Chapter

Abnormal Cervical Remodeling Early Depiction by Ultrasound Elastography: Potential Opportunities for Preterm Birth Prevention and Delay

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Abstract

Early depiction of abnormal cervical remodeling (CR) is a prime information source with days/even weeks to uterine contractions for preterm birth (PTB) risks. CR phases, softening-ripening-dilation, are distinctive by molecular, and timing in preterm/term: integrity loss in ripening and dilation. Changes in extracellular matrix, cellular content, water retention drive progressively to resistance reduction, rising elasticity, relaxing cervical smooth muscle cells (CSMCs) are organized like a sphincter at internal os. Shear Wave Elasticity Imaging-SWEI (dynamic elastography) based on objectively measurable cervical response to deformability is more accurate for early CR depiction from 8–12 weeks vs. conventional ultrasound cervical length (CL) measurements (16–23^{+6days} weeks). SWEI quantifies tissue microstructure, constant fractional stiffness reduction (~ 4%/week), and spatial gradient in Shear Wave Speed (SWS) along cervix, and CL serial measurements in addition offers better strategies for prolongation, actual/future fetal safety, when cervical softness/shortness progresses. Vaginal progesterone (P_4) from early pregnancy to complete 37 weeks is preventive: controls CR, indicated by SWS prior to CL < 25 mm, and cerclage (1–2 stiches) and/or pessary needs, adjuvant to cerclage, for CL < 15 mm after cerclage. Meta-analyses, systematic reviews proved P_4 efficacy in prolongation (>28, 34, 37 weeks) in asymptomatic cases, with characteristic history/actual abnormal CR/shortness, a small better efficacy for vaginal P_4, and cerclage vs. pessary, when separately analyzed; few retrospective studies exist on triple association efficacy for PTB prevention/delay and neonatal outcomes.

Keywords: cervical softening/ripening, human cervical smooth muscle cells, dynamic elastography—Shear Wave Elasticity Imaging, ultrasound, preterm birth, vaginal progesterone, cerclage, pessary

1. Introduction

Cervix uteri is a metabolic active organ, a "gate keeper" in normal pregnancy, an early information prime source about spontaneous preterm birth (sPTB) risks [1], besides other two pathological conditions – preterm/premature membrane rupture (PROM), preterm contractions with which cervical insufficiency/incompetence interlinks in the "Preterm Labor Syndrome" [2], which replaced an earlier five ways acting in cascade/separately: uterine cervical support structures compromise acting alone/associated to the myometrial and membranes over-distension, to decidual hemorrhage, to intrauterine infection/inflammation or to precocious endocrine fetal activation (from maternal or fetal stress) [3]. Vink and Feltovich [4] proposed a new paradigm/concept that sPTB three essential elements (cervical remodeling, myometrium activation-preterm labor, decidua-preterm membrane rupture/hemorrhage) are inextricably intertwined, all lead to each other. These factors are considered as a possible "phenotype" for sPTB [4, 5], such as those that start with premature activation of fetal membranes leading to PROM vs. myometrium premature activation leading to preterm labor vs. premature cervical remodeling leading to cervical failure, but 25% of sPTB are not associated to any phenotype [5].

Prematurity rate has not declined over the past 40 years, or a small decline was followed by a new increase, and despite significant medical and management strategies advances, it has a continuous slow rise, with high offspring morbidity and mortality, affecting 14.8 million babies annually worldwide [6]. Management with actual opportunities of assessment and medical/surgical procedures—elective, urgent, and/or emergency, consuming a substantial portion of the health budget [7, 8] is the aim of modern obstetrics/perinatal medicine societies, being imperative to improve medical understanding of normal/abnormal reproductive tissue structure and function, and how these tissues interact at cellular and biochemical level, and to act accordingly. This chapter focuses on cervix uteri involvement for sPTB prediction and prevention/delay through cervical abnormal remodeling identification by non-invasive novel approach imaging techniques.

2. Gestational cervical remodeling

"Cervical remodeling" is the collective term for progressive cervical changes and recovery during pregnancy, labor, and postpartum, involving four overlapping phases: softening, ripening, dilation, and postpartum repair [9]. Clinicians use terms such as *softening*, *shortening*, *funneling*, *effacing*, and *dilating* interchangeably [10] for gestational cervical deformations, representing tissue intrinsic material changes with anatomical transformations. Cervical insufficiency (CI) determined a lot of comments. According to the American College of Obstetricians and Gynecologists [11], it is defined as "painless cervical dilation after the first trimester with subsequent expulsion of the pregnancy in the second trimester, typically before 24 weeks gestation, without contractions or labor, and in the absence of other clear pathology (as bleeding, infection, ruptured membranes)". It was recommended the terminology "premature cervical remodeling" to describe cervical softening, shortening, funneling, and IO dilation, and to retire the anachronistic "cervical insufficiency" [4].

2.1 Cervix uteri microarchitecture

Human cervical structure was established in the 1940s when Danforth D [12] described cervical stroma—a predominantly homogenous, hydrated, collagenous structure between exo and endocervix, which over time made the distinction to the muscular structure of the uterine corpus. Stroma consists of 85–90% extracellular matrix (ECM) that contains tropocollagen, aggregating to form collagen fiber, and bundles network surrounded by a viscous substance of proteoglycans (decorin, biglycan, asporin, osteomodulin and fibromodulin, lumican, and versican), glycosaminoglycans (GAG: sulfated-chondroitin/dermatan/heparan sulfate and non-sulfated: hyaluronan-hyaluronic acid (HA) GAGs), matricellular proteins (thrombospondin 2; tenascin C); adhesion molecules (fibronectin, integrins-α, β or α-β); specific amino-acids—phenylalanine; enzymes [hyaluronidase, matrix metalloproteinases—MMP: -2, -9; lysyl oxidase-like-1 (LOXl-1), involved in ECM transformations], according to their genes, and water [13]. Cells—about 10–15%—are represented by fibroblasts, smooth muscle, glandular, immune, and vascular cells. Fibroblasts synthesize ECM, rich in collagen fibrils (type I—70%, for tensile strength; type III, 30% for elastic properties) as tropocollagen molecules self-assembled into fibrils—a nanoscale rope-like, and elastin, as tropoelastin to form the elastin fibers complex network intertwined with collagen. Collagen bundles are organized in layers, which remodel differently because of independent molecular processes and cause softening and shortening [14, 15]. Collagen content—measured as hydroxyproline, is constant along pregnancy [9]; second-harmonic generation (SHG) microscopy presents collagen preferentially aligned around the canal [16]. The triple helix arrangement allows collagen to cross-link into complex 3-D networks of fibrils, fibers, and bundles that dictate both firmness and mechanical strength during non-pregnant/pregnant states [17]. From Aspden [18] early theory on three zones in cervix structure—**Figure 1A**, actually cervical stroma is considered heterogenic, and collagen network strength depends on the degree and type of collagen cross-links between each collagen microfibril [20, 21]. Recent studies show significant collagen cross-link heterogeneity in internal OS (IO) compared to external OS (EO) [22]; IO area is the most exposed to stretch, and it is resistant to tension associated with uterine content (fetus, placenta, amniotic fluid), cervical angle in pelvis [10], and fetal membranes properties, their stiffness sustaining IO closure,

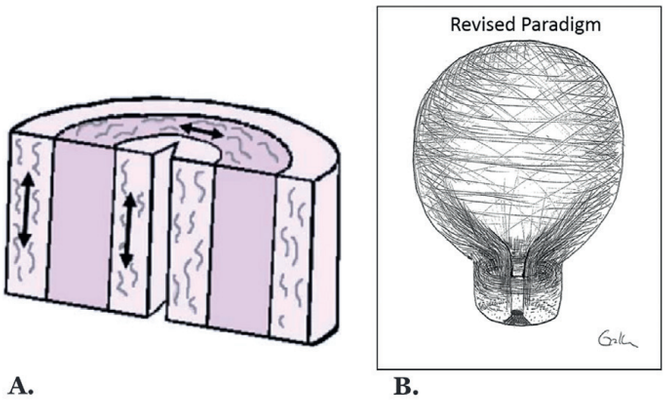

Figure 1.
A. The three zone in cervix early theory. B. New paradigm on SMCs at upper cervix half, circumferentially to IO, is like a specialized sphincter; it is not a homogenous collagenous structure, with minimal cellular content, as old paradigm. Adapted from Vink and Myers [19], free PMC article. HHS Public access.

as 3D MRI revealed [23]. Elastin is a high thermal stable protein with an 80-year half-life and a linear, compliant mechanical response to tension, permitting cervix restoration after an applied stretch. Elastic fibers are present mostly in the subepithelial stroma, aligned at early pregnancy as collagen fibers, and undergo structural changes, becoming thicker, shorter, and losing directionality [24, 25]. Proteoglycans have key roles in controlling gradients and availability of potent growth factors, chemokines, cytokines, and morphogens, important in tissue's homeostasis, mechanical strength, development, and repair, by their covalent attachment to collagen fibers surface, contributing to fiber optimal formation [26].

From early studies on cervical structure and functions [27–29], immune—staining with smooth muscle cell markers (α-smooth muscle actin, smooth muscle protein 22 calponin) in non-pregnant women <50 years old cervices proved the presence of smooth muscle cells (HCSMCs) surrounding IO, about 50–60% of tissue, like a specialized sphincter, proved by positive contractile activity tests at oxytocin/nifedipine, similar to uterine SMCs. Bundles of cervical smooth muscle cells (CSMCs) are circumferentially oriented around endocervix canal periphery. Human cervical smooth muscle cells (HCSMCs)' density is gradually reduced from the mid cervix to EO (10%), where contraction tests showed less activity. Gap junctions are present in HCSMCs for direct communication with uterine corpus SMCs [4], a cervical active involvement in pregnancy progression, as Pajntar sustained for a long time [28]. Longitudinal muscle fibers are mostly located in cervix's inner part, while circular muscle fibers are mostly in the outer part. Longitudinal muscles can contract, helping cervical dilatation, while circumferential muscle fiber contraction impedes cervical dilatation, keeping the cervix closed." Dynamic cervix" nomination is used when a short cervix appears without uterine contractions or is correlated to uterine fundus pressure [30]. This discovery changed the paradigm on HCSMCs covering cervix first half part, and their role in IO normal closure, or relaxation-inducing "funneling"/"sphincter failure", the first sign of premature cervical remodeling [19, 30], and increases the duration of latent and active phases of labor [31], according to connections of SMCs from uterine cervix and corpus. Similar CSMCs were discovered in female rats during pregnancy, being considered as a continuous muscular layer from the uterine corpus (**Figure 1A** and **B**) [32].

Resident immune cells -neutrophils, eosinophils, and macrophages are present in cervix; their location and relative number change during pregnancy [33, 34]. Rat's cervices pathological exams [35] proved macrophages role in remodeling, mainly in ripening normal/abnormal timing. There are registered reduced cell nuclei density, decreased collagen content and structure, and a greater presence of macrophages per unit area in rats under progesterone receptor antagonist (onapristone/mifepristone) or after ovariectomy on day 17 postbreeding, similar to aspects before term.

2.2 Timing of cervical remodeling

Qualitative and quantitative cervical softness/stiffness and length dynamic changes previous to uterine contractions onset are harboring different mechanisms in term/PTB regarding cervical involvement in "closing"/"opening", to prevent/delay sPTB/to induce labor, were better understood using mathematical or animal models instead of cervical biopsies because women's risks [36], and confounders by variations in timing and collected samples region [37]. Shorter pregnancy duration and mice cervices resistance to break/stretch made mice prone to study [38]. Evidences provided similarity in women, and mice regarding dynamic gestational cervical

remodeling [9, 37, 39], and simulations showed that changes are centered on stroma ECM content, which are associated to changes of SMCs surrounding IO [30], and all control gestational tissue's mechanical behavior.

2.2.1 Premature cervical extracellular matrix changes inducing preterm/premature cervical failure

Cervical stroma ensures mechanical properties [40] by ECM molecules arranged in a scaffold/matrix, and cells. One must understand mathematical models through Humphrey and Rajagopal [41] mixture theory. Updated after 20 years [42] for soft tissue growth [changing mass, solid (cells, fibers), and fluid-interstitial], and remodeling (changing microstructure) for adaption at new growth imposed functions. Microarrays and gene analysis [43] in murine models revealed two distinct phases – softening and ripening. Cervical human transcriptome is mentioned more than a decade ago [44], with some considerations on distinct cervical microRNA profiles in women destined to sPTB [45], cervical softening—an active phase, as shown in nearly daily assessment in murine models [15, 39] with rapid onset after conception, is defined as the first measurable increase in tissue distensibility versus to non-pregnant, with a precisely timed activation of slow molecular, microstructural, and mechanical changes up to term/labor [9], regarding cellularity, collagen, elastin, GAGs, proteoglycans, water. Softening drives alterations in collagen fibers organization and alignment, inducing a distinctive arrangement—short, thick, and curved, with low linearity, and change in the number and type of cross-linking between newly formed fibrils, coinciding with a marked increase in hyaluronic acid (HA), and water uptake, globally reducing mechanical resistance to tension [15, 46], an influx/activation of immune cells releasing MMPs degrading ECM [37], increase elasticity, and prepare inferior segment for delivery. Collagen waviness confers tissue isotropy and heterogeneity, destabilizing mechanical integrity with great deformations and relaxations and increased compliance necessary for uterine cavity rising pressure, with tissue integrity maintenance. Ripening—a term used first by Winkler and Rath [47]—is near labor onset, with rapid evolution, cervix marked softening and shortening. Preterm and term cervical changes are similar, not identical; sPTB can result from aberrant events timing [4]. Murine studies showed differences in the molecular mechanisms governing preterm and term ripening [38, 48]. Untimely cervical ripening in early gestation—earlier than normal (before 24–36[6] days weeks, in women), predisposes to CI, responsible for recurrent late miscarriage/sPTB, and cervical ripening inadequate in late pregnancy is associated with induction failure/prolonged labor, possible involvement of EMC and CSMCs from IO [30]. Murine preterm-induced delivery showed a dramatic reduction of mature collagen fibers, increased new fibrils synthesis, packing disorganization by the gradual replacement of mature cross-linked collagen with collagen harboring reduced cross-linking, less cross-linking between fibers of new fibrils of tropocollagen, which are shorter and thicker than in non-pregnant, besides the distinctive arrangement of fibers—from being aligned to curling, becoming isotropic [46], greater spaces between fibers, decline in thrombospondin-2 and tenascin-C [36], and ECM high hydration [49], the parallelism between water and hyaluronic acid (HA) with very hydrophilic large molecular weight-hyaluronan, increases wet weight vs. controls, and in mice at term [36, 43], affecting collagen organization [50]. The interstitial fluid controls tissue stiffness response, water content increases by 5%, modifying cervix mechanical properties, generating an unbalance/non-equilibrium state [51], and the untimed ripening initiates rapid incremental

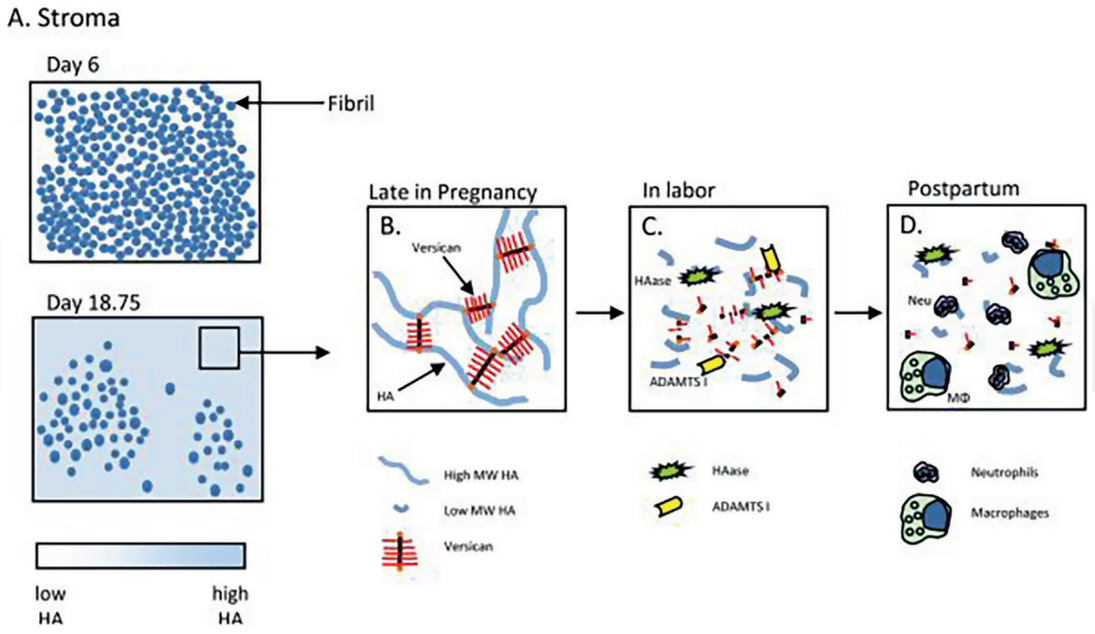

Figure 2.
Proposed model for mice cervical remodeling for mechanical resistance reduce. A. Increase of high molecular weight (MW) hyaluronan in ECM from early to late pregnancy. B. At pregnancy end, high levels HA and versican form cross-links, which are associated with water uptake, increased tissue compliance, visco-elasticity, and collagen disorganization. C. During labor, enzymes (hyaluronidase and ADAMTS enzymes) cleave HA, and versican, causing cervical tissue integrity and complete loss for dilation. D. During postpartum repair, low MW hyaluronan, versican fragments, and damaged collagen are removed by immune cells—neutrophils and macrophages. Adapted from Timmons et al. [15]. PMC free article. HHS Public access.

fall in cervical mechanical strength (**Figure 2**) [37]. As in other tissues (cartilage and blood vessels) with complex mechanical behavior, the relative ratio of collagen to proteoglycans is an important determinant of cervix visco-elasticity, compliance, and ultimate function [52].

The first presented 3D anisotropic hyperelastic model [38], based on Langevin statistical mechanics for human and mice gestational mechanical response, revealed: constant total collagen content per volume along pregnancy; mature collagen cross-link disorganization in human ripening cervix evolves by an increase in tissue acid solubility, along pregnancy; mice collagen cross-link disorganization progresses from d6 (when bundles are larger) to d15, with little change after d15. This difference explains mouse cervix resistance to break in uniaxial tensile test compared to human, in non-pregnant and pregnant state.

The stiffness progressive reduction in normal human pregnancies starts from first trimester (>2 vs. non-pregnant). It continues in the second, but not between the second and third trimester, with consistent recovery after delivery, to the level of early pregnancy, as ectocervix aspiration technique depicted [53]. Cervical shortening normally starts from mid-pregnancy and progresses until delivery, as ultrasound longitudinal studies on cervix length (CL) sustain [54], being a normal 30 mm CL at pregnancy beginning, with individual variations, from population, age, parity, and gestational outcomes, as a systematic review, and meta-analysis report [55].

EMC organization and composition aberrant regulation during softening, a tissue property, may contribute to premature shortening—a tissue deformation that results from an evolving 3D stress state, and the intrinsic constituents remodeling [56], softening plus shortening being considered as dysfunctional EMC remodeling [20], and IO "funneling" is next step, due to softness, and sphincterian HCSMCs relaxation

[30], added to dysfunctional remodeling that starts invariably from IO [57], where is the higher stress compared to EO. EO is not significantly loaded until the cervix has significantly shortened [58], and 3D hyperelastic anisotropic model, revealed relative similar stress to both orifices, and the middle part is between those in the IO and EO [38].

2.2.2 Premature relaxation of cervical smooth muscles cells promoting preterm/ premature cervical failure

One offers special attention on HCSMCs presence as a circumferential sphincter at IO level, since the new paradigm on their role in cervical closure in normal pregnancy [30]. A smooth muscle sphincter at IO, is considered one of the most important anatomic structures for pregnancy's normal progression, when it is contracted [10], and its relaxation is associated with the onset of labor, as it was supposed [59]. From Leppert [60] early studies, one knows that in early pregnancy, fibroblasts and CSMCs proliferation is at the highest level and decreases progressively, whereas apoptosis increases progressively in later pregnancy, parallel to water content in interstitial tissue, inducing mechanical imbalance [51]. Cervical contractile function contributes to cervix stiffness and closure maintenance until the latter part of the third trimester. During the ripening and dilation phases, the HCSMCs are not just a passive responder to uterine contractions; they independently initiate their own contractions, and biochemically their stretch increases MMPs [61], and proinflammatory cytokine secretions [62], representing new mechanisms on premature CR, and possible failure. Vink et al. [63] proved in pregnant women cervices with premature cervical failure (PCF) that CSMCs defect of contractility, and reduced IO sphincter tonus appear when CSMCs are exposed to a soft ECM. It is suggested that a soft ECM may lead to decreased CSMCs contractility and IO sphincter laxity, so ECM rigidity modulates HCSMCs contractility.

HCSMCs are physiological modulated by hormones (estrogens, progesterone, and oxytocin—increases intracellular calcium levels), local paracrine signals (inflammatory chemokines and cytokines), extracellular vesicles (exosomes and ectosomes), and pharmacological agents used for cervical ripening and labor induction. Vink et al. [64] demonstrated that E2 increases HCSMCs contractility by rising actin contractility, and calcium levels—less than oxytocin, and favors pregnancy progression, and

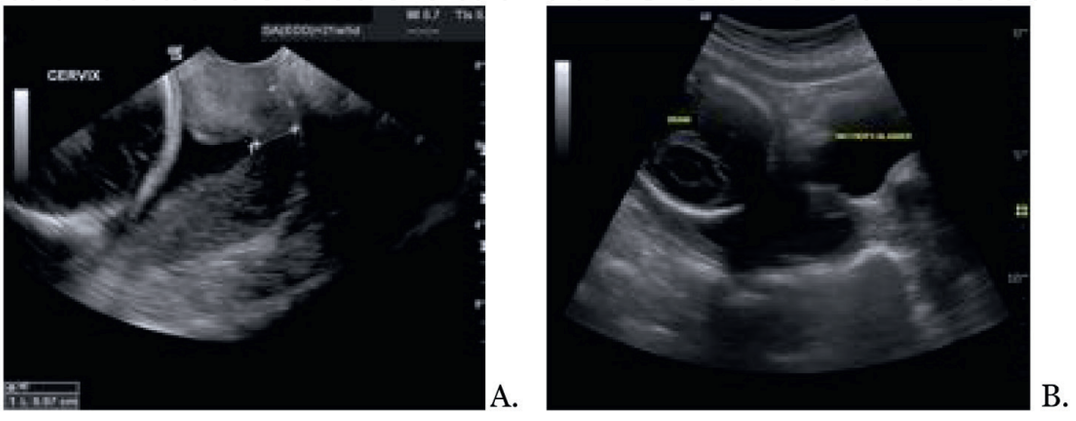

Figure 3.
A: funneling of IO, with shape of T, Y, V, or the most dangerous of U, associated to short cervix (between white markers) B: complete cervical effacement (authors proper archive: [65]).

P_4 reduces HCSMCs contractility, by oxytocin-induced contractility alterations, and intracellular calcium flux; P_4 appears favorable for IO "funneling"/dilation.

Since many years of conventional/standard sonography, starting from 16th weeks gestation, may show shortness and funneling, **Figure 3**. Funneling is considered more predictive than shortness to PTB risk, and it imposes a cervical stitch [64, 66]. Sludge association with short cervix (<25 mm) increases sPTB risks [67].

3. Hypothesis and realities on gestational cervical remodeling timing: molecular insights

Two mouse models have provided new insights into distinct processes of preterm CR: infectious/inflammatory model [68, 69] and progesterone loss/withdrawn/non-infectious model, with differences in gene pathways in preterm, and term. Analyzes of preterm mice collagen structure changes [36] showed that immune pathways system activation is sufficient but not necessary for premature ripening [33, 37, 43, 48] being differences regarding collagen fibers in preterm P_4 withdrawn/mifepristone-induced, infected-induced, and term ripening.

3.1 Inflammation in premature cervical ripening

Lipopolysaccharide (LPS) administration in amniotic sac surroundings elicits a proinflammatory response similar to infection, preterm labor progressing without P_4 concentration loss in circulation several hours after administration [68]. SHG demonstrated an interfibrillar spacing increase in collagen fibrils, with increased pores lacking collagen signal in SHG, when LPS treated mice, and preferential disruption collagen fiber architecture in the subepithelial region compared to mid-stroma region, and neutrophils infiltration. Rodents models demonstrated that ripening may be characterized as a physiologic inflammatory process, by myeloid immune cells, as neutrophils, with their chemotactic factors [69], with a temporal coincidence of decreased density of cell nuclei, decline in cross-linked extracellular collagen, and increased presence of macrophages, sustaining the immune cells hypothesis [70–72], so cervical increased biomechanic distensibility is before uterine contractile capabilities for labor. Flow cytometry and cell sorting were used to determine immune cells role in ECM remodeling before, during, and after parturition. Myeloid immune cells/leukocytes invade EMC and are activated to product proinflammatory mediators [IL-8, monocyte chemo-attractant protein-1 (MCP-1), IL-1β, and TNF-α], and proteolytic enzymes [73] altering EMC. Markers of myeloid cell differentiation and activation were used to define phenotype and function [73]. Peripheral blood does not reflect changes in cervical myeloid cell numbers. The proinflammatory mediators are ripening inducers, through prostaglandins [74], high concentrations of inflammatory cytokines (IL-6, IL-8, and MCP-1) are depicted in human PTB cervix [75]. Monocytes and eosinophils increase in cervix before birth, in a progesterone-regulated fashion, whereas macrophages number is unchanged. The cytologic conclusion is that myeloid-derived cells do not orchestrate processes required for cervical ripening initiation before birth [73].

Macrophages are analyzed in many studies. They are present in gestational cervix with different phenotypes (M1 and M2), considered to be most involved in tissue postpartum repair, sustained by increased mRNA expression of Csfr1, and markers of alternatively activated M2 in labor, or shortly after fetal expulsion [73]. Hunter

et al. [34] described a special phenotype of macrophages or phagocytic leukocytes, partially similar with blood—born monocytes, present from the 12th week in normal cases, their absence being a sPTB risk marker. These cells have critical role in cervical immunity, by preventing microbial translocation to feto-placental unit. Leukocyte insufficient recruitment to cervix is supposed to be associated with sPTB recurrence (<34 weeks). The cytokines, and MMPs are increased only in inflammation-induced sPTB, not in term ripening, where is no infection [34].

Prostaglandins are used to induce labor, according to their essential intervention in LPS-mediated preterm ripening, but not term or antiprogestin-driven preterm ripening [76].

Actually, there is a connection between amniotic fluid inflammation and imminent PTB prediction when short cervix in asymptomatic cases, starting with amniotic proteome analysis [77]; inflammation is a giant disruptor of gestational maternal-fetal homeostasis.

3.2 Premature progesterone withdrawn/progesterone receptors loss/ responsiveness in premature cervical ripening

Since 1960, from Cullen and Harkness studies on rat's cervix [78], one knows steroid hormones role in cervical stroma cells specific functions, and complex series of molecular events in pregnancy, and parturition. Steroid hormones actions are proved on HCSMCs surrounding IO [79], through their receptors (ER, PR). Progesterone exerts a dominant role for most of the pregnancy duration to initiate a loss of tissue strength yet maintain competence in the softening phase, which starts early in pregnancy when P_4 is high, E_2 is low, and normal ripening is at the gestation end [9, 80] to permit fetal delivery after active dilation. The reduced P_4/E_2 ratio—a parturition hallmark, begins the cascade of accelerated cervical ripening, responsible for the final structural integrity loss, necessary for fetus safe passage. P_4/E_2 ratio is high during softening; the highest P_4 concentration correlated with the least amount of new collagen fibril synthesis, E2 significantly rising new collagen, and progressively increased levels as P_4 decreases near term, when P_4/E_2 ratio is the lowest [81]. P_4 controls GAGs modulating ECM [82] and counteracts inflammation [83]. The studies on rats [84] showed that PR genomic receptors modulate the transition from a soft to a ripened cervix in term and PTB. Studies on many mammalian species have shown that softening shift to ripening occurs while blood progesterone is near peak concentrations, being discussed as a responsiveness loss to progesterone, a common final mechanism for mammalian cervix remodeling for term birth preparation [70]. P_4 sustains cervical softening and ripening, mediated by a stable stromal cell population, expressing PR-A, and through interactions with resident macrophages, the inflammatory ripening processes are mediated in birth preparation [85]. In ovariectomy-induced systemic progesterone loss, it was not registered hypertrophy, extracellular collagen, or macrophage number changes [35]. Yellon [70] appreciates that the structure and macrophage census in cervix is sufficient for premature ripening and birth before term; PRs are localized on other cells than macrophages, making possible interactions between cells to facilitate the loss of progesterone receptor-mediated actions, as part of a final common mechanism that ripens the cervix in certain preterm etiologies, and in term deliveries.

P_4 non-genomic membrane receptors mechanism is dominant in mice, besides genomic mechanism, mediated by isoform A [85] for prepartum softening and ripening, which start before birth, when systemic P_4 is near peak concentration [72],

through stroma fibroblasts, but not macrophages. Smooth muscles, innate immune cells, and mainly pregnancy-activated lymphocytes have P_4 membrane receptors, which fibroblasts are missing. P_4 has immune-modulatory proprieties by the 31 kDa protein "progesterone induced-blocking factor", synthesized by human lymphocytes, which inhibits natural killer cells, and modifies cervical, and decidua cytokines balance. RU486/mifepristone mice administration on the 13th day of gestation induced the onset of preterm labor within several hours [43]. This model presents significant ECM disorganization: more and larger spaces between collagen fibers, higher cervical wet weight, a 5% increased water content *vs* LPS infected mice model, with reduced edema. RU-486 group cervix weight being similar to term cervix. Premature RU-48-induced ripening results from an acceleration of the process, in place during term ripening, as well as partial activation of proinflammatory, and immune-suppressive process, which is observed during normal postpartum repair, different from LPS-induced PTB, where only the neutrophil stroma population is increased, without parallel monocytes, and eosinophils populations, or systemic blood rise; no blood systemic monocytes levels increased in any studied group [43].

Japanese studies on cultured cervical stroma fibroblasts in cases with refractory PCF and recurrent pregnancy loss [86] have a remarkable discovery: namely, cervical fibroblasts' PRs down-regulation, with impairment of P_4 inhibitory effect on lipopolysaccharide-induced inflammatory stimuli, and consecutive PCF. These results suggest that abnormal cervical ripening in CI is caused by down-regulation of P_4 signaling at receptor level and provides a novel insight into the molecular mechanism of PTB.

4. Premature/preterm cervical microstructure remodeling assessment

The current approach to evaluating cervix state relies on digital assess (with Bishop scoring [87], first proposed for labor induction success), subjective appreciation as "soft, medium, hard/firm", and conventional sonographic examination [54] being now compelling evidence that changes in viscoelastic properties are key to cervix function. Cervical length (CL) measurements are suboptimal means to assess early remodeling, providing no data on consistency [88], and because of high negative predictive value, when used as a screening test [89]. Some studies tried to give indirect data on cervical softness during conventional sonography: cervical sliding sign—assessing cervix resistance to gentle probe compression [90], cervical mucus plug distance—part of the plug nearest to vaginal microflora, as an additional sign for CR [91]. One gives much attention to CL value in asymptomatic cases: ≤15 mm before 20 weeks gestation has a dramatic and significantly higher risk of early PTB than at 20–24 weeks [92] and to differences between universal screening and selective screening in conjunction with independent risk factors for a short cervix ≤25 mm at 20–24 weeks [93]: race-ethnicity; current tobacco use; prior indicated preterm birth; a prior cervical excisional procedure. If only women with any of these variables were offered transvaginal CL screening, the specificity would increase from 62.8% for universal screening to 96.5% with a risk-based approach. The sensitivity with one variable present was 62.8%, and with two factors, 14%. The Cochrane Database Systematic Review [94] on the knowledge of CL in singleton pregnancies with symptoms vs. no knowledge up to the year 2018 showed a prolongation with only 4 days before 34 weeks gestation.

4.1 Innovative methods to objectivate premature cervical microstructure remodeling

Non-invasive methods are adopted in maternal-fetal medicine for collagen architecture assessment, clinicians collaborating with biomechanical engineers and other scientists to reveal crucial structural properties changes during pregnancy and labor. Cervical microstructure is a very good objective for new proposed *in vivo* techniques based on quantification of mechanical, optical, or electrical tissue properties: increased hydration with preconceptional collagen organization loss, and/or cervical elasticity [95].

Histopathology is the golden standard for tissue remodeling assessment. Electron/polarized light microscopy, two-photon microscopy, and fluorescence microscopy, with different dyes/antibodies for staining and with limited physicochemical changes on biological tissue, are in trend for slice sizes and collagen content accurate diagnosis. SHG scanning endomicroscopy was proposed to visualize gestational CR in many studies in Europe and the USA. There were recently three accurate collagen fiber extraction techniques (in 3D volume) for quantitative collagen content assessment [96]. SHG permits cervical collagen content longitudinal assessments along pregnancy [12 (first trimester), 23 (second trimester), and 32 (third trimester) gestational weeks], with images archive, and comparison at each visit [97].

Literature mentions numerous new approaches, depending on sites/centers resources and medical staff training, to visualize cervix microstructure: near-infrared spectroscopy and near-infrared Raman microspectroscopy (optical techniques), light-induced fluorescence by callascope—specifically designed to assess cervical ripening, and functions by measuring the natural fluorescence of cervical non-soluble collagen, other methods combine ultrasound to new capabilities to visualize cervix deformability at different levels, by devices intravaginal/intracervical introduced, capturing differences between stiff and soft cervix, as aspiration and cervical consistency index, spectroscopic photo-acoustic imaging (first used in USA, for collagen/water ratio assessment in murine cervices [88], acoustic attenuation; all these methods are not in this subchapter focus.

4.2 Ultrasound-based techniques for cervical microstructure evaluation

Different from conventional ultrasound, various imaging modalities are actually prone to visualize changes in reproductive organs as collagen fibers content and characteristics (density, size, morphology, and orientation/alignment) along pregnancy, and associated to computational tools may quantify, and predict softness abnormal progress, and shift to ripening, before cervical shortness and funneling, in asymptomatic cases, for early sPTB prevention/delay.

4.2.1 B-mode ultrasound elastography: strain/static elastography: shear wave speed estimation elastography/dynamic elastography

Elastography association with conventional/standard ultrasound aimed to bring new data on cervical stiffness, and sPTB, being two types of elastography used in clinical practice: strain elastography-SE (static elastography) and shear wave speed estimation elastography-shear wave speed (SWS) (dynamic elastography).

Strain elastography, a direct biomechanical testing, measures cervix deformation percentage, expressed as a strain value, computer analyzed on a color map (produced by a specialized software), when gentle transducer movements compress a region of interest (ROI: IO, EO, entire cervix [98, 99]) during ordinary B-mode ultrasound vaginal scanning (mid-sagital plan) for CL. Increased strain reflects increased deformation (softer tissue), decreasing strain reflects reduced deformation (stiffer tissue); dichotomous variables are usually constructed to describe whether each patient's measurement is in the bottom quartile (≤25th centile) for each cervical region [98]. The USA results sustained that strain measurements obtained in a cross-sectional view of IO were significantly associated with sPTD (<37 weeks) [98]. The strain values corresponding to 25th centile in IO were 0.19 for the endocervical canal and 0.14 for the entire cervix. Women with strain values ≤25th centile were approximately 80% less likely to subsequently deliver preterm than those whose values were > 25th centile (entire cervix: OR: 0.17; 95% CI: 0.03–0.9; endocervical canal OR: 0.2; 95% CI: 0.03–0.96). Patient's characteristics influence the results (parity, prior preterm deliveries, gestational age at examination, CL, mainly when it is <25 mm [100]).

Despite new real-time quantitative data on cervix morphology and architecture, there are some limitations regarding distance more than 1.5 cm from the transducer, if tissue is inhomogeneous, and if ROI is placed at an angle above 20° from the compression direction; it does not allow quantification of tissue stiffness, when the intensity of force (stress) is unknown, but new devices have press indicator; interobserver variability.

Shear wave speed (SWS)/dynamic shear wave elasticity image (SWEI), can differentiate between soft and firm cervices by measuring ultrasound speed through a tissue: SWS is faster through firm, and slower through soft tissues [101, 102] (**Figure 4**). SWEI assesses women's quantitative stiffness from early (5–14 weeks) to late (40 weeks) gestation, with assessment at EO [104], mid *cervix* (at bladder reflection level), at IO proximity [105], being recognized the depth importance on SWEI estimations in IO, and EO along pregnancy [106]. It is a constant fractional reduction in SWS (average 4%/week), considered the most critical value for birth timing prediction, increasing with gestational age (GA), and a spatial gradient along CL: the most important softness is at the proximal end—9%/week, and 2%/week at distal end [107]. There are differences in SWS reduction reports from first to third trimester: 52% [107], 42% [108], or only a 12% decrease [104], being demonstrated that SWS declines progressively with GA between 18 and 24 weeks, being higher at IO than at EO, at each GA, IO is the best area to assess [103], because collagen fibers longitudinal alignment along endocervical canal, and at it is proximal part, not circumferential as they are near cervix edge, as microscopy revealed on specimens collected at hysterectomy, and reduced by 1.4 at every 10 years of maternal age, not by BMI [109].

Women with a soft cervix were 3.3 times more likely to have a short cervix, and 1.5 times more likely to have had a prior sPTB than cases who did not have a soft cervix compared to a cervix that was neither short nor soft. Softness increases by 1.5 times the risk of repeating sPTB. Prevalence of sPTB is higher by 18 fold <37 weeks, and by 120 fold <34 weeks, when a soft cervix (C) (<25th percentile for GA defined by SWEI at IO) is associated with short C (cut-off <25 mm) compared to normal CL, at 18–24 weeks [103]. Study conclusion was that softness is an independent risk factor for sPTB <37 and < 34 weeks, regardless of shortness presence, or sPTB history. **Table 1** shows the superior specificity and positive likelihood ratio (LR) values for sPTB (<34 and <37 weeks), when softness and shortness are combined.

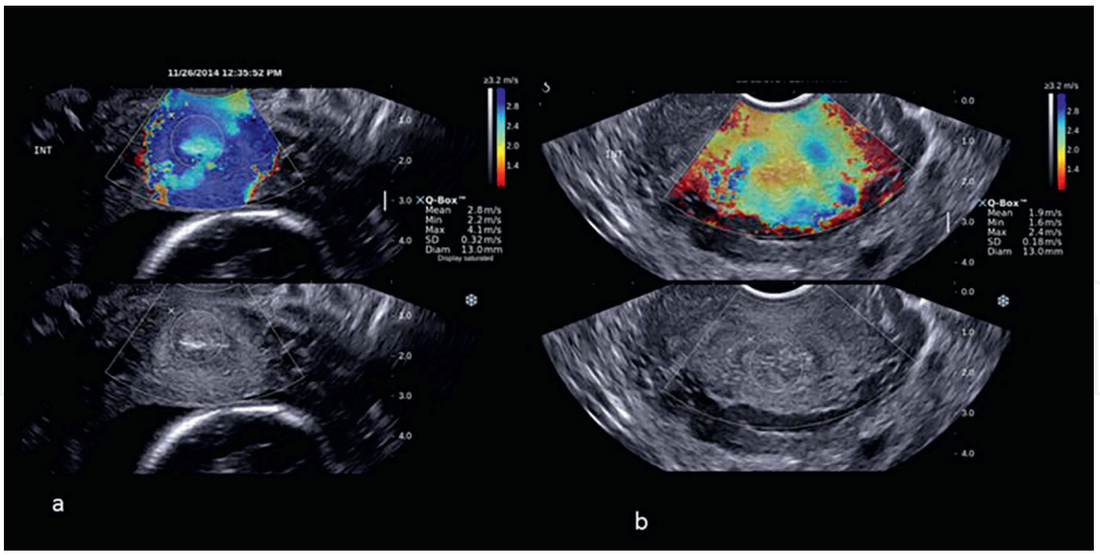

Figure 4.
Shear wave elastography in the cross-sectional planes of the external cervical OS of two patients at 21 weeks of gestation; (a) a non-soft cervix (shear wave speed (SWS) = 2.3 m/s [SWS 25th percentile = 1.72 m/s]); (b) a soft cervix with a SWS of 1.6 m/s (≤25th percentile). High SWS is shown in blue and low SWS in red. Adapted from Hernandez-Andrade et al. [103]. Free PMC article, HHS Public Access.

Sensitivity (%)	Specificity (%)	Positive LR	Negative LR
Softness shortness combination vs. short *cervix* for PTB risks <34 weeks			
33.3/33.3	98.2/96.1	18.5/12.7	0.7/0.7
Softness shortness combination vs. short *cervix* for PTB risk <37 weeks			
19.4/19.4	98.4/96.3	8.5/5.2	0.8/0.8

Table 1.
Sensitivity, specificity, LR for PTB when softness, shortness combination vs. short cervix. Results from Hernandez-Andrade et al. [103].

4.3 Anatomical computer simulation models to study human cervix uteri remodeling

The unanswered questions on premature CR, and sPTB prediction wait for answers from anatomically correct computer simulation models of pregnant human pelvis, uterus, and cervix geometry. The stress/stretch amount on the IO area depends on cervix angle/position in the pelvis [10] and on amniotic membrane strength, assessed by 3D MRI [24]. Vink and Feltovich [4] elaborated on the hypothesis that combining weak membranes to an undesirable cervical position/angle that increases the stretch on IO ultimately leads to cervical dilation and sPTB. The engineering tools will help to evaluate/predict sPTB using computational biomechanics and finite element analysis to study sPTB causes and to develop a diagnosis tool for outcome prediction [110]. One may individualize the computer models (which incorporate patient-specific tissue properties and ultrasound-derived parameters) to predict and/or identify which women with premature CR will deliver preterm.

5. Potential opportunities for preterm birth prevention/delay in preterm cervical changes

Vaginal progesterone, cervical stitch-cerclage, and pessary are actual opportunities to prevent/delay sPTB, when history, and/or current monitoring indicate gestational cervical untimed progressive changes, discussing them separately or comparing the beneficial/harmful effects of two of them cerclage *vs* P_4/pessary, and less all three therapies.

5.1 Progesterone for cervical Remodeling timing and preterm birth prevention

Progesterone works for "quiescence" or "silencing" uterine contractions [111], and exerts a dominant role for most pregnancies to initiate a loss of tissue strength yet maintain competence in softening, and delays ripening onset, maintaining stiffness [103], and CL > 25 mm, as was proved by RCT [112], a meta-analysis [113], and meta-analysis on individual data [114]. A retrospective study on P_4 administration when short cervix <25 mm at 24^{+0} and 33^{+6} weeks gestation revealed P_4 efficacy in pregnancy prolongation after 34 weeks (**Figure 5**) [115].

Natural micronized progesterone vaginal administration (200 mg/capsule, or 90 mg gel), from early pregnancy up to 36^{+6} weeks, induced an sPTB<33 weeks reduction of 38% in singleton and 31% in twins, and for sPTB recurrence in cases with sPTB history [11, 116], and sPTB prophylaxis in cases with short cervix (OPPTIMUM study) [117]. Actual data in twins pregnancy prolongation >34 weeks are contradictory on P_4 beneficial effects [118]. There is only a 0.02% reduction of sPTB absolute risk in singleton, when short cervix [119]. It is important to add that only vaginal P_4 has anti-inflammatory effects on the murine maternal-fetal

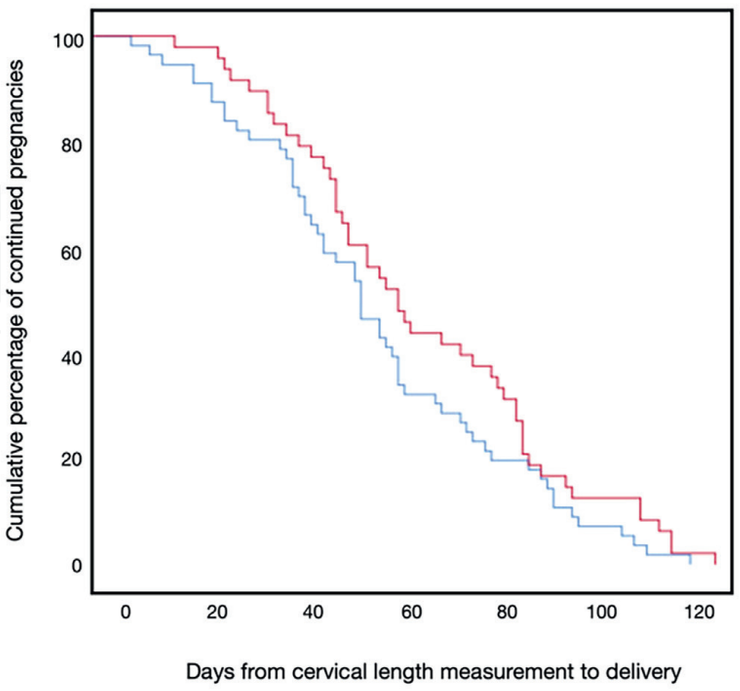

Figure 5.
Kaplan-Meier plot for days to delivery. The Kaplan-Meier plot of delivery by days from cervical length measurement of the progesterone treatment group (red line) and the non-treatment group (blue line). Adapted from Luxenbourg et al. [115]. Free PMC article. HHS Public access.

interface [120, 121], which are absent when comparing weekly i.m. 17α-hydroxyprogesterone caproate to vaginal P_4 for short cervix [122], and has more side effects, FDA does not recommend it since 2022.

Cervical static/strain elastography (with E-cervix™ program) for entire cervix – IO, EO, ECI was proposed to continue cervical strain assessments, together with conventional ultrasound, 1 week after P_4 treatment for CL ≤25 mm at 18–24/16–32 weeks, for PTB <32 weeks better prediction [103, 123]. The South Korea study showed that one could differentiate cerclage needs to avoid delivery <32 weeks by pretreatment elastography IO, and 1 week post-P_4 treatment EO assessment, showing significantly higher area under curve (AUC) than CL alone (0.858; P = 0.041), with sPTB delay/avoidance. P_4 direct contact with cervical matrix structures explains the better effects on EO at 1 week post-P_4 treatment, and women with no improvement in IO and EO after 1 week, possibly undergo progressive cervical softening.

5.2 Cervical cerclage, cervical pessary for preterm birth prevention

The mechanical support for an incompetent cervix has a long history, Cochrane Database of Systematic Reviews [124] presents three decisions for cervical cerclage, based on late miscarriages/sPTB history, short cervix ≤25 mm or open/dilated, with bulging membranes risking PROM, at ordinary ultrasound. Cerclage reduces sPTB risk by about 30% in women with a history of sPTB and short cervix, although at least one in three of these women still delivers preterm, as meta-analysis on individual patient-level data shows [125]. Cochrane Database of Systematic Reviews [126] showed that cerclage vs. no cerclage reduced the number of birth <37, <34 weeks (average RR: 0.77, 95% CI: 0.66–0.89) and <28 completed weeks gestation; and probably perinatal death risks.

Stiffness assessment favors a test for cerclage decision by "missing effect of vaginal P_4 on cervical stroma remodeling", and continuous cervical softness and shortness (even when CL < 10 mm) with sPTB high risks. A USA multicenter study revealed a gestational age at delivery significantly increased after 34 weeks, when CL < 10 mm while on P_4 and cerclage vs. without cerclage, but on vaginal P_4 (34^{+3days} weeks vs. 27^{+2days} weeks; P < 0.001), and lower rate sPTB at <37, 35, 32, 28, and 24 weeks in cerclage group (44.1 vs. 84.2%; 38.2 vs. 81.6%; 23.5 vs. 78.9%; 14.7 vs. 63.2%, and 11.8 vs. 39.5%; better neonatal outcomes parallel recorded) [127]. Cerclage decreases local levels of proinflammatory cytokines, adjuvant to P_4, and offers a biochemical barrier to membranes, effects added to mechanical support [128]. Post-cerclage CL assessment is very important, according to PTB high risk after elective cerclage vs. general population [129], and a CL ≤ 25 mm after cerclage can make the prognosis differences for the delivery before or after 32 weeks: 91.0% sensitivity, and 30.0% specificity [130], shortening progresses on softness, imposing P_4 continuation postcerclage, and a second/"reinforcing" cerclage to delay delivery [131], being mentioned also when amniotic membrane prolapse to or past the level of initial cerclage placement (visualized by direct speculum/ultrasound) [132]. Second/reinforcement stitch placement is an attempt to prolong pregnancy, imposing much caution, for maternal safety, and to avoid fetal inflammatory syndrome risks [133]. FIGO recommends stitch removal at 36–37 weeks [134] when everything is under medical control.

Vaginal microecology and immunity have great importance for cerclage outcomes [135], the epithelium protective effects on cervical stroma premature progression to ripening is not sufficient when abnormal vaginal microbiota, being recommended

vaginal probiotics/eubiotics, according to *Lactobacillus acidophilus* known protective effects [136], topic therapy against bacterial vaginosis.

Cervical pessaries have a long history since 1961 [137], being reloaded according to new materials and technologies. A meta-analysis for PTB prevention <34 weeks vs. controls revealed that pessary shows a lack in prevention efficacy [138], without the possibility of determining inferiority, when compared to other methods because of studies heterogeneity. When short cervix (<25 mm), P_4 works as cerclage [124], confirmed by meta-analyses on singleton [139], and twins, with sPTB history [140], and better than pessary [141], that sustains utero-cervical angle [20]. P_4 was added to pessary when a shorter cervix (below 10th percentile) than in a previous sPTB (below 3rd percentile) was not more efficient, did not reduce birth rate <28, <32, <34, or <37 weeks gestation compared with pessary alone, in a German cohort study [142]. The Canadian Soc. Obstet. Gynecol. (2020) Guideline No. 398 [143] specifies that in women with a short cervix (<25 mm), when P_4 is used for PTB prevention, additional therapies as cervical cerclage (with the exception of a rescue cerclage for an examination-based diagnosis) or a pessary are not recommended (strong/moderate). The choice of treatment in Canada depends on adverse events, interventions cost-effectiveness, and patient/physician's preferences.

RCT systematic review from Cochrane Pregnancy and Childbirth's Trials Register, Clinical Trials.gov and WHO International Clinical Trials Registry Platform to 22 September 2021, evaluated pessaries benefits and harms in preventing sPTB (<34, 37 weeks gestation) compared to no treatment, vaginal P_4, cervical cerclage or bedrest [144] revealed that pessary may reduce sPTB risks vs. no treatment [<34 weeks (RR: 0.72, 95% CI: 0.33–1.55); <37 weeks (RR: 0.68, 95% CI: 0.44–1.05), with low-certainty evidence for studies]; or vs. P_4 [<34 weeks (RR: 0.72, 95% CI 0.52–1.02); <37 weeks (RR: 0.89, 95% CI 0.73–1.09), with moderate certainty evidence for studies]; has little or no effect on maternal infection/inflammation risks (RR: 1.04, 95% CI 0.87–1.26); the comparison to cerclage was unclear, from o single study.

5.3 Progesterone, cerclage, pessary for reducing preterm birth risk

Perinatal outcomes of all three therapeutic opportunities are compared by a multination study [145], on three patient cohorts treated with cerclage (142, USA), vaginal P_4 (59, UK), and pessary (42, Spain). The results showed no statistic significant difference in perinatal loss, neonatal morbidity, and sPTB among the three groups, apart from a higher rate of sPTB < 34 weeks, after vaginal P_4 treatment in comparison to pessary (32% vs. 12%; RR: 2.70; 95% CI: 1.10–6.67). When one compared only subgroups with CL < 25 mm, irrespective GA, the difference between these two cohorts was not statistically significant (RR: 2.21; 95% CI: 0.83–5.89).

In sPTB < 34 weeks, the three procedures are recommended mainly when cervical softness is not accurately known, when vaginal P_4 fails, when CL after cerclage is below 15/10 mm, and one tries pregnancy prolongation by pessary adding, fetal high prematurity avoidance, without maternal and/or fetal risks. A Romanian retrospective study [65] compared PTB reduction, neonatal and maternal outcomes when short cervix (11–25 mm, and <10 mm at 14–23^{+6} weeks), in cases treated with vaginal P_4 and cerclage (8 patients, no CL < 10 mm), vaginal P_4 and pessary (62 cases, 10 with CL < 10 mm), and vaginal P_4, cerclage, and pessary (13 cases, 3 with CL < 10 mm). P_4 was administered from 6 weeks gestation, 200 mg/day, up to 36^{+6} weeks, bedtime, with supplementation at 16 weeks, or when was considered necessary, pessary was

added when postcerclage CL was ≤25 mm. It was recorded pregnancy prolongation >34 weeks ($P < 0.0001$) when early administered vaginal P_4, and P_4 increased cerclage or/and of pessary benefits, better neonatal outcome (fetal weight, Apgar score ≥ 7, $P < 0.05$), reduced neonatal morbidity (only RDS, $P < 0.05$). All three methods were applied in sPTB before 34 weeks very high risks; average gestational age at delivery was 34.91 weeks vs. 37.37 weeks—P_4 and cerclage; 36.58 weeks: P_4 and pessary, when late administration of vaginal P_4, or when P_4 did not influence cervical remodeling. A retrospective cohort multicenter study [146] compared three therapeutic opportunities in four groups of pregnancies, at 15–29 weeks gestation, with CL ≤ 25 mm: A—P_4 vaginal, cerclage, pessary ([18], with highest risk for sPTB, and shortest cervix; median (range) 14.5 (0–25)); B—P_4 vaginal, and pessary ([141], CL: median (range) 15 (0–25)]); C—P_4 vaginal, and cerclage ([38], CL: median: 15.5 (0–25)); D—P_4 vaginal ([110], CL: median: 19 (2–25)). The rate of sPTB < 37 weeks was similar: 44.4% vs. 32.5% vs. 36.8% vs. 32.7% ($P = 0.665$). Their conclusion was that a combined rescue therapy involving vaginal P_4, cerclage, and pessary emerges as a promising management strategy when a short cervix and background history for sPTB high risk; pregnancy prolongation was safe.

5.4 New proposals for premature cervical remodeling prevention

There is still much work to understand how well therapy may better influence cervix consistency at cellular and microscopic level, what drug/material can restore stiffness, and sustain IO, ECM, and CSMCs/sphincter on increasing stress/stretch up to 37^{+1day} weeks. There were proposed/tested biomaterials, biodegradable and biocompatible for intracervical injection in rats—purified silk protein-polyethylene glycol, for intrinsic cervix reinforcement/augmentation with no other interference on structure, and to permit labor dilation, not extrinsic as pessary or cerclage [147], or to propose adult stem cells with their bioactive molecules to reinforce cervix, IO sphincter, as intraurethral/intrasphincterian use for postpartum stress urinary incontinence.

6. Conclusions for everyday practice

Gestational CR is difficult to accurately predict for individual cases. Usage of serial ultrasound (16–23^{+6} weeks) for CL, resistant CL when funneling, cervical mucus plug distance, and cervical sliding sign are beneficial, when new techniques for cervical stiffness assessment (such as ultrasound strain elastography, SWEI) are not available for sPTB risk accurate prediction, or for vaginal progesterone failure in short cervix syndrome. Softness may be an alert sign for fetus abnormalities, membranes, and amniotic fluid, not only per se. Vaginal microbiota evaluation and correction for Döderlein bacilli presence is very important, associated with vaginal progesterone, with positive effects on vaginal and cervical inflammations reduction. Actually, vaginal micronized progesterone is a strong recommendation for short cervix <25 mm, in asymptomatic cases, for history of recurrent late pregnancy loss, sPTB <34, and < 37 weeks, with singleton/twins, being proved no adverse effect on offspring, cerclage (one/two stitches) and pessary are second or third line option, with CL, and stiffness monitoring after procedure, without stopping progesterone up to 36^{+6} weeks, to avoid prematurity, if mother's health permits; all three therapies may be used when high risks for sPTB, with advanced cervical changes, even when CL < 10 mm.

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