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REVIEW

Evolving cervical imaging technologies to predict preterm birth

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Abstract

Preterm birth, defined as delivery at less than 37 weeks' gestation, increases maternal-fetal morbidity and mortality and places heavy financial and emotional burdens on families and society. Although premature cervical remodeling is a major factor in many preterm deliveries, how and why this occurs is poorly understood. This review describes existing and emerging imaging techniques and their advantages and disadvantages in assessing cervical remodeling. Brightness mode (B-mode) ultrasound is used to measure the cervical length, currently the gold standard for determining risk of preterm birth. Several new B-mode ultrasound techniques are being developed, including measuring attenuation, cervical gland area, and the cervical consistency index. Shear wave speed can differentiate between soft (ripe) and firm (unripe) cervices by measuring the speed of ultrasound through a tissue. Elastography provides qualitative information regarding cervical stiffness by compressing the tissue with the ultrasound probe. Raman spectroscopy uses a fiber optic probe to assess the biochemical composition of the cervix throughout pregnancy. Second harmonic generation microscopy uses light to quantify changes in collagen fiber structure and size during cervical maturation. Finally, photoacoustic endoscopy records light-induced sound to determine optical characteristics of cervical tissue. In the long term, a combination of several imaging approaches, combined with consideration of clinical epidemiologic characteristics, will likely be required to accurately predict preterm birth.

Keywords Preterm birth · Cervical remodeling · Ultrasound · Elastography

Introduction

Each year, preterm birth, defined as delivery before 37 weeks' gestation, affects approximately 15 million babies and causes roughly one million deaths in children under the age of five [1, 2]. Those who survive face a lifetime of adverse health outcomes, such as blindness, deafness, respiratory difficulties, and cognitive disabilities [2]. As of 2005, more than $26 billion is spent each year to hospitalize premature infants in the USA [2]. The financial burden of preterm birth would likely be even more staggering if the long-term costs to caregivers and families were quantified [3].

One of the largest hurdles to reducing preterm birth is our lack of knowledge regarding the underlying mechanisms. Although several preterm birth risk factors have been identified—such as previous preterm birth, short cervix, infection/inflammation, race, environment, genetics, nutrition, and socioeconomic stressors—more than half of preterm births occur in women with no known risk factors [4–6]. Several recent research endeavors focus on inflammation as a biomarker for PTB. In fact, a number of studies have shown that evidence of immune activity is present long before the onset of PTB symptoms [7–10].

Preterm birth is often subtyped into two broad categories, spontaneous and indicated. Spontaneous preterm birth typically includes preterm labor (painful contractions leading to cervical change and delivery) or preterm rupture of membranes. Indicated preterm birth includes deliveries that are performed for maternal, placental, or fetal factors such as preeclampsia,

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fetal growth restriction, oligohydramnios, placental abnormalities, or maternal medical conditions [11]. Spontaneous preterm births result from multiple, likely overlapping, pathologies, all eventually converging in a final common pathway of cervical change and delivery [5, 11]. In a paradigm put forth by Vink and Feltovich, the path to PTB includes three main elements: preterm premature rupture of membranes, premature myometrial contractions, and cervical remodeling [5]. In this model, the three components happen in no particular order and may all lead to one another.

Cervical remodeling, in which the cervix transitions from a firm and closed structure to a soft and open structure, is essential for both term and preterm birth. Additionally, much of cervical remodeling occurs several weeks before contractions begin [6]. Thus, the biomedical community has focused extensively on cervical remodeling both to understand the underlying mechanisms and pathologies and to identify markers of remodeling that can be used to predict, and potentially prevent, preterm birth. In this review, we describe the microstructural changes that occur during cervical remodeling and several of the evolving imaging technologies for measuring these changes.

Gross and microscopic cervical anatomy

The cervix is contiguous with the lower segment of the uterus, forming a cylindrical canal that connects the uterus to the vagina. The cervix is in the true pelvis between the bladder and the rectum. Laterally, its ligamentous and peritoneal extensions project onto the pelvic wall encasing important structures, such as the ureters and the uterine vessels. A dense vascular network supplies the cervix, with the principal blood supply arising from uterine and vaginal artery branches. The cervix is held in place by the uterosacral and cardinal ligaments. The opening from the body of the uterus to the endocervical canal is termed the internal os, and the opening from the canal into the vagina is termed the external os.

On the microscopic level, the cervix has two components: two epithelial layers surrounding a stroma. The endocervix epithelium is composed of columnar cells, whereas the exocervix epithelium is composed of squamous cells [12]. These epithelia undergo significant proliferation and differentiation throughout pregnancy. The stroma consists mostly of collagen and extracellular matrix (ECM), with minimal smooth muscle, vascular cells, and immune cells [5, 6, 13, 14]. In contrast, the uterine body is composed mostly of smooth muscle [15].

The cervical collagen network comprises three layers [5, 16]. The inner and outermost layers are composed of longitudinal fibers that run parallel to the endocervical canal and help prevent the cervix from separating from the uterus during dilation [5]. The middle layer, composed of circumferential collagen fibers, is thought to control cervical dilation [5]. In addition to the structural heterogeneity of these three layers, the internal os and external os may also have different amounts of collagen solubility and crosslinking [5, 17]. Thus, in studies of the cervix, it may be valuable to measure properties in multiple regions to best predict preterm birth. For example, as the internal os usually dilates first, it may be especially important to understand the mechanical and biochemical properties of this region [14].

The cervix provides an important mechanical barrier to prevent ascending infections between the uterus and the microbe-rich vagina [18–21]. Additionally, the cervical mucus plug, which forms during pregnancy, constitutes another physical and antimicrobial barrier [11, 21]. The cervical mucus, cervical epithelium, and pericellular matrix all serve as physical, mechanical, and immunologic barriers [18–22]. An intact, healthy epithelium creates a physical and immunologic barrier with innate and adaptive qualities. Antimicrobials in the epithelium can sense viral and bacterial antigens, and then mount an appropriate immune response that protects against ascending infection [18, 19, 21].

Cervical remodeling during pregnancy

Cervical remodeling, in which the cervix transitions from a rigid structure to a highly compliant tissue, involves dynamic and complex changes to the collagen network. There are four overlapping phases: softening, ripening, dilation (aided by uterine contractions), and postpartum recovery [5, 13, 23]. Softening, defined as a decrease in tensile strength or tissue compliance, begins in the first trimester and progresses slowly [4, 5, 13, 22]. This phase is characterized by a decrease in cross-linked collagen and an overall decline in collagen concentration due to water absorption [5, 15, 24]. The next phase, ripening, occurs in the weeks or days before parturition and involves accelerated softening and shortening of the cervix [5]. During this phase, the cervix experiences the greatest loss of competency [13]. Ripening is characterized by additional hydration and further collagen degradation as collagen fibers increase in diameter and become disorganized and dispersed [4, 5, 15, 24]. The final phase of remodeling is known as postpartum repair. This process is key to recover the integrity and competency of cervical tissue. Timely repair is also essential to block environmental insults to the reproductive tract and ensure successful future pregnancies. Similar to cervical maturation, postpartum repair is believed to be multifactorial involving epithelial modifications, specific inflammatory changes, and ECM remodeling [15, 21].

Evolving technologies

By the time a physician recognizes that a woman's cervix has ripened, the microstructure has already transformed. Thus, the transition from softening to ripening is a critical time point to predict PTB and target interventions to prevent PTB. Because increasing gestational length by even a few days can improve maternal and fetal outcomes, many researchers are focused on developing and testing methods to detect the microstructural changes that precede cervical ripening.

Here, we focus on both traditional and experimental methods to assess cervical changes during pregnancy. We describe the uses of these tools in improving our understanding of cervical remodeling and discuss their advantages and disadvantages. We will group these techniques into categories which detect morphologic versus molecular changes in cervical tissue.

Morphologic

B-mode ultrasound

Brightness mode (B-mode) ultrasound-measured cervical length (CVL) in the second trimester is the best clinical tool to assess risk of spontaneous preterm birth [1]. A normal CVL is defined as 30–40 mm, and a short cervix is less than 25 mm [25–27]. Regardless of obstetrical history, the shorter the CVL, the higher the risk for preterm birth [1]. However, those with a history of preterm birth and a short cervix are at the highest risk [1]. To evaluate CVL, transvaginal ultrasound (TVUS) is the "gold standard" method (Fig. 1). TVUS measurements are highly reproducible and more sensitive to measurement cutoffs than abdominal ultrasound measurements [1]. Additionally, they are unaffected by obesity, position, and shadowing from the fetal head [1]. The current protocol for obtaining CVL measurements is summarized in Table 1. Ideally, a sonographer or practitioner with advanced training should measure CVL once between 16 and 24 weeks' gestation. Routine screening is not advised past 24 weeks' gestation in asymptomatic women because of the limited therapies and interventions available at that time [1]. Clinical guidelines are varied and are often unique to patient history and clinical circumstance. For those with a history of preterm birth, serial assessments (every 1–2 weeks) are often performed between 16 and 22 weeks' gestation. Guidelines do not currently recommend CVL screening in women with cervical cerclage, multiple gestation, preterm premature rupture of membranes, or placenta previa [1]. Although CVL is presently the only clinically used imaging tool to assess preterm birth risk, its use is limited by its modest predictive values. In two studies, most women with a preterm birth did not have a short cervix in the early second trimester, and nearly half of patients with a short cervix delivered at term [28, 29]. Additionally, the sensitivity of CVL in predicting preterm birth is only 9–11% [4, 30].

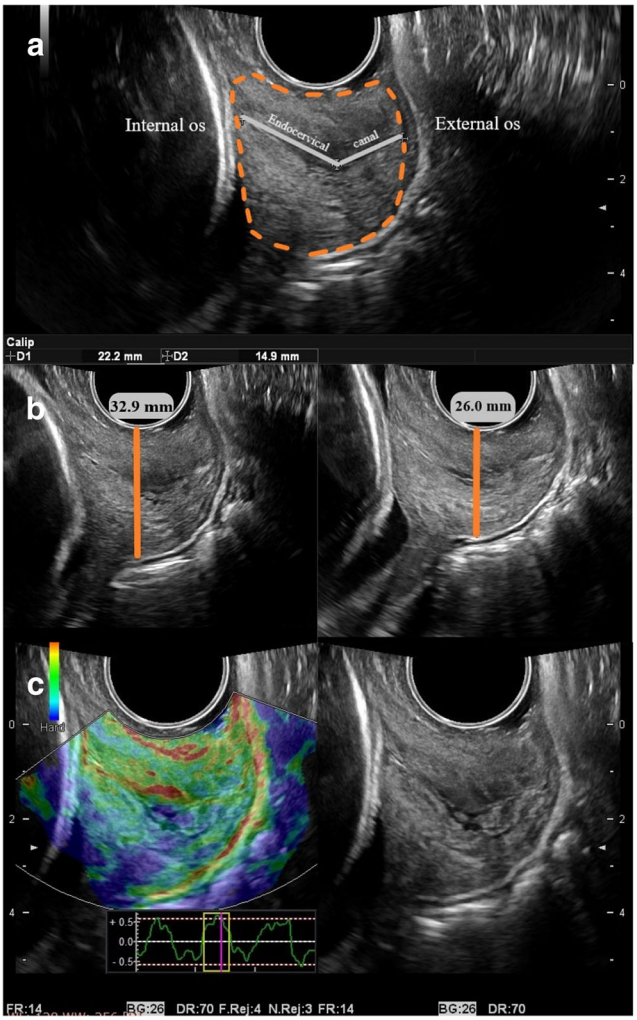

Fig. 1 **a** B-mode image demonstrating a cervical length measurement. Cervical tissue is outlined with the dotted line. The endocervical canal is demonstrated with a solid line. Two contiguous segments are often used when the cervix is not straight. The cervical length on this patient is 37.1 mm, which is in the normal range. **b** Ultrasound images illustrating the assessment of cervical consistency index (CCI). The left image is without pressure applied to the cervix. The right image is with pressure applied to the cervix by the transducer. CCI = 26 mm/32.9 mm × 100 = 79%. A smaller CCI is consistent with a softer cervix. **c** Strain elastography makes conclusions regarding tissue stiffness through observing deformations caused by probe pressure. Each color represents the difference in compressibility relative to the adjacent area. Softer tissue appears red while firmer tissue is assigned to blue

During sonographic evaluation, the presence of intra-amniotic sludge is also evaluated. Amniotic sludge is defined as echogenic matter near the internal os; it can be free floating or dense. Sludge is thought to be a poor prognostic finding for the risk of PTB especially when it was associated with a short CVL [31–33]. It has been suggested that this sludge is a biofilm and could represent ascending microbial infection into the amniotic cavity. Unfortunately, by the time amniotic

Table 1 The Cervical Length Education and Review (CLEAR) Program was created in 2011 by the Perinatal Quality Foundation. It was developed in order to standardize measurements of the cervix. It is accepted as protocol by several organizations, such as the American College of Obstetrics and Gynecology (ACOG), the Society of Maternal Fetal Medicine (SMFM), and the Society of Diagnostic Medical Sonographers (SDMS) [55]

Criteria:	Things to consider:
1. Measurement is taken on a transvaginal image.	• Transvaginal measurements are the gold standard. • Short cervix can be missed on transabdominal scans.
2. The transvaginal image is filled primarily with the cervix and the field of view is optimized for measurement.	• The cervix occupies approximately 75% of the image.
3. The anterior width of the cervix equals the posterior width.	• The anterior cervical thickness and echogenicity is equal to the posterior cervical thickness and echogenicity. • There is limited concavity created by the transducer.
4. The maternal bladder is empty.	• The maternal bladder has a variable effect on the cervical length. • The bladder is visibly empty, or nearly so.
5. The internal os is seen.	• The internal os is a small triangular area at the superior portion of the endocervical canal, adjacent to the uterine cavity.
6. The external os is seen.	• The external os is a small triangular area at the inferior portion of the endocervical canal.
7. The endocervical canal is visible throughout.	• The endocervical canal is a linear echogenicity created by the interface between the anterior and posterior walls, and extends between internal and external os.
8. Caliper placement is correct.	• Calipers are placed at the internal and external os. • If the cervix is curved, two or more linear measurements are performed and the values are added together. • Do not trace the cervical length.
9. Shortest best of three acceptable measurements is reported	• Total exam time is about 3–5 min.

sludge is present, preterm delivery may be imminent. In one study, the mean interval from ultrasound with sludge present to delivery was 21.7 ± 30.1 days [33].

We next describe three additional B-mode sonographic measures that researchers are developing to refine preterm birth risk assessment. The diagnostic performance of the techniques described below does not have the same well-defined predictive values as CVL, due to their limited use in clinical populations.

The first such B-mode ultrasound method is attenuation, which is a reduction in signal amplitude as depth increases. Attenuation measurements are useful in other fields, such as diagnosing diffuse liver disease and differentiating between benign and malignant tissues. McFarlin et al. hypothesized that, because attenuation should decrease with increased tissue hydration, this method could be used to assess tissue changes associated with cervical ripening [34]. To test this idea, they first used an ultrasound system that allows access to radiofrequency image data to measure a tissue-mimicking phantom. They developed and validated an algorithm to acquire an attenuation coefficient of 0.64 dB/cm-MHz. They then used this same ultrasound system to perform TVUS imaging on 40 women scanned once between 10 and 41 weeks' gestation [34]. An investigator chose regions of interest in areas of homogenous tissue and used the algorithm to assess attenuation in those regions [34, 35]. Regression analysis revealed that attenuation data reliably predicted the time interval from examination to delivery but did not reliably predict gestational age or CVL at the time of examination [4, 34]. The advantages of attenuation are that the measurements are non-invasive and use standard ultrasound technology. One primary limitation of the attenuation algorithm is that it assumes that the tissue is homogeneous, which may not be the case in the cervix. Additionally, attenuation measurements require standardized settings that may be not practical across multiple care settings, varied ultrasound platforms, and heterogeneous patient-level sonographic characteristics.

Another method utilizing B-mode ultrasound is assessment of cervical gland area (CGA). In past studies, the detection rate of the CGA decreased after the 31st week of pregnancy [36]. This absence of glands may signify the beginning of the cervical ripening process [36–38]. In one study assessing the utility of this measure, 600 pregnant women were examined by TVUS between 16 and 19 weeks' gestation [38]. Women with a short cervix (< 25 mm) were excluded from the study. There was no significant difference in age, CVL, or obstetrical

history between subjects. Visually, absence or presence of cervical glands was determined by whether the images demonstrated hyperechoic or hypoechoic tissue surrounding the endocervical canal. Mucosal glands were detected in 77% of the women who delivered at term and in 55% of women who delivered preterm ($P = 0.002$) [4, 38]. The primary advantages of this measure is that it is easy to perform. However, establishing whether mucosal glands are present can be subjective [37, 38]. Additionally, cervical gland assessment may not predict preterm birth significantly better than traditional CVL measurements [38].

A third B-mode ultrasound method is the cervical consistency index (CCI). This technique involves obtaining the ratio of the anterior-posterior diameter of the cervix before and after applying pressure to the cervix (Fig. 1). The rationale behind this approach is that softer tissue will deform more and have a lower CCI than stiffer tissue. To test this method, 1031 women were imaged once between 5 and 36 weeks' gestation [30]. CCI decreased with increasing gestational age. The sensitivity for predicting preterm birth by CCI was 45–67%, which is greater than the 9–11% sensitivity of CVL measurements [4, 26]. Additional advantages to this method are that it is relatively simple, it can be implemented into standard ultrasound protocols without special software, and results are reproducible between operators. However, a significant limitation of CCI is the absence of an objective way to measure the force used to deform the cervix.

Shear wave speed

Shear wave speed (SWS) is an ultrasound-based approach used to quantify the mechanical properties of tissues, such as stiffness. A shear wave is defined as the transverse particle displacement that results from ultrasound propagating through tissue [23, 39, 40]. The speed of this wave reflects tissue stiffness; softer tissue has a lower SWS, and firmer tissue has a higher SWS [23, 39, 40].

To assess the utility of SWS as a measure of the cervix, investigators performed ex vivo studies of hysterectomy specimens collected from 22 non-gravid women with benign conditions that did not involve cervical pathology [4, 23, 39]. They divided the women into three groups. The first group, defined as having "unripened" cervices, was not menstruating at the time of surgery ($n = 9$). The second group was bleeding or expecting menses at the time of surgery ($n = 6$). The third group softened their cervix by applying vaginal misoprostol 10–12 h before surgery ($n = 7$). Ultimately, the second and third groups were combined into a single "ripened" group because results did not significantly differ between the two. After excision, specimens were brought to the pathology lab, where SWS was measured with a prototype transducer. In both "ripened" and "unripened" specimens, SWS was higher at the internal os than at the external os and was higher in the posterior than in the anterior [23]. Additionally, SWS was higher in "unripened" cervixes than in "ripened" cervices. Finally, the difference between the SWS of the interior and exterior os was greater in the unripened cervixes than in the ripened cervixes, suggesting that ripening increases cervix homogeneity [23].

After this in vitro study, Muller et al. examined SWS of the cervix in 157 pregnant women [41]. Women with no symptoms of preterm labor ($n = 76$) and women hospitalized for preterm labor ($n = 81$) were imaged once during their anatomy or other clinically indicated ultrasound exam. All patients in the preterm labor group were between 24 and 35 weeks' gestation and received tocolytic agents, as well as corticosteroids for fetal maturity. The exam was performed transvaginally with an 8-mm region of interest in the distal, anterior cervix. In the preterm labor group, the average SWS was 1.3 ± 0.1 m/s, which was significantly lower than the SWS in the non-preterm labor group (1.5 ± 0.15 m/s) [41]. Additionally, the authors noted that SWS was lower in patients in the third trimester than in the first or second trimester. Together, these data indicate that SWS reflects cervical softening.

Advantages of SWS are that it is non-invasive and is operator-independent with reproducible results [23, 39–41]. Additionally, shear wave imaging is regarded as safe and has been used in sonographic evaluation of the liver for over a decade. Due to biological and spatial variability, standardizing sample location in SWS is an important consideration. One limitation to this method is the need for specialized transducers and software, which could be an obstacle to widespread clinical implementation.

Elastography

Elastography provides qualitative information regarding mechanical properties of materials, including stress, strain, and deformation [42, 43]. Stress is defined as the pressure applied to a tissue with an ultrasound probe, and strain is quantified as changes in the overall shape of the tissue. Conclusions regarding tissue stiffness are made by observing the relationship between stress applied and strain observed, with higher strain reflecting softer tissue. Elastography has been used to diagnose breast tumors, lymph nodes, thyroid tumors, prostate cancer, and liver disease [42]. The key principle is that pathology can be identified because certain tissue areas (e.g., breast tumors) are stiffer than the surrounding normal tissue. Elastography has been applied to the cervix in ultrasound imaging. The ultrasound machine provides both a grayscale ultrasound image and an overlapping heat map (Fig. 1) in which, for example, warm colors reflect softer tissue and cool colors reflect firmer tissue.

In one study assessing the utility of elastography of the cervix, 112 women were imaged with TVUS by a single operator between 12 and 40 weeks' gestation [43]. A second

operator performed another elastography measurement in 50 of the patients. In each case, probe pressure was applied to produce four or five compression/decompression cycles. Four regions of interest were selected for analysis: anterior-distal, anterior-proximal, posterior-proximal, and posterior-distal. The rate of change (strain) for tissue displacement, as a function of imaging depth, was computed for all regions. Distribution of elastography measurements suggested that the anterior-distal part of the cervix was significantly softer than the posterior-proximal part [43]. Importantly, the only significant differences in elastography measurements between the two operators were in the area in direct contact with the transducer.

Another study with 262 patients, between 8 and 40 weeks' gestation, aimed to evaluate variation in tissue strain, investigate potential patient characteristics that modify these measurements, and propose a method for estimating changes in tissue stiffness during pregnancy [42]. TVUS was performed to obtain three elastography images: one longitudinal (in the same plane as used to measure CVL) and two transverse (external and internal os). Six strain measurements were calculated in each patient. The authors concluded that the endocervical canal had higher strain than other areas of the cervix, strain was greater in parous women than in nulliparous women, strain was greater in cervixes between 25 and 30 mm long than in those greater than 30 mm long, and cervical tissue strain more strongly associated with cervical length than with gestational age [42].

The main advantage to elastography is that it can easily be performed with a TVUS probe. The central limitation of elastography is that measurements are dependent on operator force with no way to control for variability and standardize pressure. Additionally, elastography has only been clinically successful in applications where the stiffness of the region of interest is compared with normal surrounding tissue. In evaluating cervical tissue, elastography is limited by the lack of a reference tissue. Current elastography research is working to address these limitations by adding reference material with a known Young's modulus to the transvaginal probe, as well as using aspiration to objectively determine the amount of pressure applied [44, 45].

Molecular

Raman spectroscopy

Raman spectroscopy uses light to assess biochemical data in real time. The Raman effect refers to the differences in energy produced by vibrations from scattered photons when light is introduced [6, 46, 47]. The Raman spectrum is represented by peaks that each correspond with a defined molecule, such as water, lipids, and carbohydrates [6, 46, 47]. Therefore, the entire Raman spectrum provides information about the biochemistry and structure of tissues [6, 46, 47]. Raman spectroscopy has been used to detect cervical dysplasia [6, 46, 47]. Initial research revealed that Raman spectra of the cervix are sensitive to hormonal changes, leading investigators to use this method to examine the cervix longitudinally in pregnant mice and humans.

O'Brien et al. used Raman spectroscopy to compare the cervix in wild-type mice and mice lacking the enzyme cyclooxygenase-1 (Cox-1). In humans, Cox-1 is required to produce the prostaglandins that promote cervical ripening [46], and Cox-1 null mice exhibit delayed parturition but normal uterine contractility [46]. In wild-type mice, Raman spectra of the cervix changed as gestation advanced, and these changes occurred later in Cox-1 null mice. Specifically, the lipid-to-protein ratio changed just before parturition in both types of mice, but this change occurred later in gestation in Cox-1 null mice. These formative studies indicate that Raman spectroscopy can be used to identify biochemical and physical properties of the cervix during softening and ripening.

In preliminary human studies, O'Brien and colleagues recruited five women expected to have healthy pregnancies. Patients were measured monthly during their first two trimesters, weekly during the third trimester, and once again at their postpartum visit [6]. Cervical alterations were analyzed longitudinally with a generalized linear model. The authors noted significant changes throughout pregnancy, with four Raman spectral peaks of interest: phenylalanine (near 1000 cm^{-1}), Amide III (near 1300 cm^{-1}), CH_2 (near 1440 cm^{-1}), and Amide I (near 1650 cm^{-1}). The Amide I peak was analyzed with a generalized estimating equation to compare values from each patient across gestation. The peak intensity decreased throughout pregnancy, with the most rapid change in the last month before delivery. This decrease in the Amide I peak likely represents ECM breakdown, as Amide I bonds (C=O) are widespread in ECM proteins [6].

In a study of 68 pregnant patients, the Raman spectra changed significantly throughout pregnancy. For example, the peaks associated with ECM proteins decreased as gestation progressed and returned to early pregnancy values by the time of the postpartum exam [47]. O'Brien and colleagues also found that the peaks associated with blood (1530–1600 cm^{-1}) increased over gestation. However, multiparous patients' baseline measurements were significantly higher than those of nulliparous patients, suggesting the development of a permanent vascular network [47]. Additionally, actin peaks and ECM protein peaks both decreased more over gestation in patients with low BMI (< 25 kg/m^2) than in patients with high BMI, suggesting that cervical ECM remodeling occurs more slowly in patients with high BMI than in patients with low BMI [47].

One major advantage of Raman spectroscopy is the ability to capture multiple biological and biochemical features with a single non-invasive measurement. Combining biochemical

data from Raman spectroscopy with data from other imaging modalities may provide a more complete picture of cervical remodeling.

Second harmonic generation imaging

Second harmonic generation (SHG) microscopy has recently emerged as a superior imaging modality for assessing collagen architecture. SHG refers to the specific photons that are emitted by tissue proteins, such as collage type I, after excitation by light [48]. These photons are exactly half the wavelength of the excitation light. For example, collagen type I generates a SHG signal from 350 to 532 nm with an excitation wavelength between 700 and 1064 nm [49, 50]. Using the spatial information obtained from individual collagen fibers, SHG has the potential to provide data concerning the physiologic changes of the cervix.

Over the past decade, several ex vivo human and murine studies utilizing SHG imaging have been conducted. In one murine study, cervices were collected from non-pregnant mice, mice on days 6, 12, 15, and 18.75 days of gestation, and from mice treated with mifepristone. Mifepristone is a progesterone receptor antagonist and, thus, results in premature cervical ripening [15, 17, 21, 24, 51]. Cervical tissue samples were then frozen and imaged with SHG microscopy. The laser was tuned to 900 nm, which resulted in an SHG signal detected at 450 nm [50]. Quantifiable changes in SHG intensity, collagen fiber size, and matrix porosity were observed.

SHG signal intensity doubled between day 6 and day 12 of gestation, and increased another 35% from day 12 to day 15 [50]. Collagen fiber size increased progressively by 30–40% from non-pregnant mice to early gestation mice (day 6) to late gestation mice (day 18) [50]. Porosity can be quantified in a number of ways: number of pores, pore-to-pore spacing, pore size, and pore fractional area. The number of pores decreased throughout gestation, while pore-to-pore spacing increased [50]. Pore size decreased significantly from the non-pregnant mice to the early gestation mice, and then progressively increased throughout the remainder of the pregnancy [50]. Pore fractional area decreased significantly from the non-pregnant mice to day 6 and then remained constant at that level [50]. SHG was able to differentiate collagen organization in mice treated with mifepristone from normal gestation mice as well. Mifepristone-treated mice had a small decrease in SHG intensity in comparison with the control mice, but saw a twofold increase in pore size. Collagen fiber size was moderately larger in the treatment group [50]. The information obtained by SHG imaging gives researchers a greater understanding of how the cervix is ripening during the normal progression of pregnancy, as well as in a preterm birth model.

The properties of SHG make it highly sensitive to collagen fiber size and structure. Even without a detailed understanding of how and why these collagen changes occur, SHG could be developed into an effective tool for identifying women at risk for preterm birth. Translation of this technology into human studies is ongoing and offers promise for early detection of cervical ripening.

Cervical photoacoustic endoscopy

Photoacoustic endoscopy (PAE) uses a small probe consisting of a light-guiding optical fiber and a single-element ultrasonic transducer in a stainless steel tube. The optical fiber delivers light pulses into a tissue, and the single-element transducer receives light-induced ultrasound waves (i.e., photoacoustic waves) [52, 53]. Light is optimal for imaging human tissue because it does not require sample preparation and does not cause damage to the structure it is interrogating [52]. The

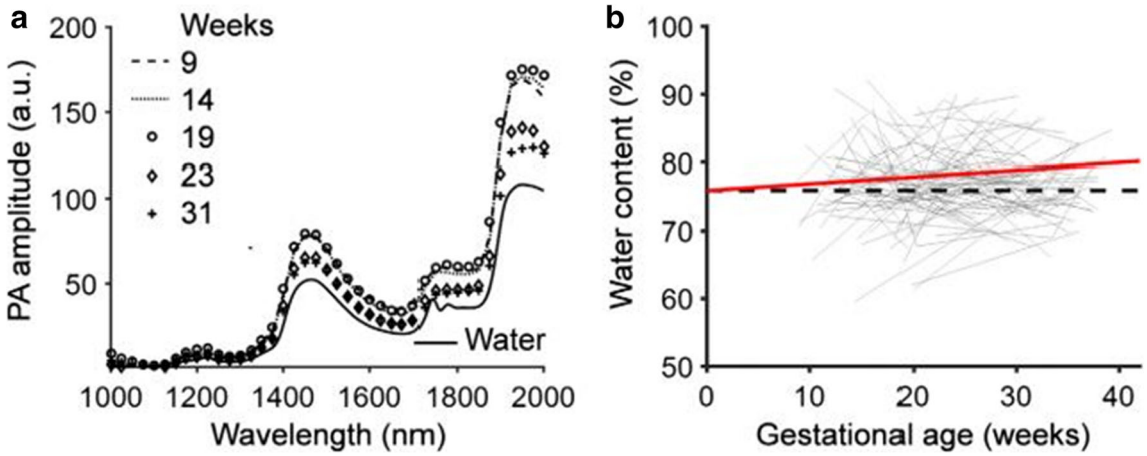

Fig. 2 **a** Cervical PANIR spectra measured at 5 different gestational ages in one patient. The solid line represents water. The closer the spectra get to the water reference line, the greater the water content. **b** Longitudinal measurements in all 205 women. The red solid line represents the generalized linear model, while the black-dashed line represents the level of the intercept [52]. Permission granted to reprint figure on February 13, 2020, from the publisher and original authors

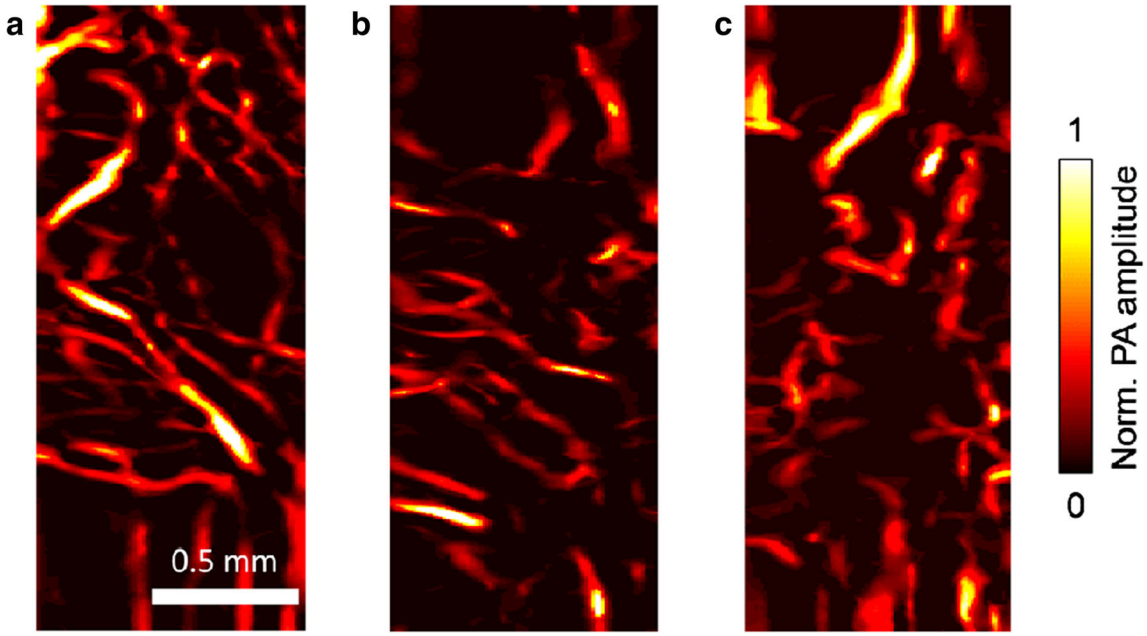

Fig. 3 Fast-scanning optical-resolution photoacoustic endoscopy (fsOR-PAE) images acquired in vivo. **a** and **b** were obtained from the same patient at 32 and 36 weeks, respectively. **c** was acquired by a second patient at 36 weeks [53]. Permission granted to reprint figure on February 13, 2020, from the publisher and original authors

photoacoustic signals recorded by the probe provide optical signatures of molecules in the tissue.

PAE probes can be specialized to evaluate different tissue characteristics, such as water content and vascularity, by using different wavelengths of light and mechanical configurations. For example, multi-wavelength PAE in the near-infrared region can assess tissue hydration of the cervix. Currently, near-infrared (NIR) spectroscopy is used industrially to quantify the water content of numerous substances [52, 54]. In 2018, photoacoustic near-infrared (PANIR) spectra were measured

Table 2 Summary table of current biotechnologies being developed to assess preterm birth risk

Imaging technology	Advantage	Disadvantage
Cervical length (TVUS) [1, 4, 25–27, 30]	• Reproducible	• Incomplete picture • Limited predictive value
Attenuation algorithms [4, 34, 35]	• Easily incorporated into prenatal care	• Assumes homogeneous tissue • Need to standardize ultrasound settings
Cervical gland area [4, 36–38]	• Can be performed on any standard US machine	• Subjective • Predictive utility may not be superior to CVL
Cervical consistency index [4, 26, 30]	• Superior sensitivity compared to traditional CVL • Can be performed on any standard US machine	• Force applied not quantified
Shear wave speed [23, 39–41]	• Reproducible • Operator-independent	• Specialized software required • Standardization of anatomic location needed
Strain elastography [42–45]	• Easy to perform	• Specialized software required (compatible with most existing equipment) • Dependent on operator force • No reference tissue
Raman spectroscopy [6, 46, 47]	• Abundant biochemical information in a single measurement	• Biochemical components only (not tissue architecture)
Second harmonic generation [15, 17, 21, 24, 48–51]	• Directly visualizes collagen microstructure • Quantitative	• Development for in vivo use is needed
Cervical photoacoustic endoscopy [52–54]	• Provides information about tissue hydration and vasculature • Quantitative	• Specialized equipment needed • Accessibility to technology

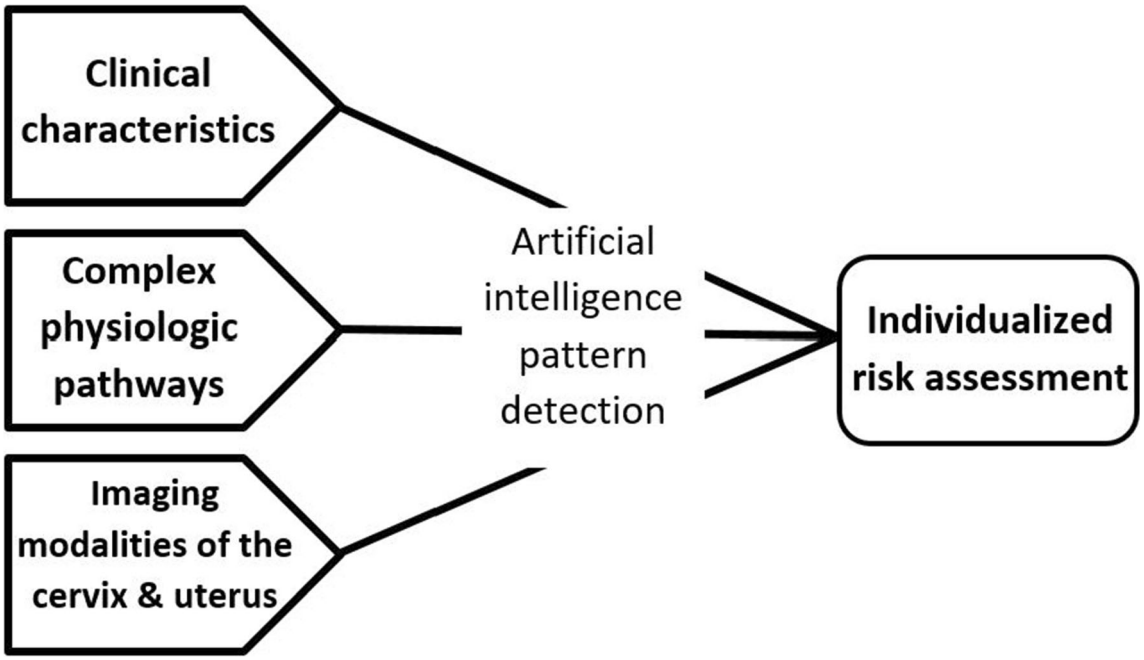

Fig. 4 Comprehensive risk assessment will require the help of artificial intelligence to detect patterns of clinical characteristics and complex physiologic pathways while incorporating novel imaging risk assessment tools

and analyzed in phantoms made of hydrogel to mimic connective tissues [52]. The water contents of the gels ranged from 70 to 100%. The PANIR system was tested at two wavelengths, 1460 and 1940 nm [52]. At both wavelengths, the system was able to provide a similar and accurate prediction of water content of the phantoms. Next, the PANIR probe was used to examine the cervix in pregnant women longitudinally across pregnancy. The probe was placed on the anterior portion of the cervix during a speculum exam, and three or four measurements were obtained [52]. Analysis of the PANIR spectra showed little change in tissue hydration before 20 weeks' gestation. However, by the end of the second trimester, the water content began to increase. Overall, increasing water content had a significant linear relationship with advancing gestational age (Fig. 2).

Another PAE system has been developed to examine cervical vasculature. A fast-scanning optical-resolution PAE probe was custom designed to execute mechanical scanning [53]. Sector scanning in this probe is achieved by a rotating mirror that is steered by a micromotor. The coupling medium for the acoustic waves is provided by deionized water within the tip of the probe. This probe was first validated in tissue-mimicking phantoms and then in ex vivo cervix specimens obtained from hysterectomies. Finally, this probe was used for in vivo cervix imaging in two pregnant women, one at 32 and 36 weeks' gestation, and the other only at 36 weeks' gestation (Fig. 3). With this method, the investigators detected morphological vasculature differences between the ectocervix, uterine body, and sublingual mucosa and observed longitudinal differences in cervical vasculature.

One limitation of cervical PAE is accessibility, as it requires complex hardware and software that are not commercially available. Nevertheless, given the large amount of data it can provide, PAE holds promise as a non-invasive cervical imaging technology.

Conclusion

Table 2 provides a summary of current and developing technologies to assess cervical remodeling**.** Due to the predominant experimental use of some of these technologies, clinical diagnostic performance in specific patient populations is not well described at this time. Although there have been major breakthroughs in the last decade, many questions remain about cervical remodeling and preterm birth risk assessment. For example, how and why does the cervix shift from softening to ripening? Researchers must focus on developing non-invasive tools that can produce reliable repeated measurements in the same patient over time and provide between-patient comparisons. It is also important to note that these studies are restricted to singleton pregnancies, and therefore, any conclusions cannot necessarily be applied to multiple gestations. Given that preterm birth is a result of multiple, complex pathways, a combination of approaches will likely be required for comprehensive risk assessment. Additionally, clinical and obstetric characteristics will likely need to be incorporated into novel imaging risk assessment tools. With future studies, innovative imaging techniques combined with artificial intelligence analytic techniques may reveal new

phenomena that are important in human cervix remodeling (Fig. 4). Ultimately, continued collaboration across disciplines is required to improve our abilities to predict, and hopefully prevent, preterm birth.

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Compliance with ethical standards

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