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Contractile function of the cervix plays a role in normal and pathological pregnancy and parturition

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Abstract

The cervix plays an integral part in ensuring the proper timing of pregnancy and parturition. It maintains the fetus within the uterus and protects it from pathogens present in the vaginal canal. The cervix undergoes extensive remodeling during pregnancy and parturition. This process is associated with collagen degradation, an increase in immune cell response and inflammation in the cervix. However, our understanding of the role of cervical smooth muscles and their contribution to cervical remodeling is still lacking. In this paper, we propose that the active contractile function of the cervix influences cervical remodeling during pregnancy and parturition. Contraction of the cervical smooth muscles helps the cervix to remain firm and closed during early pregnancy, while relaxation of the cervical smooth muscles help facilitate cervical dilatation during labor. This contractile function of the cervix can be influenced by endocrine signals, such as estrogen, progesterone, and oxytocin; local paracrine signals, such as inflammatory chemokines and cytokines, as well as extracellular vesicles, such as exosomes and ectosomes; and by pharmacological agents used for cervical ripening and the induction of labor. A deeper understanding of the role of smooth muscles in cervical remodeling can help us elucidate the cellular processes in the cervix during pregnancy and parturition. This can also help in finding critical signaling pathways and therapeutic targets in the cervix that may decrease the rates of premature cervical ripening and preterm birth.

Keywords

cervical ripening; cervical remodeling; labor; oxytocin; prostaglandins; smooth muscle

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Introduction

The cervix plays an important role during pregnancy by serving as a protective barrier to keep the growing fetus *in utero* until term delivery [1]. Maintenance of the protective function during pregnancy is achieved by the cervix undergoing extensive remodeling. The cervix stays firm and closed until the latter part of the third trimester of gestation. During this period, the cervix undergoes gradual softening, which involves remodeling of the extracellular matrix (ECM) in the cervical stroma [2]. The onset of labor is associated with collagenolytic degradation of the cervical ECM, and an increase in the influx and activation of immune cells and pro-inflammatory mediators. This inflammatory process eventually leads to cervical dilatation, which allows the safe passage of the fetus during delivery [3].

The cervix has been considered to just respond passively to uterine contractions at the onset of labor. However, a few studies have shown the possibility of active cervical contraction during pregnancy and labor. The cervix is composed of a cervical epithelial layer and a stromal layer [4]. The cervical stroma is composed mainly of collagen and ECM. However, smooth muscle fibers also account for 10 – 45% of the cervical stroma [5,6]. The presence of smooth muscle cells in the cervical stroma may functionally contribute to a contractile function in the cervix. During pregnancy, contractile function may be involved in cervical remodeling and may contribute to the development of cervical pathologies, such as cervical insufficiency, short cervix which increases the risk of spontaneous preterm birth, premature cervical ripening, and non-compliant cervix.

Coordinated cervical remodeling is important to ensure successful term delivery. This process involves the rearrangement and realignment of collagen fibrils, changes in the ECM, and an increased inflammatory cytokine production [7]. Naturally, cervical ripening happens spontaneously in preparation for parturition. However, there are pathological conditions that may affect the timing of cervical ripening. There are instances where the cervix ripens early in the pregnancy, and this usually leads to preterm birth [8]. There are also conditions that may compromise the health of the pregnant women or the fetus, which require hastening of the cervical ripening process; hence, the need for labor induction [9].

Around 25% of term pregnancies undergo labor induction [10]. The goal of induction of labor is to have a successful vaginal delivery [9]. However, some patients experience failed induction of labor, culminating in a Cesarean delivery [11]. Oxytocin is the most common labor induction agent used worldwide. It can be used alone, in combination with amniotomy, or after administering other cervical ripening agents [12]. Prostaglandins, such as dinoprostone and misoprostol, are the most common pharmacological cervical ripening agents. These agents cause prostaglandin-mediated cervical remodeling and the generation of uterine contractions [13]. Since the cervix also contains smooth muscles, these labor induction agents and other physiological stimuli may also affect smooth muscle contraction in the cervix. This may have implications in the cervical ripening and dilatation phases during pregnancy and may deepen our understanding of the underlying process of cervical remodeling during pregnancy.

The hypothesis

We hypothesize that the active contractile function of the cervix influences the cervical remodeling process during pregnancy. From the start of pregnancy until the latter part of the third trimester, cervical contractile function helps to maintain a firm cervix, which remains closed. During the ripening and dilatation phase, the cervical smooth muscle cells are not just a passive responder to uterine contractions but independently initiate their own contractions.

Contraction and relaxation of the cervical smooth muscle may be involved in cervical dilatation during labor and delivery. This contractile function of the cervix may be influenced by endocrine signals, such as estrogen, progesterone, and oxytocin; local paracrine signals, such as inflammatory chemokines and cytokines, as well as extracellular vesicles, such as exosomes and ectosomes; and by pharmacological agents used for cervical ripening and the induction of labor.

Evaluation of the hypothesis

Cervical smooth muscle cells promote cervical contraction

There are several existing studies showing the possible contractile function of the cervix [14–16]. A previous study showed that cervices from mice in late pregnancy or in non-pregnant mice exhibited spontaneous contraction [17]. Cervical tissues collected from non-pregnant women showed that smooth muscle cells are present in the internal and external os of the cervix. They are arranged circumferentially in the stromal layer, which resembles a sphincter-like pattern. Oxytocin was shown to promote contraction in the cervix, while nifedipine inhibited cervical smooth muscle contraction [5]. Another study also stated the presence of longitudinal and circularly-arranged muscle fibers in the cervix. The longitudinal muscle fibers are mostly located in the inner part of the cervix, while the circular muscle fibers are mostly in the outer part. The longitudinal muscles can contract and help in cervical dilatation, while the contraction of the circumferential muscle fibers impedes cervical dilatation and keeps the cervix closed [5,15,18].

Cervical contractions may also serve as a sphincter, which may regulate the closing and opening of the cervical canal during pregnancy [6]. The cervix from term pregnancy contains both endothelial and inducible nitric oxide synthase. This nitric oxide system is responsive to nitric oxide, which can cause relaxation of the cervical muscle during labor and delivery [19]. Electromyography (EMG) studies reported that contractions in the cervix were independent of the myometrial contractions [14,16]. This independent activity of the smooth muscle of the cervix suggests an active role in labor rather than a passive response to myometrial contractions [14]. This is supported by clinical studies, which showed that pregnant women with cervical contractions experienced a lesser degree of effacement and tended to have an undilated cervix during parturition [20]. Cervical contractions may also explain the problems with cervical compliance during pregnancy. Compliance is a biomechanical term that reflects how easily a tissue can distend and increase in volume with increasing pressure [1]. In the cervix, compliance is associated with disarrayed collagen fibers, elastic extension, increased water content, and increased proteoglycans [21,22].

Cervical contractions may alter tissue compliance, which can result in poor rates of dilatation and can generate high intrauterine pressure that can also eventually contribute to fetal compromise requiring Caesarean section [20].

Cervical ripening is a slow physiological process occurring throughout pregnancy and is completed with the onset of labor [8]. Cervical ripeness is usually determined by Bishop's score, which classifies the cervix as ripe or unripe based on the cervical dilation, effacement of the cervix (length), station of the fetus, consistency of the cervix, and position of the cervix [23]. A ripe or favorable cervix is considered if the Bishop's score is 9 or more, and this indicates that labor will most likely commence spontaneously [13]. Cervical ripeness is also associated with the contractile function of the cervix. Cervical EMG studies showed that the unripe cervix exhibited a higher electrical activity compared to the fundus of the uterus. However, this is reversed in the ripe cervix, especially at the onset of labor, which is characterized by regular strong uterine contractions [6]. Ripe and unripe cervices also have differential responses to oxytocin during pregnancy. A ripe cervix is stimulated to contract immediately when exposed to oxytocin. However, it takes a longer time for the unripe cervix to contract upon treatment with oxytocin [15]. It is also possible that oxytocin differentially stimulates the fibers running longitudinally and circumferentially in the cervix during parturition. It can potentially stimulate the fibers running longitudinally and relax the fibers running circumferentially, in order to promote cervical dilatation.

Steroid hormones, such as estradiol and progesterone, may also influence the contractile function of the cervical smooth muscles. Animal studies showed that estradiol can activate oxytocin receptor (OTR) gene expression and active OTR synthesis in rat cervical smooth muscle cells, while progesterone antagonized the estradiol's effect on OTR expression [24]. In myometrial studies, progesterone inhibited the binding of oxytocin to its receptors whereas estradiol increased the affinity of oxytocin to its receptors [25,26]. In the gastric smooth muscle cells, progesterone promotes relaxation through the nitric oxide (NO)/cyclic guanosine monophosphate (cGMP) pathway. Progesterone may also exert its relaxation effect on the cervical smooth muscles via this pathway, since the cervix also has an endogenous nitric oxide system and is responsive to nitric oxide [19]. This evidence shows that hormones that are elevated during pregnancy may also affect the contractile function of the cervix through regulation of receptors that are involved in contraction [27]. This may have implications on the role of the cervical smooth muscles in cervical ripening and dilatation.

Prostaglandins (PGE2, PGI2, and 6-keto-PGF1) can regulate the contractile ability of the cervix. However, the exact mechanism and function of prostaglandins on cervical contractions are still unknown. Studies on cows showed that PGF2α can affect the EMG activity of the two smooth muscle-containing layers of the cervix [28]. PGF2α and PGE2 inhibited the smooth muscle contraction of the cervices of women undergoing elective caesarean section in the 38 – 40th week of pregnancy [29,30]. This inhibitory effect on cervical contractility was not observed in non-pregnant and early pregnant patients. The inhibitory function starts to exert its effect on the cervix during late pregnancy, in preparation for labor [29,30]. Four types of PGE2 receptors, E prostanoid (EP) 1 – 4, mediate the effects of PGE2 on the contractility of the cervix [31]. EP1 and EP3 are

associated with calcium influx and the inhibition of adenylate cyclase, which can lead to smooth muscle contraction, while EP2 and EP4 can stimulate adenylate cyclase and more likely lead to smooth muscle relaxation [32–34].

These receptors may also be responsible for the mechanism of action of prostaglandins in the cervix. A previous study demonstrated a lower expression of the relaxatory EP4 and an increased expression of the contractile EP3 in the cervix of pregnant women who did not respond to prostaglandins for cervical ripening [35]. An unfavorable balance in the contractile and relaxatory prostaglandin receptors may explain why some women cannot be induced, even with treatment with cervical ripening agents. This temporal effect of naturally occurring prostaglandins to cervical smooth muscle contractility may further support the role of smooth muscles in cervical ripening and dilatation. This tells us that prostaglandins may not just promote collagen degradation but may also influence the cervical smooth muscles to facilitate cervical dilatation. Future research on prostaglandins that specifically act on the relaxatory prostaglandin receptors as cervical ripening agents may be an effective therapeutic option.

Infection and inflammation may also affect the cervical smooth muscle cells and impair the contractile function of the cervix. Animal studies showed that infection with *Chlamydia muridarum* decreased the contractile force of spontaneous contractions of the cervix but not in the uterus [36]. Inflammatory mediators may bind to receptors on smooth muscle cells and can change the activity or number of ion channels, the intracellular signaling molecules. or affect the expression of genes involved in smooth muscle function [37]. Inflammation also causes the inhibition of contractions in other types of smooth muscle cells [38]. Inflammation can cause smooth muscle remodeling, which can lead to hyperplasia, hypertrophy, and fibrosis. Inflammation also increases the secretion of signals that may affect smooth muscle contraction, such as oxytocin, which may cause uterine and cervical smooth muscle contraction [39]. The smooth muscle remodeling caused by inflammation has not yet been studied in the smooth muscles of the cervix. This warrants more studies to deepen our understanding of the pathological effects of infection and inflammation on cervical smooth muscle function.

Exosomes, which are 30 – 160 nm extracellular vesicles released from cells, may also affect the contractile function of the cervix [40]. Previous studies in our laboratory have shown that exosomes serve as paracrine signalers that can initiate labor and delivery [41–44]. In mouse models, maternal plasma exosomes collected on day 18 of gestation contained high levels of inflammatory cargo and injection of these exosomes on gestational day 15 induced preterm birth. A greater inflammatory response was also seen in the cervix compared to the uterus when treated with day 18 exosomes. The top biological functions associated with the cargo of these maternal plasma exosomes include chemotaxis, inflammatory response, leukocyte activation, and neutrophil infiltration [44]. These physiological changes are associated with cervical remodeling and initiation of labor.

Fetal cell-derived exosomes can also increase the inflammatory response in the maternal cells [41,43]. Amnion epithelial cell (AEC)-derived exosomes contain p38 mitogen-activated kinase, a marker of inflammation and a key mediator of senescence induction and sterile

inflammation [41]. Uterine and decidua cells treated with AEC exosomes exhibited higher secretion of different pro-inflammatory mediators, such as interleukin-6, interleukin-8, and PGE 2, and activation of NF-κβ [43]. These studies show that both maternal and fetal exosomes can serve as important contributors to the pathogenesis of human parturition. This includes the possibility of promoting inflammation, which can affect the contractile function of the cervix.

The contractile function of the cervical smooth muscle cells can also be affected by the rigidity of the ECM. The collagen cross-link maturity ratio, which measures the ratio of mature (deoxypyridinoline and pyridinoline) to immature (hydroxylysinonorleucine and dihydroxylysinonorleucine) collagen cross-links, is associated with cervical tissue stiffness. It was previously established that the collagen cross-link maturity ratios decrease as the cervix softens [45]. Recent evidence has shown that cervical tissue from patients with premature cervical failure have decreased collagen concentrations and collagen cross-link maturity ratios. Moreover, exposure to soft ECM decreased the contractility of cervical smooth muscle cells. This suggests that having a softer, remodeled cervical ECM may impair the contractile tone of the cervix and may eventually lead to cervical sphincter laxity and spontaneous preterm birth [46].

Cervical smooth muscle cells also promote inflammation and extracellular membrane remodeling

Aside from the contractile function, cervical smooth muscle cells can also influence pregnancy and parturition by promoting inflammation cascades that can hasten the cervical ripening process. The cervical smooth muscle layer experiences a cyclical stretch due to its contractile ability. The cyclical stretch in the cervical smooth muscle cells derived from pregnant women with a history of asymptomatic premature cervical remodeling (PCR) led to an increase in the secretion of pro-inflammatory cytokines (IL1RA, EGF and IL4) [47].

Cervical smooth muscle cells can also influence cervical remodeling by secreting enzymes that can degrade the extracellular membrane. Cervical smooth muscle cells from pregnant women with a history of PCR secreted higher levels of matrix metallopeptidases (MMP)-2 in response to stretching compared to the control [48]. Treatment of cervical smooth muscle cells with tumor necrosis factor-α (TNF-α) increased the mRNA expression of elastinolytic enzymes, cathepsin S, and the collagen degrading enzymes MMP-1, -3, and -9 [49]. PGE and TNF-α also increased the expression of tumor necrosis factor-stimulated gene-6, a hyaluronic acid (HA)-binding protein, in the cervical smooth muscle cells. HA-binding proteins are thought to mediate the function of HA in tissue hydration, the release of collagenase, and leukocyte migration. These activities are important in the cervical ripening process [50].

Testing the hypothesis

To verify this hypothesis, *in vitro* studies may shed light on the molecular mechanisms of cervical contraction and are thus needed. An *in vitro* collagen gel contraction assay can be used to evaluate the contractile function of human cervical stromal cells. The underlying principle of this assay is that cervical stromal cell attachment to type I collagen can produce

mechanical tension as a response to specific stimuli or conditions, and consequently leads to tissue contraction [51]. We can utilize this assay to study the effects of different labor inducing drugs and cervical ripening agents given to pregnant patients in cervical stromal cell contraction. This can also help us investigate the role of cellular transitions in physiological and pathological cervical contractions.

We can also test this hypothesis by doing *ex vivo* studies on cervical tissue explants from patients undergoing hysterectomy for benign gynecological conditions. While cervical tissues from pregnant patients are ideal, removal of the cervix or even cervical biopsy are rarely done for pregnant patients. While there are patients who undergo post-partum hysterectomy due to uncontrolled bleeding, the majority of cases involve supracervical hysterectomy, which spares the cervix. It is important to have a standardized method for collecting cervical tissues from patients. The majority of the smooth muscles are located in the internal os of the cervix; thus it is ideal to obtain samples from this area of the cervix [52]. This region contains almost 50 – 60% smooth muscles while the lower part of the cervix contains less smooth muscles and a higher connective tissue component [5]. Isolated tissue bath experiments can be conducted using the cervix tissues to determine their contractile function under normal conditions and in response to steroid hormones, inflammatory signals, and pharmacological agents, such as oxytocin and prostaglandins. Parallel experiments can also be performed using uterine myometrial tissue so that we can compare the contractile function of the cervix and uterine smooth muscles under normal conditions and in response to exogenous factors.

Animal studies using pregnant mice can be useful in understanding the contractile activity of the cervix and how they respond to different stimuli. Experimental treatments with steroid hormones, inflammatory signals, and pharmacological agents, such as oxytocin and prostaglandins, can be undertaken, and EMG studies can be performed during the parturition phase to monitor the contractile function of the cervix and the uterine muscles. Similarly, EMG studies of the cervix and uterus can also be carried out in pregnant humans throughout the entire course of labor. This can be synchronized with uterine pressure measurements to determine the individual contractile function of these muscle tissues and the extent of their response to the aforementioned stimuli.

It is also worth studying the effects of different pathological conditions, such as infection and inflammation, and oxidative stress on the contractile functions of the cervix. Infection and/or inflammation are associated with premature cervical ripening and preterm birth. They can compromise the cervical epithelial barrier and can cause collagen degradation and excessive inflammation in the cervical stroma. However, their exact impact in cervix centrality has not yet been studied. Oxidative stress has been shown to cause smooth muscle relaxation in different tissues. Endogenous hydrogen peroxide was shown to induce smooth muscle relaxation in the endothelium [53]. A high level of oxidative stress was shown to decrease the tone of smooth muscles in the anal sphincter [54]. However, the effects of oxidative stress on cervical smooth muscles are yet to be elucidated.

Consequences of the hypothesis and discussion

Current treatment options, designed to prevent preterm labor, have not reduced the incidence of preterm birth [55,56]. This may be due to the large gap in our understanding of the causes and mechanistic events leading to preterm birth. Data on molecular mechanisms of cervical remodeling during pregnancy and parturition are still lacking. The majority of studies in premature cervical ripening have focused on the roles of ECM remodeling, the influx of immune cells, and inflammation [2,57,58]. There is still a paucity of data on the contractile function of the cervix and how the remodeling of these smooth muscle cells contribute to the cervical remodeling process during pregnancy and parturition. To better understand pathological labor, we need to further understand the molecular mechanisms involved in the cervix during parturition. This entails understanding the importance of smooth muscles in the cervix and identifying their response to hormones, inflammatory mediators, and pharmacological agents. Understanding this new paradigm in cervix biology can help guide the clinical use of cervical ripening agents and may also help in finding targeted treatments that can be used to prevent premature cervical ripening and preterm birth.

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