction, and with correction

	1	2	3	4	5	6	7	8	9
Correction	114.5	118.4	121.0	115.9	117.0	107.7	109.3	113.2	107.1
Bergman	192.5	199.3	202.4	192.8	199.4	196	165		
UMC Utrecht	151			165	146.5	141.4	147.8	144.5	152.5

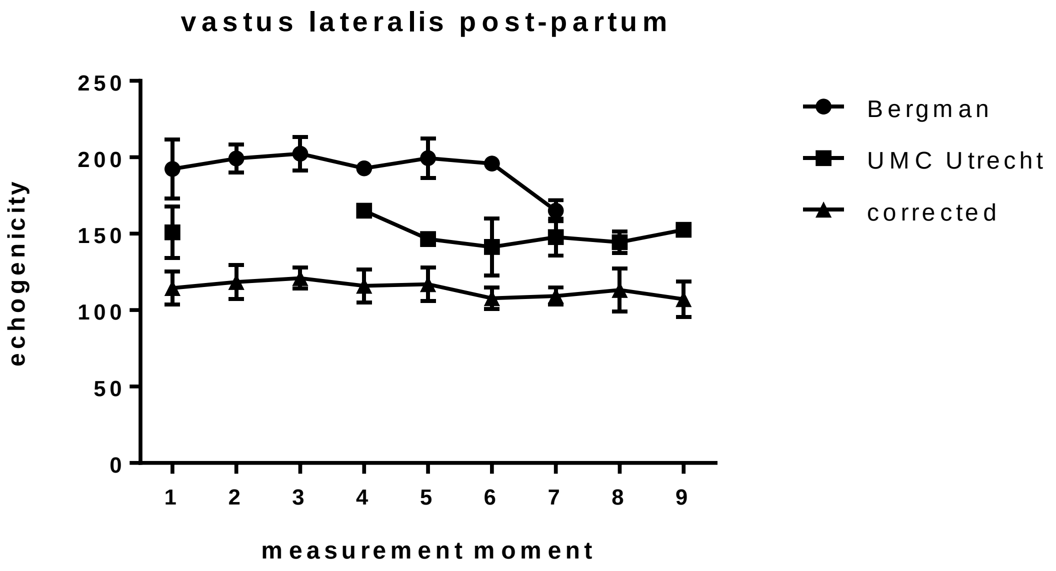


Figure 18, echogenicity of the vastus lateralis post-partum, with and without correction

The results are also shown in a linear time course due to non-linearity of the follow up measurements.

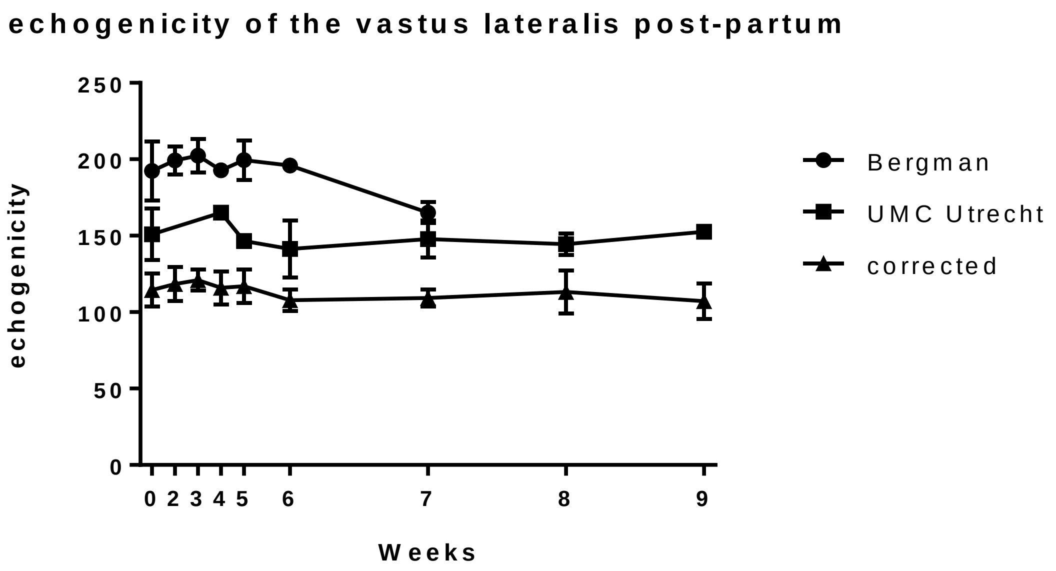


Figure 19, echogenicity of the vastus lateralis post-partum, in a linear time course.

In Figure 18 and 19 it can be seen that the corrected values have a lower echogenicity than the original measurements of the UMC Utrecht device. A reason for this is the correction of the reinforcement over depth compared to the back level. This caused a weakening of the signal in the region of interest. As expected, there is no effect over time visible, as before there is a relatively straight line over time.

2.3. Discussion

Based on the results, it can be concluded that the method complies with the previously stated requirements. In this method, the location of the LUT, the device or location and the region of interest will have to be entered by the observer. Now, there is a semi-automatic method to assess the echogenicity of a structure whatever the origin of the images. Clear instructions and default settings make the procedure easy to use. The accuracy based on the phantom data, are varying from 1.0% till 9.1% with an average of 4.5%. The differences in clinical data between the two devices are 5.5%, so quite similar to the phantom data. The method accuracy (around 5.5% and 4.5%) is very close to the desired accuracy (5%). It is to be expected that the small difference in accuracy will have little or no effect in further analyses, given the accuracy and reproducibility of ultrasound images, echogenicity measurements and its clinical relevance.

Both clinical images and phantom images have been used to validate. Together they seem strong enough to confirm the expected outcomes; equal echogenicity values of the lesions and similar echogenicity of the vastus lateralis muscle during recovery post-partum. Despite the fact that the method seems to work, there is still room for improvement. Four out of five different contrast lesions (-6, -3, 0 and +6 dB), scanned with the UMC Utrecht device, were scanned exactly in the centre of the image. Measuring contrast lesions not in the centre at 30 mm depth, gives unreliable data due to differences in distance travelled from the signals. The longer the distance, the more attenuation can occur. Scanning all lesions in the centre could lead to an improved correction method. The clinical data of the vastus lateralis still has an unknown possible effect over time, because on each measurement moment, there is only one device used. Despite the fact that it is unlikely, considering the collagen study with pregnant rats of Alperin and co-workers[19], it can not be eliminated.

The method could be optimized by adding different devices. As a result, data from other hospitals can also be analysed. So far, it has been taken into account in the method with the ability to add different devices. But it is not suitable for analysing yet.
In addition to adding different devices, the method can be optimized by adding different probes options. Whereby the research area can be expanded. Instead of analysing only images made with the abdominal probe, so the puborectalis muscle in this case, the cervix can be analysed by adding the vaginal probe. Because the lack of a suitable phantom for this frequencies, this was not suitable. For example, the phantom model ATS 550 is suitable for frequencies above the 7.5 MHz. In this phantom, the contrast lesions are at 20 mm depth instead of 30 mm in the used model ATS 570. The method is only suitable for the calibration of the puborectalis image and unfortunately not for the cervical images because of the lack of a suitable phantom.
Another improvement would be to add a method that would include the signal and noise through the depth analyse in Matlab. This makes it easier to add other devices or probes and reduces dependency of different software programs.
Furthermore, the distance straight down to a certain depth is shorter than the distance to the side of the image at the same depth. Therefore, a backscan is necessary. In a backscan the image is converted from a curved shape to a square shape, for compensation of distances. Hereby the determination of echogenicity on different depths or locations is more reliable. Whereby corrections based on square shapes are applied at the correct height and depth on the image. On the phantom data, this limitation has no effect, since the depth and locations of the lesions are the same.

The calibration of the different ultrasound devices were based on trial and error. For the development of the semi-automatic analysis, sometimes later obtained information has been used to improve earlier parts of the analysis.

2.3.1. Conclusion

It can be concluded that the developed method is suitable for the calibration of images of the Bergman and UMCU device, based on the accuracy (varying between 4.5% and 5.5%). There is a semi-automatic method to assess the echogenicity of a structure whatever the origin device of the images.

Chapter 3. Echogenicity measurement protocol

3.1. Introduction

In order to determine the association between echogenicity of the cervix and MOD, it is important to determine the echogenicity in a reproducible and reliable fashion. Therefore, it is required to investigate which region of the cervix is most suitable for this purpose.

3.1.1. Cervix anatomy

The cervix is located in the pelvic region and forms the connection between the uterine cavity and vagina. The cervix act as a barrier between the external environment and the uterus, as defender of infection, but also serves as an entry route for sperm to the uterus.[2, 20]

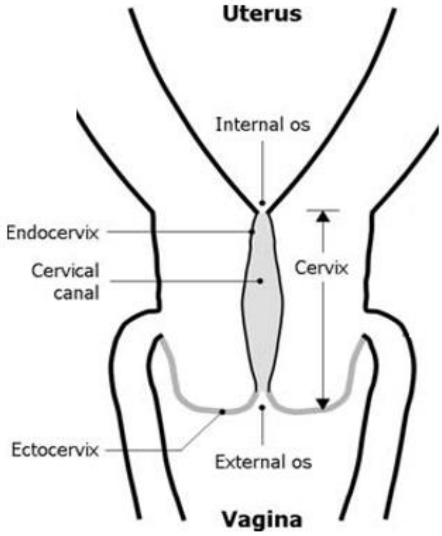


Figure 20, schematic cervical anatomy.[1]

The cervix consist of the ectocervix and endocervix, and has average length of 3-4 cm and is 2.5 cm wide. The length and shape differs between women. Aging, hormonal changes, parity and surgical treatments of the cervix are all variables that may influence the size and shape of the cervix. The ectocervix is the portion of the cervix on the side of the vagina, with the external os as opening into the vagina. The endocervix is the part of the cervix on the side of the uterine cavity. The cervical canal is connected to the uterus cavity with the opening into the uterus called the internal os.[20, 21]
This canal is important for the anatomical orientation of the cervix on ultrasound images. It is the separation between anterior and posterior lip. The anterior lip is the cervical part that is directly between the probe and cervical canal. The posterior lip is the part opposite of the canal, e.g. most distal from the probe. The distinction between anterior and posterior lip is purley based on this anatomical position based on 2D ultrasound images. In reality, the cervix is cylinder shaped and there is no difference in and the histology of the anterior and posterior lips. To illustrate this, Figure 21 is added.

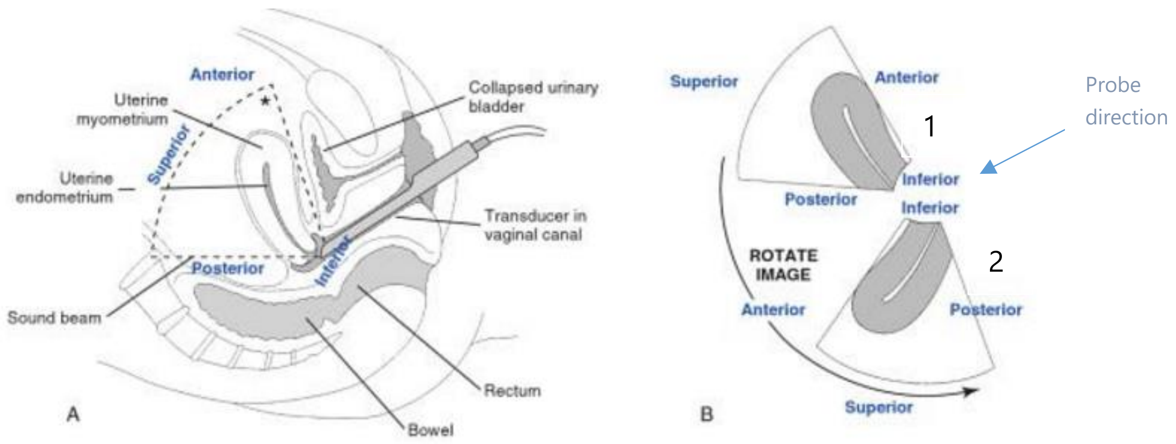


Figure 21, A: anatomical overview, B: forward and backward tilted uterus, the arrow indicates the probe location[22].

Histologic, the major component of the cervix is fibrous connective tissue. The ECM in the cervix consist predominantly of collagen, elastin and proteoglycans. Collagen is the major component of ECM, non-pregnant cervical dry weight consist for 54%-77% of collagen protein. Furthermore, smooth muscle fibroblast, epithelium and blood vessels are present. There are differences in distributions of these cells. The internal os has a higher ratio of smooth muscle cells to connective tissue than the external os, the percentage of smooth muscle cells varying from 29% till 6%, with an average of 10% in non-pregnant women.[20, 23, 24] As will be explained later in this chapter, this difference in composition between ECM and muscle cells is of importance because it will influence echogenicity as measured with ultrasound.

3.1.2. Region of interest

Differences in tissue composition will influence echogenicity. There are different regions of interest, see Figure 22.

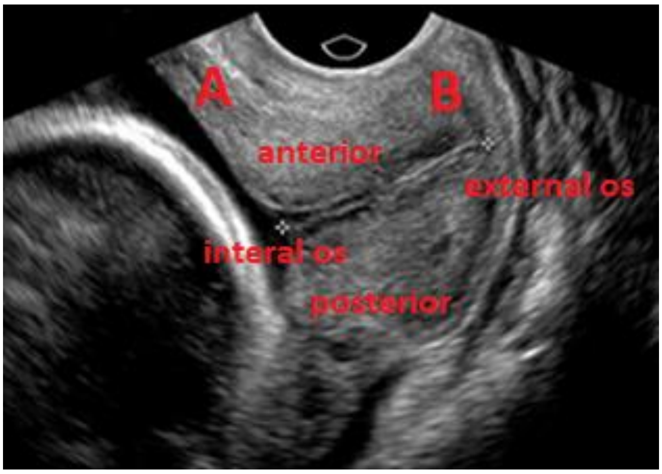


Figure 22, locations within the cervix

Both, internal os en external os, differ in tissue composition. The internal has a high smooth muscle cell/ECM ratio and therefore it would be dark on the US image. Given that muscle cells are more hypo-echoic compared to connective tissue and fat cells. The external os has a lower smooth muscle cell/ECM ratio and should therefore have higher echogenicity. Measuring echogenicity in both these regions may provide important information, but may have a limited reliability because the region is not clearly defined and variation in measurement site may occur between investigators. Therefore, measuring the echogenicity of the entire anterior or posterior lip may provide more reliable information, because the size of the region of interest is larger.

The effect of boundaries of two tissues on the echogenicity is important for selecting the anterior or posterior lip. Boundaries such as those between the cervix and cervical canal or the intestines, influence the echogenicity, because of differences in acoustic impedance. This difference in acoustic impedance is proportional to the intensity of the reflected echo. The acoustic impedance is determined by the density and the speed of sound in a tissue. A large difference in acoustic impedance causes that most of the signal will be reflected back to the transducer. Thus, less signal passes through the deeper tissue. This decrease in intensity results in a decreased echogenicity of the deeper tissue. The cervical canal consist mainly of water. The acoustic impedance of water is smaller than the one of soft tissue, see Appendix 1. Because of this, the intensity and thus the brightness of the deeper tissue differs in women, depending on the amount of water in the canal. Because the intensity changes has nothing to do with lower tissue composition, a biased echogenicity of the deeper tissue can be obtained. Obtaining non-representative data also applies on the boundary of the cervix with deeper tissue, air or intestines. Furthermore, the phenomenon of acoustic enhancement can occur at boundaries between tissue and fluid. Hereby, an area of increased brightness in the deeper tissues can occur. This could occur in the posterior lip, given the histology of the canal. Considering the physical effects on the echogenicity of the anterior and posterior lip, it is important to investigate which lip provides the most reliable and reproducible information.

The aim of this chapter is to obtain a reliable and reproducible method for measuring the echogenicity of the cervix. In order to do so we examined the echogenicity of the different regions of the cervix (partial part or entire lip, the anterior or posterior lip), and tested the reliability of these measures.

3.2 Method

3.2.1.Data acquisition

For this part of the study, data from the PURE study (protocol see Appendix 3) is used. These are images of the cervix at 12 weeks pregnancy from 84 patients of St. Antonius hospital and 184 from UMC Utrecht. The cervix images are made with GE ultrasound devices. The pre-set Cervix is used (har-mid, pwr 100, a gain of -4, C8/M5, E3 and SRII 3/CRI 1 settings (see Appendix 4)). The vaginal scans are made with the vaginal probe type RIC 5-9H/GYN.

3.2.2. Measurements

The echogenicity is measured with the aid of MATLAB R2014b. A manual selection of the region of interest is required, so that the average pixel echogenicity value can calculated.
There are several measurements done on these images to develop a reliable method. First, different sizes of regions of interest are measured. Two measurements of small (around 0.8 cm x 0.8 cm) regions (respectively low echogenicity region and high echogenicity region) and a measurement of the entire lip are done. These analyses of 3 regions are done in 35 scans and are repeated three times. With several weeks between the measurements to avoid a recall bias. Thereafter, the echogenicity of both, the anterior and posterior lip, are measured in 176 scans. For inter-observer differences two analysis per hospital are compared. There were 50 scans of UMC Utrecht and 84 scans of St. Antonius hospital used for the analysis.

3.2.3. Analysis

The statistical analysis is done with SPSS (IBM SPSS Statistics). A paired-sample t-test is performed to analyze the differences between two analyses per device, with a significant level of $p=0.05$. These measurements are done by one observer to test the agreement between repeated measurements.
To test the agreement between different observers, interclass correlations (ICC) and a Bland-Altman are done. Observer A did 3 times the analysis of 35 sans and observer B ones. Observer A had no experience as opposed to observer b, which collected all data for the study. ICC values <0.8 are moderate, above the 0.8 are classified as good, above 0.9 as very good. For the Bland-Altman test, the mean of the three measurements of observer A are taken and compared to the measurement of B. Based on the inter- and intra-observer results, the optimal region will be determined.

3.3 Results

3.3.1 Region on interest

The ICC for the comparison of the regions of interest varying from 0.66 to 0.99, see Table 8. The results of the entire anterior lip showed very good correlations. The results of the partial lip show for both, low and high echogenicity region of interest, lower correlations, varying from moderate till very good correlation.

Table 8, ICC of three different regions, based on a triple analysis of 35 scans. Measurements of low echogenic region, high echogenic region and the entire lip.

Measurement	Low	High	Entire lip
1 vs 2	0.66	0.77	0.97
1 vs 3	0.73	0.83	0.97
2 vs 3	0.73	0.97	0.99

The echogenicity of the entire anterior and posterior lip are determined by measurements of 176 scans from both St Antonius and UMC Utrecht patients. The standard deviation and standard error mean are both smaller for the anterior lip in comparison with the posterior lip values. The mean of the echogenicity of the posterior is significant higher compared to the echogenicity of the anterior lip, see Table 9 and 10.

Table 9, descripted data of anterior and posterior measurements. With n is the number of measurements, the mean, standard deviation and standard error mean.

	n	Mean	Std. Deviation	Std. Error Mean
Anterior	176	45.9	11.7	0.88
Posterior	176	63.2	20.1	1.52

Table 10, paired t-test of the anterior and posterior lip measurements. Statistical significant ≤0.05.

<table>
<tr><th rowspan="3"></th><th colspan="5">Paired differences</th><th rowspan="3">t</th><th rowspan="3">df</th><th rowspan="3">Sig. (2 – tailed)</th></tr>
<tr><td rowspan="2">Mean</td><td rowspan="2">Std. deviation</td><td rowspan="2">Std. Error Mean</td><td colspan="2">95 % confidence interval of the difference</td></tr>
<tr><td>Lower</td><td>Upper</td></tr>
<tr><td>Posterior vs. Anterior</td><td>17.304</td><td>20.436</td><td>1.540</td><td>14.264</td><td>20.344</td><td>11.234</td><td>175</td><td>.000</td></tr>
</table>

3.3.2. Reliability

Inter-observer

In case of St. Antonius hospital, two times 84 scans are analysed, see Table 11. The cervical echogenicity of 50 images obtained from the UMC Utrecht is determined two times, see Table 11. The results of these analyses are almost similar, based on the standard deviation and the mean. The difference (1.6 and 1.8) in mean between the two measurements are very small in comparison with the standard deviation (±19 and ±12.3).

Table 11, two analysis of the anterior lip, St. Antonius device. With n is number of measurements and statistical significant ≤0.05.

St. Antonius	**n**	**Mean**	**Std. Deviation**	**Significance**	**ICC**
Measurement 1	84	51.4	19.4	P=1.07	0.921
Measurement 2	84	53.0	18.4		

Table 12, two analysis of the anterior lip, UMC Utrecht device. With n is number of measurements and statistical significant ≤0.05.

UMC Utrecht	**n**	**Mean**	**Std. Deviation**	**Significance**	**ICC**
Measurement 1	50	46.6	12.5	P=0.60	0.941
Measurement 2	184	44.8	12.1		

The paired sample t-test for the measurements per hospital did not show any significant differences between the measurements. Furthermore, there are very good correlations between the two measurements per device, see Table 11 and 12.

<u>Intra-observer</u>
Three different analysis of 35 scans of observer A is correlated to one measurement of observer B, see Table 13. All correlations are classified as good. Between the different measurements of observer A en B there are very small differences, see Table 13 and Bland-Altman in Figure 23.

Table 13, ICC of observer A and B

	Observer B
Observer A 1	0.848
2	0.871
3	0.856
Mean (of 1,2 and 3)	0.869

Bland Altman
The Bland Altman shows a good correlation, a Pearson correlation of 0.88, and a squared correlation coefficient of 0.88.

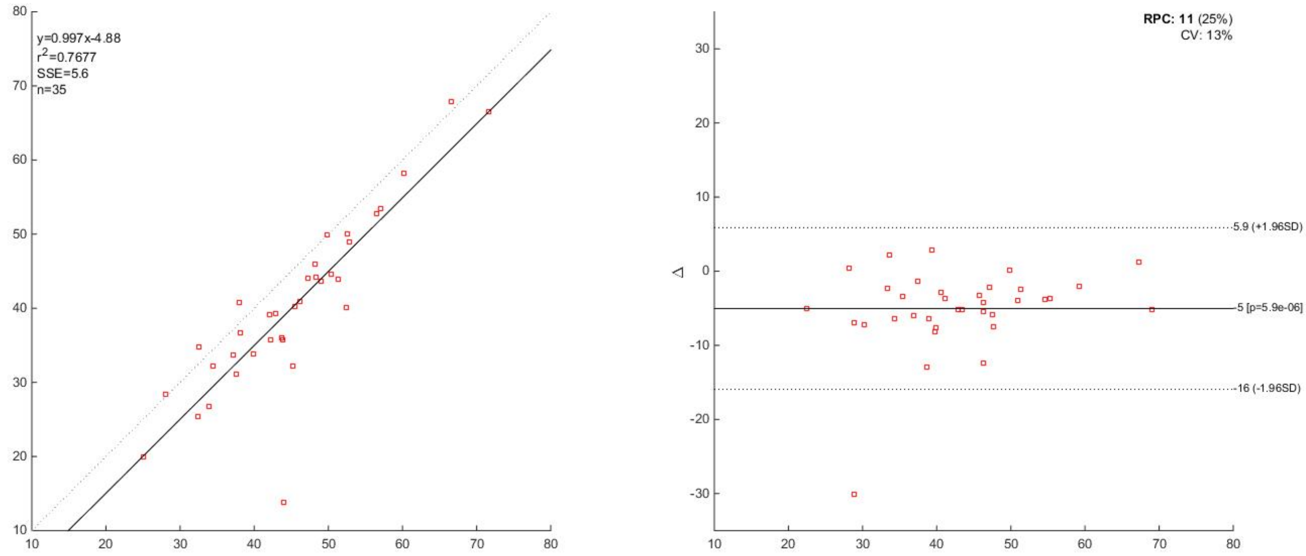


Figure 23, Bland Altman of the mean measurements of observer A and measurement observer B

There is an outlier visible in both graphs. This outlier can be explained by another interception of the cervical canal location on the echo between the observers. Therefore, different areas are classified as anterior lip. There is a sum of squared error of 5.5. The linear equation, y=0.997x-4.88, shows a consistent difference of 4.9 between observer A and B. Reason for this difference can be a different conception of artefacts. However, a difference of 5 on the scale from 0 to 255 is not very big.

3.4 Discussion

3.4.1. Region

The aim of this chapter was to obtain a reliable and reproducible method for measuring the echogenicity of the cervix. The highest correlation coefficients was found for the entire lip, see Table 8. There is a significant difference between the echogenicity of the anterior and posterior lip. So, for reproducibility it is important which lip is measured. Given the higher standard deviation and standard error from the posterior lip, it is preferable to measure the anterior lip.

The measurements of the partial and entire lip were performed only in the anterior lip to avoid influences of signal processing of ultrasound devices, which occur in the posterior lip. The advantage of this method is that it is corrected for different anatomical composition per location, since it averages the echogenicity over the entire lip. There was chosen for an area with high and low echogenicity because of the quality of the images. A specific area based on the anatomy, like internal os or external os region, was difficult. Since not all images contained an indication for an anatomical position within the cervix. For example, it is difficult to differentiate in location without any part of the uterus on the image, because it is not known where internal os and external os are located is. The area with the highest and lowest echogenicity is less dependent on the content of the image; there is always an area with the highest or lowest echogenicity. The results of the region with low echogenicity shows the worst results, the assessment of high echogenic region shows better results than low and worse than the entire lip. This could be explained by artefacts and signal processing. It could be difficult to differentiate a low echogenic region caused by anatomic variation from a low echogenic region caused by artefacts. Besides, it is difficult to distinguish the origin of a high echogenic area; anatomic or physical origin. Sometimes, a region with reinforcement of echogenicity due to the effect of acoustic impedance difference between two tissue is measured instead of an anatomical high echogenicity area of the cervix. So, it seems to be the best choice to use the entire lip of the cervix.

The higher echogenicity levels of the posterior lip can be explained by the depth of the cervix on the image. The signal processing of the ultrasound are mainly focused and hence the strongest in the mid-field, the area of the posterior lip. In the near field, the region of the anterior lip, is less influence of the

signal processing of ultrasound devices, most of the reinforcement is located in the mid-field, as seen in Chapter 2 (Figure 13 and 14). A reinforcement in the mid-field causes the higher echogenicity levels of the posterior because of signal processing. This processing reduces reliability, because this effect has probably nothing to do with the anatomy of the lip. This reduced reliability also applies because the influences of acoustic impedance differences between tissues can influences the intensity of the reflected and passes signal. For example, the boundary between posterior and air causes a major intensity change and thus the echogenicity of the posterior lip. There is a change of acoustic enhancement, which is the attenuation phenomenon resulting in an area of increased brightness. This occurs mainly during a boundary with water. For example, this can occur in the cervical channel to posterior lip and affect echogenicity of the posterior lip. Thus, these reinforcements are caused signal processing and physics, and probably not necessarily due to changes in tissue composition. This, and given the higher standard deviation and standard error from the posterior lip, it is preferable to measure the anterior lip.
Summary of the suitable region, we can conclude that the measurement of the entire anterior lip gives the most reliable results. There is less strain of artifacts because these artifacts can not occur throughout the entire cervix. When selecting a partial specific region, there may be an obstacle because the artifacts, mainly if the artifact occur in the specific region.

3.4.2. Reliability

Since, it is obvious which region is suitable, the reliability and reproducibility have been investigated. Therefore, the agreement per device between different analyses done by one observer are investigated. The Table 10 and 11 show similar results between two analyses per hospital. The ICC between the two measurements are classified as very good, 0.941 and 0.921, and there are no significant differences between the two measurements ($p=0.941$ and $p=0.921$). So, the measurements can be repeated by one observer, without obtaining significant differences in echogenicity, for both devices.
Thereafter, the agreement between different analyses done by two observer are investigated.
The ICC of intra-observer differs from 0.85 to 0.87, also qualified as a very good correlation. The absolute differences were visualized by the Bland Altman test. The mean of all measurements of observer A is used for this test, because of the experience. Observer A had no experience as opposed to observer b, which collected all data for the study. The graph shows an outlier under in the graph. This is the result of another interception of the cervical canal location on the echo. Therefore, different regions of interest are measured. The measurements are done with excluding areas influenced by artefacts. As previously mentioned, sometimes it is difficult to separate the reason of low echogenicity; artefact of anatomically. This result indicates that the assessment of echogenicity can be done reproducible by different observers.
Summary, the analyses can repeated per device or by different observers without obtaining remarkable differences in echogenicity, given the agreement of the measurements.

3.4.3. Conclusion

It can be concluded that reproducible assessment of echogenicity during pregnancy by 12 weeks is possible with the pre-sets and the use of the entire anterior lip.

Chapter 4. Cervical echogenicity in relation to mode of delivery

4.1 Introduction

Since, it appears that the echogenicity can be measured reliable and reproducible (chapter 3), the association can be made with the MOD. However, there are many variables, which influence the echogenicity or the MOD. The influence of the variables that effect the echogenicity, the associated variables, are described. These are the different regions, duration of pregnancy, induction and augmentation. In addition, the influences of the variables on the MOD are described, the unassociated variables; like the birthweight, duration of labour, fetal head position and pain medication. There will be verified if there is a significant univariate association between echogenicity and associated variables. Wherein the primary association between echogenicity and MOD the most important is.

4.1.1. Mode of delivery

In 2015 in the Netherlands, 75.534 nulliparous women gave birth. There are several options for the delivery in the Netherlands; women can give birth in primary (under supervision of a general practitioner or obstetrician) or secondary care (in a hospital). Of the nulliparous, 19.9% gave birth in primary care and 80.1% gave birth in the secondary care. However, 54.0% of these women started the labour in the primary care, meaning that they needed to transfer during labour. The main reasons for transfer during the first stage of labour is pain medication (30.7%) and failure to progress (<1cm/hour) (20.5%). During the second stage of labour, 64.4% of the women are transferred because of failure to progress in full dilatation.[25]

Failure to progress or dystocia is characterized by an abnormal slow labour progress. There are three types of causes. First, the power or expulsive forces of the uterus may be abnormal. For example: uterine contractions may be inappropriately coordinated to efface and dilate the cervix (first stage) or insufficiently strong during second stage. Secondly, the fetal abnormalities may be the origin of the problem. Fetal abnormalities; presentation, position or size, could affect the labour. The last option is the passage, caused by maternal abnormalities, like obstruction of the fetal descent.[26, 27]

In case of an abnormal progress, management of labour can be done with augmentation medication, with an assisted-delivery or secondary CS. Of the 75.534 deliveries, 64.9% of the nulliparous had a spontaneous vaginal delivery, 16.4% had an assisted-delivery and 18.7% had a CS. In the Netherlands, preference is given to a vaginal delivery, CS will only be done with a medical indication, because of the risks.[25, 28]

Failure of vaginal delivery results in higher costs and emotional burden.[29-31] An unplanned intervention during labour, both assisted-delivery and CS, are associated with a more negative maternal childbirth experience. This effect is larger in women who underwent intervention due to arrest of labour, than in women who underwent intervention due to risk for fetal or mother.[31] By a vaginal delivery, which ended in a secondary CS is the post-partum risk of haemorrhagia greater compared to primary CS.

Considering the negative experience by an unplanned management of labour, it is interesting to investigate the possibility to predict the MOD. Prediction of the MOD may lead to good preparation of the mother and father, causing a less negative experience. In addition, the MOD is important for a subsequent pregnancy, because the adverse risks of assisted delivery and CS on the long term. The long-term risks of faecal and urine incontinence, pudendal nerve damage and dyspareuania are increased with an assisted-delivery and there is a higher risk of uterine rupture after a CS in a subsequent delivery.

Considering the influence of collagen on the dilatation and thereby on failure to progress, it could interesting to investigate the association of the echogenicity to the MOD. Given the study of Grob and co-workers [12] and the above-mentioned influences of collagen, it is expected that women with an assisted delivery or CS have a lower echogenicity of the cervix compared to women with vaginally labours.

4.1.2. Region of interest

In addition to the echogenicity of the anterior lip in relation to the MOD, the echogenicity of other areas of the cervix could be interesting. Although selecting a small region of the cervix is difficult as described in Chapter 2. As described earlier, the distribution of cells is different per location in the cervix. It is conceivable that the ratio between the echogenicity of internal and external os can provide information of the MOD. It could be that women with a lower level of echogenicity at external os compared to internal os need more often management of the labour. This could be because the dilation of the distal part of the cervix takes a long time. Maybe there is less collagen, causing a more difficult softening and dilation. In this case you could get an prolonged labour, which is again correlated with a higher risk of assisted-delivery or CS. These hypotheses will be investigated in this study.
The interest in the ratio of different areas also applies to the ratio between anterior and posterior lip. Research of Kuwata and co-workers [32] described the association between the cervical consistency as measured with the Bishop sub-score and with the mean grey-level (MGL) on ultrasound at 27-30 weeks gestation. They determine the MGL of the anterior and posterior cervix. They showed that the cervix is stiffer when the difference in echogenicity is larger between anterior and posterior. They also found a significant association between the anterior-posterior difference in echogenicity and the Bishop sub-score.[32] This can be explained by the ease of the echo beam transfer to the anterior cervix by different consistencies. There is less absorption in soft tissue compared to denser tissue. An echo beam propagates easier through a soft cervix, whereby the posterior cervix becomes more echogenic.[32]
So, the ratio between internal os and external os regions within the anterior lip are determined, considering the histological differences in the cervix. The ratio between anterior and posterior lip are determined, considering the effect of the channel and depth gradient, which could information about the weakness. These ratios of the echogenicity will be associated with the MOD.

4.1.3. Variables

The echogenicity can be influenced by several variables, which are determined during the pregnancy or delivery. The influence of these variables; the duration of pregnancy, augmentation, induction and the regions, on the echogenicity are described per variable below. After these associated variables, the influences of the unassociated on the MOD will also be described. These are mainly relevant for an association between the associated variable and the MOD. Both are important, because of the influences on the primary association between echogenicity and MOD.

<u>Duration of pregnancy</u>
The first variable that influences the MOD is the duration of pregnancy. 90% of all women gave birth a term (37 – 41.6 weeks), 1,3% after 41 weeks and 7,1% pre-term.[25, 28] Only 4% of the estimated due dates turns out to be correct. 70% Of the women deliver within 10 days of their estimated due date.[33] pre-term delivery is defined as delivery before the 37th week. Although it occurs in less than 10% of pregnancies, it is the major cause of perinatal mortality and results in morbidity for survivors.[34, 35] pre-term birth is the leading cause of death in children.[35, 36] Research has shown that premature softening, shorting and dilation of the cervix can lead to critical problems, sometimes causing of spontaneous pre-term birth.[2, 37] Still the underlying pathophysiology for the reason of adaptations is unknown. Lurie and co-workers [38] showed that longer pregnancy is related to a longer duration of dilatation.
There seems to be a connection between dilatation and the duration of pregnancy. Therefore, the relationship between duration and echogenicity is interesting. Perhaps, at twelve weeks pregnancy, there are indications for premature softening, shortening and dilatation because of the composition of the muscle. Premature softening and/or dilatation could be caused by collagen synthesis in a cervix with less organized collagen network or amount of collagen, compared to a-term dilatation and softening. This makes it interesting to investigate the association of echogenicity with pre-term birth. Hereby it is expected to find a higher echogenicity associated with pre-term delivery, compared to the echogenicity of labours a term.

Induction

Induction is related to a higher risk of assisted-delivery or CS by nulliparous.[39-44] A study of Sharma and co-workers [42] showed a 2-fold increased risk of CS for induced nulliparous compared to women with spontaneous labour. There is more common failure to progress at the first stage, maybe due the unfavorable cervix at moment of induction.[40, 42, 44] The collagen synthesis could be a possible reason for an unfavorable cervix, and therefore related to the echogenicity. Given the unfavorable cervix by many inductions, it is possible that there is an association with the echogenicity between women who get an induction and women without. It is thought that women without induction have a cervix, which is soft and dilated enough to prepare for delivery. It could be that women with an unfavourable cervix have a bad ratio with many muscle cells compared to collagen. Presenting as a lower echogenicity on ultrasound. Therefore, it is expected to find an association between a lower echogenicity in women with induction, than the ones without.

Augmentation

The last associated variable is the use of augmentation. The use of augmentation of labour can indicate inadequate expulsive effort. Whereby the risk of assisted-delivery and CS is higher than in women without ineffective labour. The uterine contractions are initiated by calcium, which activates the smooth muscle cells.[45] In the cervix at internal os, consists of more smooth muscle cells compared to external os.[21, 23] It could be that the inadequate expulsive effort is related to the proportion between smooth muscle cells and other cells, such as fat and connective tissue cells. This is reflected in the echogenicity in relation to the MOD. However, it is also possible that the inadequate expulsive effort is totally independent of the composition of the cervix. Therefore, it is interesting to look at the echogenicity of women with augmentation and women without augmentation.

Furthermore, there are also unassociated variables that influences the MOD, like birthweight and duration of stage 2 of labour. It is important to take this variable into account when finding an association between one of the associated variables and the echogenicity.

Birth weight

First, the birth weight is related to a higher risk of emergency CS. Ju and co-workers [46] showed that macrosomia is associated with a nearly two times higher risk of emergency CS. According the guidelines of the Dutch association for obstetrics and gynaecology (NVOG)[28], macrosomia is a birth weight ≥4000 gram. Macrosomia is a risk factor for dystocia, and is one of the clinical finding in women with ineffective labour. The birth weight of the baby was also a significant factor in vaginal delivery. Babies whose weights were within the normal range of birth weight were more likely to be delivered vaginally compared with macrosomic infants. Compared with normal weight babies, macrosomic infants had also been associated with unengaged fetal head, malpositioning and prolonged labour in nulliparae.[47] Birth weight is an important determinant of MOD because of shoulder dystocia, Primary PPH and anal sphincter injury.[48, 49]

Because of several complications, such as shoulder dystocia, birth weight is a determinant of the MOD.[48] The reason for ineffective labour and thus higher risk of assisted- delivery or CS by marcorsomia does not seem to convert to echogenicity. There are no indications that the distribution and amount of collagen, muscle and fat cells influence MOD, depending on birth weight. The influence of tissue composition will logically belong to the inadequate cervical dilation as clinical finding in ineffective labour. Foetopelvic disproportion is one of the other clinical findings in women with an ineffective labour.[50] So, the birthweight is not directly associated with the echogenicity but it can maybe influence the outcome because the influence on the MOD. Besides, birth weight is related to a prolonged stage 2, which has also influence on the MOD, as will be described hereinafter.[51]

Duration of labour

Labour can be divided into two stages, first stage is dilatation till full dilatation, the second stage is from full dilatation till birth. Only the duration of the second stage is known and therefore interesting. A prolonged second stage of labour is associated with less favorable outcomes for mother and child. There is, among other things, a higher risk of fluxus post-partum and assisted-delivery.[25, 28] The second stage is after reaching full dilatation, thus the influence on echogenicity has been eliminated. But, it can maybe influence the MOD, and therefore an interesting variable to taken into account.

Fetal head position

In addition to the weight and duration of second stage, the fetal head position is associated with the MOD. Normally, 92% of the fetuses are located in left occiput anterior (LOA).[25] The persistent occiput posterior position (OP) is the most common malposition and is associated with higher rate of complications during delivery. The malposition has a significant lower rate of spontaneous delivery and often assisted-delivery or CS are necessary.[52] Besides, the head position is a risk factor for dystocia.[53] Such as birthweight and duration, the fetal head position is not directly related to the echogenicity but has influence on the MOD. Therefore, an interesting variable to taken into account.

Pain medication

Lastly, the pain medication is an important variable. It is the most common reason for a transfer from primary to secondary care, as described earlier. The common used pain medication in the included population is an epidural analgesia. Unfortunately, there are many contradictions in literature about the effects of this medication on the MOD. There are indications that epidural analgesia increases the risk of a prolonged labour by nulliparous. Furthermore, there could be an association with a higher rate of vacuum use and not necessarily with CS.[54, 55] However, Patel showed that epidural analgesia is related to CS.[56] Despite, the contradiction, it could be interesting to see the effects between the different MOD. Without unambiguity of the effects of epidural analgesia, the results of the association between echogenicity and MOD can be influenced by medication use.

The aim of this chapter is to associate the echogenicity with the MOD. Furthermore, the relation between echogenicity and several associated variables on the echogenicity will be determined.

Hopefully, more insight in the way of giving birth can be obtained with the association between echogenicity and the several variables. Possible early prediction of the MOD could decrease the number of transfers from line 1 to line by estimation of failure to progress. Hereby, the emotional burden could decrease by counseling in an early stage. Furthermore, the extra cost could decrease because the extra time spent in the delivery room.

4.2 Methods

4.2.1. Data acquisition

For this part of the study, data from the PURE study (protocol see Appendix 3) is used. These are images of the cervix at 12 weeks pregnancy from 84 patients of St. Antonius hospital and 184 from UMC Utrecht. The cervix images are made with GE ultrasound devices. The pre-set Cervix is used, consisting har-mid, pwr 100, a gain of -4, C8/M5, E3 and SRII 3/CRI 1 settings (see Appendix 4). The internal scans are made with the vaginal probe RIC 5-9H/GYN.

Furthermore, information about the delivery is obtained from the patient files or obstetrician clinic. From all patients the following variables are obtained: the MOD, birth weight, duration of pregnancy and duration from stage 2 of labour, the fetal head position, induction and the use of pain medication.

4.2.2. Measurements

The echogenicity will be measured with the developed method described in Chapter 3 using MATLAB 2014b. From all images, the echogenicity of the anterior lip is determined. For the ratio of internal os compared to external os, the echogenicity is determined using a small area (around 0.8cm x 0.8cm). 32

images were useful for determining the ratio, based on of the quality of the images. Whereas 176 scans were useful for the ratio between anterior and posterior lip, where both entire lips are used.

4.2.3. Analysis

The statistical analysis is performed using SPSS (IBM SPSS Statistics). The one-way ANAOVA was performed to determine the significance differences in echogenicity between delivery groups. The groups of MOD were classified (see Table 14) based on the classification used in the study of Grob.[12] For the other variables, there was an independent samples t-test performed. Based on the Levene's test for equality of variances, the p-value was determined. The classifications of these groups (see Table 15) was based on the pathology according the guidelines of the NVOG, the use of medication or intervention.[28]

Table 14, classification of the mode of delivery

Group (mode of delivery)	Number
Vaginal	0
Assisted-delivery	1
Primary CS	2
Secondary CS, because of fetal distress	3
Secondary CS, because of failure to progress	4

Table 15, classification of the several variables

Variables	Normal	Pathological
Duration of pregnancy	pre-term, <37 weeks, ≤ 258 days	≥37 weeks, ≥ 259 days
Augmentation	Without	Used, any kind of
Induction	Without	Used, any kind of
Child weight	< 4000 gram	≥ 4000 gram
Duration of stage 2	no reference values	no reference values
Fetal head position	Occiput anterior	Occiput posterior
Epidural analgesia	Without	Used

4.3 Results

The mean echogenicity of the 146 scans of UMC Utrecht and 59 scans of St. Antonius are similar, given the standard deviation, as described in Table 16.

Table 16, descripted data of the analysis of the cervical echogenicity per hospital. With n is the number of measurements, the mean and the standard deviation.

	n	Mean	Std. Deviation
UMC Utrecht	146	44.7	11.8
St. Antonius	59	46.8	12.2

4.3.1. Mode of delivery

Most of the women delivered vaginally 147 out of 211 women. The women who delivered by a primary CS or secondary CS because of fetal distress, respectively 5 and 7 patients are in the minority. The mean echogenicity varying from 43.1 till 53.9 between groups, with an overall standard deviation of 12.5.

Table 17, the descripted values per mode of delivery. With n is number of measurements, the standard deviation and standard error.

	n	Mean	Std. Deviation	Std. Error	min	max
Vaginal	147	46.8	13.2	1.09	22.4	87.6
Assisted-delivery	30	43.1	10.3	1.88	25.1	65.7
Primary CS	5	53.9	11.0	4.92	35.5	63.0
Secondary CS, Fetal distress	7	52.2	16.7	6.32	31.8	82.7
Secondary CS, failure to progress	22	43.7	6.8	1.45	30.2	55.7
Total	211	46.3	12.5	0.86	22.4	87.6

The ANOVA did not show any significant differences between the delivery groups.

Table 18, ANOVA of the mode of delivery. With statistical significant ≤0.05.

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	649.966	3	216.655	1.404	0.243
Within Groups	31944.552	207	154.322		
Total	32594.518	210			

4.3.2. Region

The mean echogenicity of the internal os region is higher compared to the region of external os, as can be seen in Table 19. Besides, the posterior lip shows a higher echogenicity compared to the anterior lip. The standard deviation of the posterior lip is the largest of all regions.

Table 19, the descripted values of the different regions. With n is the number of measurements, the mean echogenicity and the standard deviation.

	n	Mean	Std. Deviation
Internal os	32	60.1	17.0
External os	32	42.1	13.9
Anterior	176	45.9	11.7
Posterior	176	63.2	20.1

The ANOVA for the different ratios of the regions did not show any significant differences in echogenicity for different delivery groups.

Table 20, ANAOVA for both ratio's, internal/external os and anterior/posterior. With n is number of measurements, the mean, standard deviation and p-value. Statistical significant p≤0.05.

	n	Mean	Std. Deviation	p-value
Internal : External	32	1.56	0.60	0.519
Anterior : Posterior	176	0.79	0.3	0.480

4.3.3. Variables

The associated variables; duration of pregnancy, induction and augmentation, were analyzed with an independent t-test. p-value depends on Levene's test for equality of variances. No variable showed a significant difference between groups for the Levene's test (see Appendix 5).

Table 21, result of the t-test per variable. With n is number of measurements, the mean, standard deviation and p-value. Statistical significant p≤0.05.

Variable		n	Mean	Std. dev.	p-value
Duration of pregnancy	Normal	195	45.6	12.1	0.456
	Pathologic	17	43.4	11.4	
Induction	Normal	156	45.5	12.0	0.719
	Pathologic	49	44.8	11.9	
Augmentation	Normal	98	44.0	11.7	0.702
	Pathologic	107	46.5	12.1	

Based on the results of these associated variables, it has not been necessarily to do a multivariate analysis. Therefore, the unassociated variables were not analyzed.

4.4 Discussion

The aim of this chapter was to associate the echogenicity with the MOD. There was no significant difference found in the echogenicity between the different MOD groups. Furthermore, there was no significant relation between echogenicity and several influencing variables on the MOD.

4.4.1. Mode of delivery

The echogenicity per MOD did not show any significant differences between delivery groups. The mean echogenicity for the different groups varied from 43.1-46.8 with a standard deviation of 12.5 overall. Except for the primary CS group, which had a mean of 53.9. An explanation for this could be the small number of patients included in this group. Therefore, the statistical analyses was not considered reliable. Group 2 was too small and therefore not normal distributed (Appendix 5); thus ANOVA is not applicable for this group.

Because inadequate cervical dilation is one of the clinical findings in women with ineffective labour and ineffective labour can managed with assisted-delivery of CS, it was expected that there are significant differences in echogenicity per delivery group. This finding corresponds to the findings of Grob and co-workers.[12] They show a significant higher echogenicity of the puborectalis in contraction state from women who deliver vaginal compared to women who underwent a CS. This difference not visible in the rest state of the puborectalis.[12] Given there are no contractions of the uterus at 12 weeks, the cervix can be considered as at rest.

Most of the changes take place after 28 weeks in a-term pregnancy. It is therefore possible that 12 weeks is too early to see changes in at the echogenicity and associate it to the MOD. Most of the variables were related to dilatation, and that solely occurs at the late pregnancy.

4.4.2. Region

The ratio between echogenicity of internal os and external os is determined considering the histological differences. There was no significant difference in ratio between different groups per MOD. The results showed a higher mean of echogenicity at the internal os region compared to the external os region. This indicated more collagen content relative to muscle and fat cells at that area. This does not correspond to the literature, which describes more smooth muscle cells in internal os compared to the external os.[57, 58] An explanation for the non-corresponding results could be the quality of the images. Only 32 scans were useful, due to the orientation and the both areas had to be on the image. Many images showed both regions, however without the presence of the uterus, thus making orientation impossible. Despite the selection of suitable images, artefacts made it difficult to select region of interest. Especially the many images with artefacts at external os, probably because of the improper contact between probe and cervix. Furthermore, for the PURE study, the cervix was not the region of interest, which places the focus on the quality of other regions. Besides, the internal os is more often located in the mid-field compared to the external, which is always located in near-field. As described in chapter 3, the effects of the ultrasound devices were greater in the mid-field whereby reinforcement of the signal can occur. Resulting in a higher level of echogenicity. Another possible explanation could be that literature is based on data of non-pregnant women. It was not possible to find an article about the distribution of smooth muscle cells during pregnancy. Given the changes during the first weeks of pregnancy, it is plausible that the ratio in smooth muscle cells changes at 12 weeks pregnancy.

Similar results were visible in the ratio of the echogenicity of the anterior and posterior lip; there were no significant differences relative to the MOD. Kuwata and coworkers [32] showed that the cervix is stiffer when the difference in echogenicity are larger. Furthermore they showed a significant association between the anterior-posterior difference in echogenicity and the Bishop sub-score. The results of this study does not correspond to the study results of Kuwata and co-workers.[32] Those differences between anterior and posterior can be caused by several reasons. It is possible that the difference occur due to anatomic variations (mentioned by Kuwata and co-workers).[32] Next, it can be caused by artifacts and it is possible that settings of the device influenced the differences. The posterior part is located in the mid-field, while the anterior lip is in the near-field. Effects of the signal processing ultrasound devices are greater in the mid-field whereby reinforcement of the signal can occur. Resulting in a higher level of echogenicity. Besides, the effects of the surrounding tissues with major difference in acoustic impedance are more often located around the posterior lip, such as the cervical canal and the intestines. In addition to the density, the speed of sound determined the acoustic impedance. The difference in acoustic impedance is greater by the interface from water or air to soft tissue than soft tissue to soft tissue interface. The greater difference in acoustic impedance can result in a higher intensity of the reflected signal, and therefore in a lower echogenicity of the deeper tissue. Prevention of measuring tissues near an interface with major difference in acoustic impedance makes echogenicity more reliable.

4.4.3. Variables

For the duration of pregnancy, Table 21 show no significant difference in all associated variables between delivery groups. The expectation that women who deliver pre-term have a higher echogenicity compared to women who deliver a term was not visible in the results. There was no significant difference between both pre-term and a-term delivery. A possible reason for pre-term softening and/or dilation, is

not visible in the echogenicity at twelve weeks in the different MOD groups. After ten weeks of gestation, the uterus is twice its non-pregnant size. This enlargement is among others, caused by increased vascularity, hyperplasia and hypertrophy. These adaptations are also visible in the cervix resulting in softening of the cervical at the sixth week in a normal cervix (called the Goodell sign).[7] Thus, the cervix underwent some changes at twelve weeks pregnancy. However, the cervical ripening occurs not until the late pregnancy (from 28 weeks) in normal situations. In this phase, the collagen fiber alignment decreases and separation of collagen fibers occurs.[9, 59, 60] This will affect the echogenicity. Therefore, measurements of cervix echogenicity later during pregnancy may provide more information. A suggestion of this measurements would be at 32 weeks, when ripening occurs.
There was no significant difference in echogenicity between the women with the use of induction compared to the spontaneous deliveries, see Table 21. There is no indication that the unfavorable cervix in women with induction differs in echogenicity compared to those without induction. An explanation could be the distinction of indication and method of the induction. No distinction has been made between the indications and methods. It is possible that different methods of induction have different effects on the MOD. As example, misoprostol increases the risk of assisted-delivery compared to the use of dinoprostone.[61] Induction can be performed because of maternal (e.g. diabetes mellitus or hypertensive disease) or fetal (growth restriction) indications.[62] Induction because of approaching serotinity is not associated with a higher risk of CS, according to a Cochrane-review.[63] Parkes concluded in his research [43] that an indication for induction increases the risk of CS. This variable is interesting considering the relationship with MOD but has no association with echogenicity. Distinguishes between the many methods and indications of induction, with this small number of patients, will not lead to more representative data.
As the previous associated variables, the echogenicity between women with and without augmentation was not significant different. There are many more muscle cells at internal os than external os. Given the location of smooth muscle cells within the cervix, the method of the region with ratio of internal and external os are similar. Probably the histology is not necessarily different and visible to distinguish both groups using the echogenicity. With regard to calcium and signal transmission trough cells are reasons that are more obvious instead of the amount of smooth muscle cells. Such as a non-functioning uterus fundus as a pacemaker.[64]
The influences of the unassociated variables on the MOD were described, however not taken in to account in the analysis. Due to the influence of the variable on the MOD, the MOD is biased. It would be more reliable if these influences were taken into account. However, distinguishing between different variables is unrealistic, given the sample size. In addition, there is no association between echogenicity and MOD. Without significant association, it is not necessary to perform a multivariate analysis.

There are several limitations in this study. Ultrasound imaging of the cervix is difficult, since it does not always show the entire cervix. Therefore, the orientation of anatomical structures is more difficult. Making the scans by the observer itselfs makes orientation much easier. Due to the lack of the feeling of orientation during scanning, it is difficult to assess the orientation. Maybe the entire lip on the images appears as a really small part of the entire cervix anterior lip. Therefore, data can be not representative and it is recommended to make guidelines of the quality of the image for the orientation.

The mental health is important for the (subsequent) MOD.[29] As described earlier, the unplanned management of labour has a negative impact on the experience. Besides, preparation of any CS is recommended by Guitter.[30] Therefore, prediction could contribute positively to the experience and subsequent pregnancy. However, it is also suggested that prediction can have a negative impact, because of fear. The results of a study of Wangle [65] showed that mental health status could have a predictive bearing on delivery outcomes. Handelzalts [31] showed that fear of childbirth (FOC) pre-partum was correlated with MOD, whereby a higher FOC is associated with instrumental delivery and emergency. There is an increased risk of emergency CS when a pregnant woman suffers from FOC.[66, 67] Therefore, the mental aspects around the childbirth should be considered in the future, both positive (preparation) and negative (FOC).

The descripted data in Table 16 of St. Antonius and UMC Utrecht showed almost similar results in terms of mean echogenicity and standard deviation. The major differences between devices, described in Chapter 1 did not occur, because the images are recorded with the same depth and frequency. Despite the almost similar data, it is preferable to separate the data based on location. However, this is not possible given the amount of data, especially the amount of data of the different MOD groups. Separation per hospital results in very small groups of assisted-delivery and CS, whereby the outcome of the association between MOD and echogenicity, is not representative.

4.4.4. Conclusion

Concluding from all results, the echogenicity per MOD did not show any significant differences between groups. Based on the echogenicity of the cervix at 12 weeks pregnancy, it is not possible to predict the MOD for all variables.

Chapter 5. General discussion

The goal of this study was to associate the echogenicity of the cervix to the MOD. So that in future, the way of giving birth can be predicted with, inter alia, the echogenicity of the cervix at 12 weeks pregnancy. This could contribute to improvement of emotional experience of childbirth and decrease of additional health costs. There were differences between the images per device, despite the pre-set per probe. To properly compare the data of different hospitals in this study, calibration of images was done. This calibration was done based on phantom data and data of the vastus lateralis, which functioned as control muscle in the PURE study. Three different corrections were applied; images were corrected for the scaling of grey-values, the strength of the signal over the depth and the scaling of density. These corrections have been processed in a semi-automatic method with an accuracy around 4.5% a 5.5%. This method is suitable for the images obtained with the abdominal probe with the Bergman device or UMC Utrecht device.

Furthermore, there is a method developed to achieve the goal to associate the cervix echogenicity with the MOD. For this, a reliable and reproducible measurement of the cervix was needed. The optimal region to comply with this is the anterior lip. Partial regions and posterior lip showed less good results, based on correlations, standard deviations and standard error, compared to the anterior lip. The inter- and intra-observer correlations (respectively ICC of 0.97 and 0.87) and the squared correlation coefficient (r= 0.88) indicated a reliable and reproducible method.

With the developed method, the echogenicity was determined and the association with MOD is investigated. In this study, the MOD is divided into five groups; vaginal, assisted delivery, primary CS, secondary CS because of fetal distress and secondary CS because failure to progress. Thereafter, 211 scans of the cervix from St. Antonius and UMC Utrecht were analysed. The results of both hospitals are merged, due to corresponding results (based on mean and standard deviations). As a result of the merge, a larger sample size was obtained. Which means that only one group was too small to reliable take part in the analysis; since there were only 5 patients with a primary CS. No significant differences in echogenicity between the groups were found. This also applied for ratios of different regions of the cervix, considering histological and physiological differences. Furthermore, other associated variables to the echogenicity are investigated. For all variables as, duration of pregnancy, induction, use of augmentation and the use of pain medication, no significant differences in echogenicity between groups were determined. Because the lack of an association, there is no multivariate analysis performed where the unassociated variables would play a role.

Various limitations have been noted while these results were achieved, mentioned in the different chapters. Ultrasound is a convenient and appropriate method to obtain an impression of tissue structures; however, it is less suitable for the quantitative analysis of echogenicity. Notwithstanding, that it is possible; many factors are taken into account, like the system settings, device and transducer.[68] Considering the results of chapter 4, echogenicity obtained with this method can not be used as a single parameter. It is expected that echogenicity can be a good contribution of a prediction model of the MOD. The recently started research (GYNIUS) into strain and the relation with the MOD is promising. Use of the echogenicity within this project seems an addition. Especially because the role of tissue composition (collagen fat and muscle cells) is related to strain, and given the significant differences in echogenicity between the MOD for the puborectalis during contraction, showed by Grob.[17]

Given the time course of changes in the cervix during pregnancy (see Appendix 2), it is plausible that 12 weeks is too early to see a difference within groups. The dilatation is important during preparation for delivery, and plays a role in complications during labour. Besides that, changes in collagen are related to echogenicity. Measurements in dilatation phase could give more information. Nowadays, the cervical length is used as predictor of pre-term (see Appendix 2). Combining both methods seems a good option to obtain more information about the moment of delivery. The cervical length can be used for predicting pre-term. Women without a prediction of pre-term could be measured around the 37 weeks. While women with an indication for pre-term can be measured more often. Given the time course of the adaptations, could this measuring shortly before the delivery lead to more useful information.

Furthermore, calibration of the images of the cervix could lead to more representative results. Hereby, less influence of signal processing of the devices on the echogenicity are expected. Therefore, adding the vaginal probe into the calibration method is necessary and seems an addition. This may lead to more representative echogenicity data of the cervix.

Next, mental health seems important. As described, FOC has a negative aspect on the MOD. The possible predicted MOD could possibly lead to lower costs and better preparation for women. However, it is the question of whether it will contribute to fear and therefore on the MOD. It has to be consider or the prediction or preparation does not cause to much fear, whereby the MOD is negatively affected.

The whole process from gestation to delivery is a complex process. Many factors have influence on the outcome for mother and child. Even the way of giving birth is influenced by many factors, such as induction, augmentation and pain medication, duration of labour but also birthweight. These factors also interact with eachother. Physiological and mental aspects must be taken into account. All of these factors make predicting the MOD difficult and complex. More research is needed to obtain more insight into the process of pregnancy, step by step. Echogenicity can maybe play a role in this, but only as additional parameter or after improvement of the calibration method by adding the vaginal probe.

5.1. Conclusion

With the use of a developed semi-automatic calibration method, the conversion of ultrasound images obtained with different devices was possible with an accuracy around the 4.5% a 5.5%. The most reliable region of interest is the anterior lip, based on the inter- and intra-observer correlations. There are no significant differences in echogenicity between different MOD for 211 women measured at UMC Utrecht and St. Antonius hospital. This also applies on other associated variables, like duration of pregnancy, augmentation, induction and region.

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Appendix

1. Ultrasound background

The wavelength depends on the speed of sound and frequency following the formula: $\lambda = \frac{c}{f}$

Speed of sound and acoustic impedance of different materials:

Table 22, density, speed of sound and acousitc impedance for different tissues or materials [69]

Tissue or material	Density [g/cm^3]	Speed of sound [m/s]	Acoustic impedance [kg/(s $\cdot$ m^2] x 10^6
Air	$1.3 \cdot 10^{-3}$	330	0.0004
Water	1	1480	1.48
Blood	1.055	1575	1.66
Fat	0.95	1450	1.38
Muscle	1.065	1575	1.68
Bone	1.9	4080	7.75

Acoustic impedance

The acoustic impedance is determined by the density and speed of sound. The difference in acoustic impedance of two tissues determined the balance between reflection and transmitting of the energy. The difference is proportional to the amplitude.

Attenuation

The amount of attenuation depends on the frequency of ultrasound.[70] High frequency signals are absorbed more, compared to lower frequency signals. Attenuation occurs in four forms:

- reflection
- refraction
- absorption
- scattering

Scattering is the interaction between the wave and the boundaries, whereby the physical properties are different from those of the surrounding medium. Scattering causes speckle structure of ultrasound images of soft tissue. Because the density and compressibility of scatters are close to those of the surrounding medium. Therefore, the contribution of scattering to overall attenuation is relatively small of the total amount of attenuation at low frequencies (10-15%).

2. Cervix background

Adaptations of the cervix during pregnancy

In preparation for childbirth, the cervix undergoes various changes, called remodelling. There are several processes of remodelling during pregnancy; softening, ripening, and dilation. The whole remodelling process by human pregnancy is still not entirely clear.

Softening

Softening is mainly the first 32 of importance. Non-pregnant cervix consist of densely and irregularly packed collagen bundles. During pregnancy, there is decorin secreation. Presumable, decorin causes disorganization of the collagen fibers. There is an increased secretion of decorin during late pregnancy. In literature, the working of decorin has so far been assumed. In any case, there is collagen synthesis during pregnancy. The hyalorinic acid concentration increases whereby the water concentration increases as well. Hyaluronic acid is secreted by fibroblasts. Increased water concentration and collagen synthesis cause a decrease in collagen concentration during pregnancy; the cervix feels softer. Furthermore, there are more cells which infiltrates the cervix (e.g. macrophages, neutrophils). All those cells interact and allows the cervix to soften and prepare for dilation.[20, 71]

Ripening

After the cervix has become softer ripening occurs. The cervix changes as a load-bearing structure due to combination of external loading conditions. The load-bearing region of the cervix is the cervix stroma and the properties arises from the organization of collagen network.
One of the mechanism of ripening is decorin production, as explained above. Other mechanisms are the enzymatic degradation of ECM, the influence of cytokins (activity of collagenases) and hyaluronic acid (stimulate the syntheses of proteolytic enzymes) and hormonal manipulation (collagenase).[20] When bundles disperse, the collagen fibers lose strength and thus effacement begins. Cytokines, hyaluronic acid, collagenases, and elastase possibly work together to allow effacement. Thereafter, the elastin extents and allows dilation due to the mechanical forces of uterine contractions.[20]

Dilatation

Dilatation occurs just prior to delivery. Elastin plays a role in maintaining the fetus in the cervix. The cervix can allow to dilate for parturition because elastin can distend twice its length.[20]
Smooth muscle cells and fibroblasts forms the cellular component of the cervix. The changes of the components during pregnancy in the human cervix are poorly understood. It is thought that these cells proliferate to a phase with cell death and decorin becomes up-regulated, based on animal study. Decorin has influence on the organization of collagen fibers, so on the the softness of the cervix.[20]

In summary, there are four phases of cervical adaptations during pregnancy. The first 32 weeks is the softening phase of the cervix. During this weeks there is collagen reorganization, growth edema and an increased vascularization. Between 32 weeks till uterine contractions the ripening takes place. There is a decrease of collagen concentration, due to an increase in synthesis in collagen, hydrophilic glycosaminoglycan's and proteoglycans. This phase is followed by the dilation phase. During this phase, a release of proteases and collagenases into the ECM, leukocytes infiltration and a decrease of collagen concentration occur. The fourth phase is taking place after delivery and is characterized by repair of the tissue. The decrease in collagen concentrations is the consequence of increase in tissue hydration.[10]

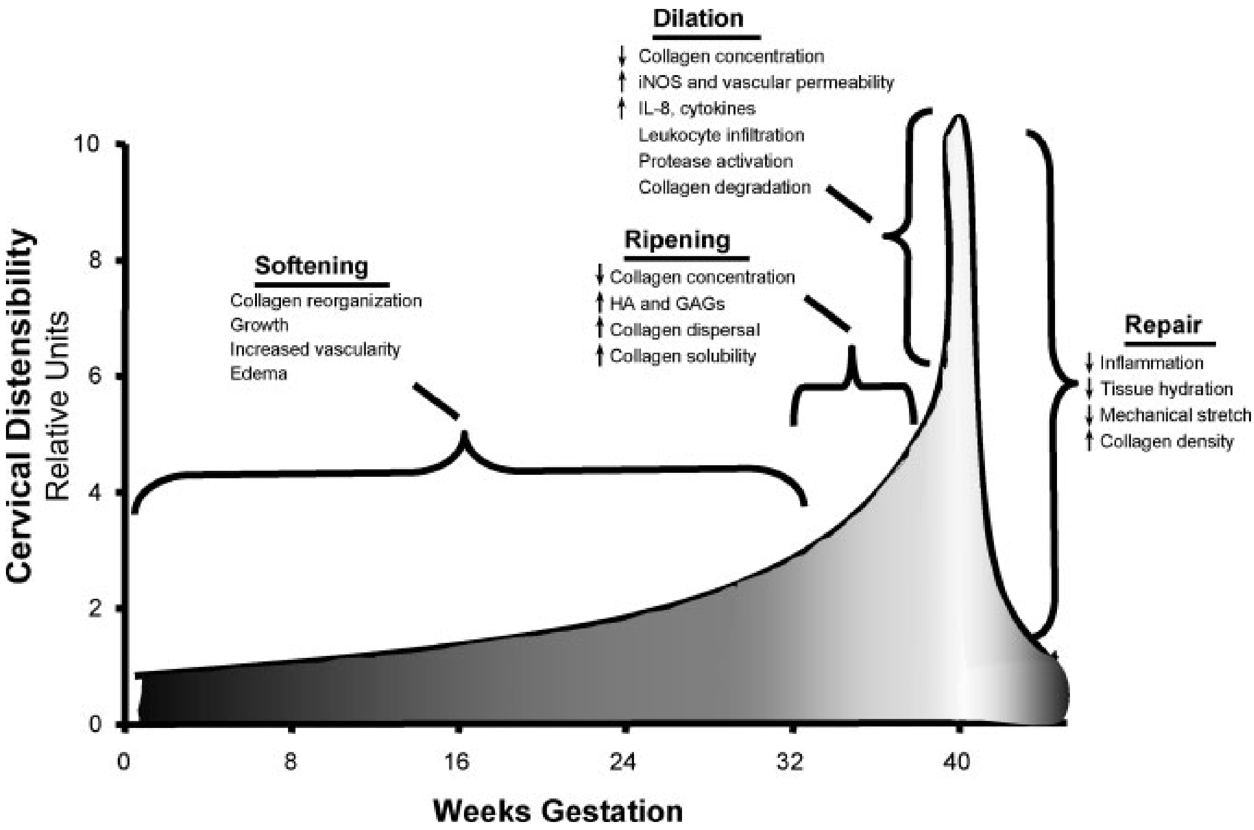

Figure 24, cervical distensibility [10]

<u>Cervical length</u>
Nowadays, the cervical length plays a role in the prediction of when a women will give birth.
There is a lack of understanding of the deformation shortening of the cervix.[72] Lot of previous studies of predicting of pre-term is based on the cervix length. The length of the cervix can be determined with the use of ultrasound. A short cervix is associated with spontaneous pre-term birth, possibly explained by an intrinsic weakness of the cervix. Weakness due to among other things; connective tissue disease, surgical damage, traumatic damage.[1] The generally dimensions of a cervix is 3 cm long and a diameter of 2.5 cm.[1, 21] By funnelling cervical shortening begins at the internal os, here the cervix starts to dilate, leading to funnelling.[23]

3. Protocol PURE study

Data acquisition:
Data from the PURE study is used. The Pure study is a multicentre study from UMC Utrecht, including four different hospitals; UMC Utrecht (Utrecht), Reinier de Graaf (Delft), Antonius (Nieuwengein), Radboud (Nijmegen), all-in the Netherlands.

Population
All the participated women of the PURE study meets the following criteria:
- Nulliparous
- Singleton pregnancy
- Good knowledge of Dutch language
- Signed informend consent

Women with the following criteria are excluded from participation in this study:
- Aged <18 years
- History of pelvic organ prolapse or incontinence surgery
- History of surgery in the uterus
- Connective tissue disease
- Not allowed to do a maximum Valsalva manoevre ecause of cardiac or pulmonary disease

Database
The PURE study forms a suitable database with ultrasound images of 306 women at 12 weeks pregnancy. Per women, there are images of the cervix, myometrium and the muscle vastus lateralis. Furthermore, there is post-partum data of 20 women, consisting several perineal ultraound images over time. The follow up is 1 day, 1 week, 2 weeks, 3 weeks, 4 weeks, 6 weeks, 12 weeks, 18 weeks and 24 weeks after delivery.

4. Pre-set Cervix

Table 23, settings of the pre-set per probe

	Abdominal probe	**Vaginal probe**
Frequency	23 Hz	32 Hz
Gain	15	-4
Power	100	100
Harmonic	Mid	Mid
Contrast	8	8
Grey-map	4	5
Persistence	8	-
Enhance	3	3
Location TGC-bars	Middle	Middle

The frequency is the number of cycles per second.[73]

The gain allows to adjust the brightness.[73]

Power is the energy transmitted by the transduces.[73]

There is a harmonic signal used, concentrated op the mid-field. It improves the imaging technique by taking the second harmonic, which is two times the emitted frequency. Hereby, the effect of grating lobes is excluded. The second harmonic is generated in the tissue, so the wave travelled only from the tissue to the transducer. The emitted wave travelled from the transducer to tissue and back to the transducer, whereby more noise can occur. Resulting in enhanced contrast and grey tone differentiation in comparison with the standard mode.[11]
In the mid-field, the distance to the transducer is far enough to generate a harmonic wave. In the near-field region, the distance is too small to build up a harmonic wave. In the far field, the intensity and sensitivity of the signal is lost, so there is no harmonic wave generated.

Grey-map adjust the brightness of each echogenicity value.[73]

Persistence influences the temporal resolution used in grey-scale imaging. It is smoothing by average of different images, so reducing the variation in the images between different frames. [73]

Enhance attempts to make a sharper image by combining adjacent signals.[73]

The location of TGC-bars determines the reinforcement of the signal on different depths. It is intended for compensation of attenuation in the depth.[73]

Speckle reduction is based on algorithms evaluating each pixel to identify tissue and eliminate speckle. The weak signals are eliminated, while strong signals are enhanced/brightened. It provides smoother and cleaner images. It makes identifying of tissues easier. It is comparable with frame averaging or persistence. Whereby multiple frames are combined and averaged into a single image.

Dynamic range increases or decreases the number of grey shades displayed.[73]

5. Statistical data

- Test for normality for group 2 (primary CS) because the small number of 5 patients.

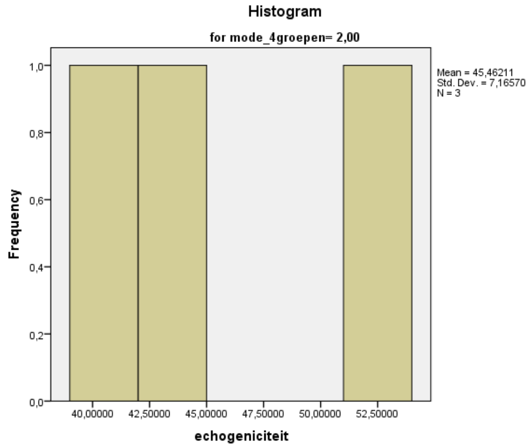

Figure 25, histogram of distribution of group 2

- Independent T-Test results of the associated variables

Duration of pregnancy

Group Statistics

	Days_group	N	Mean	Std. Deviation	Std. Error Mean
Echogenicity	a-term	195	45,62442	12,053197	,863147
	Pre-term	17	43,35746	11,397575	2,764318

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
Echogenicity	Equal variances assumed	,314	,576	,747	210	,456	2,266958	3,035781	-3,717553	8,251468
	Equal variances not assumed			,783	19,26	,443	2,266958	2,895941	-3,788849	8,322764

Induction

Group Statistics

Induction		N	Mean	Std. Deviation	Std. Error Mean
echogeniciteit	No	156	45,4709389	12,00393962	,96108434
	Yes	49	44,7645880	11,88857889	1,69836841

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
									Lower	Upper
echogenicity	Equal variances assumed	,003	,955	,360	203	,719	,70635085	1,96135420	-3,16088819	4,57358989
	Equal variances not assumed			,362	81,089	,718	,70635085	1,95144520	-3,17634838	4,58905007

Augementation

Group Statistics

Augmentation		N	Mean	Std. Deviation	Std. Error Mean
echogeniciteit	No	98	44,0415482	11,68172157	1,18003208
	Yes	107	46,4566313	12,13224327	1,17286823

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
									Lower	Upper
echogenicity	Equal variances assumed	,147	,702	-1,449	203	,149	-2,41508307	1,66653810	-5,70102766	,87086152
	Equal variances not assumed			-1,452	202,484	,148	-2,41508307	1,66375947	-5,69559910	,86543296