OBSTETRICS

A new paradigm for the role of smooth muscle cells in the human cervix

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BACKGROUND: Premature cervical remodeling resulting in spontaneous preterm birth may begin with premature failure or relaxation at the internal os (termed "funneling"). To date, we do not understand why the internal os fails or why funneling occurs in some cases of premature cervical remodeling. Although the human cervix is thought to be mostly collagen with minimal cellular content, cervical smooth muscle cells are present in the cervix and can cause cervical tissue contractility.

OBJECTIVE: To understand why the internal os relaxes or why funneling occurs in some cases of premature cervical remodeling, we sought to evaluate cervical smooth muscle cell content and distribution throughout human cervix and correlate if cervical smooth muscle organization influences regional cervical tissue contractility.

STUDY DESIGN: Using institutional review board—approved protocols, nonpregnant women <50 years old undergoing hysterectomy for benign indications were consented. Cervical tissue from the internal and external os were immunostained for smooth muscle cell markers (α-smooth muscle actin, smooth muscle protein 22 calponin) and contraction-associated proteins (connexin 43, cyclooxygenase-2, oxytocin receptor). To evaluate cervical smooth muscle cell morphology throughout the entire cervix, whole cervical slices were obtained from the internal os, midcervix, and external os and immunostained with smooth muscle actin. To correlate tissue structure with function, whole slices from the internal and external os were stimulated to contract with 1 μmol/L of oxytocin in organ baths. In separate samples, we tested if the cervix responds to a common tocolytic, nifedipine. Cervical slices from the internal os were treated with oxytocin alone or oxytocin + increasing doses of nifedipine to generate a dose response and half maximal inhibitory concentration. Student *t* test was used where appropriate.

RESULTS: Cervical tissue was collected from 41 women. Immunohistochemistry showed cervical smooth muscle cells at the internal and external os expressed mature smooth muscle cell markers and contraction-associated proteins. The cervix exhibited a gradient of cervical smooth muscle cells. The area of the internal os contained 50-60% cervical smooth muscle cells that were circumferentially organized in the periphery of the stroma, which may resemble a sphincter-like pattern. The external os contained approximately 10% cervical smooth muscle cells that were randomly scattered in the tissue. In organ bath studies, oxytocin stimulated the internal os to contract with more than double the force of the external os (1341 ± 693 vs 523 ± 536 integrated grams $\times$ seconds, respectively, $P = .009$). Nifedipine significantly decreased cervical tissue muscle force compared to timed vehicle control (oxytocin alone) at doses of 10^{-5} mol/L (vehicle 47% $\pm$ 15% vs oxytocin + nifedipine 24% $\pm$ 16%, $P = .007$), 10^{-4} mol/L (vehicle 46% $\pm$ 16% vs oxytocin + nifedipine $-4\% \pm 20\%$, $P = .003$), and 10^{-3} mol/L (vehicle 42% $\pm$ 14% vs oxytocin + nifedipine $-15\% \pm 18\%$, $P = .0006$). The half maximal inhibitory concentration for nifedipine was 1.35×10^{-5} mol/L.

CONCLUSION: Our findings suggest a new paradigm for cervical tissue morphology—one that includes the possibility of a specialized sphincter at the internal os. This new paradigm introduces novel avenues to further investigate potential mechanisms of normal and premature cervical remodeling.

Key words: cervix, premature cervical remodeling, smooth muscle cells

Introduction

Spontaneous preterm birth (sPTB) is a significant obstetric dilemma affecting approximately 10% of US pregnancies.[1,2] Etiologies vary, but sPTB must eventually involve premature remodeling and dilation of the cervix to allow for delivery of the premature fetus.[3] Although the pathophysiology of premature cervical remodeling is not fully understood, sonographic findings and computational modeling suggest that in some cases, the process starts with dilation of the internal os (the top aspect of the cervix where the uterine arteries insert into the uterus), which is clinically termed "funneling"[4,5] (Figure 1). Clinicians also describe a "dynamic cervix" where the cervix appears shortened in the absence of uterine contractions, which can be seen if transvaginal ultrasound is performed for several minutes. A dynamic cervix has also been described as cervical shortening in response to fundal pressure. Despite these clinical findings, we still cannot explain why the internal os weakens first in some cases of premature cervical remodeling. Our overall goal is to study whether premature cervical failure at the level of the internal os is due to regional differences in cervical tissue morphology and function.

Since the 1940s, the cervix has been characterized as a mostly collagenous structure (90% collagen/extracellular matrix [ECM]) with minimal cellular content (10% smooth muscle cells [SMC]).[6-8] However, these early studies suffered from technical limitations of the time and used immunohistochemical methods (Masson trichrome staining and subjective evaluation of SMC morphology)[6-8] that do not specifically identify SMC. Decades later, investigators questioned why SMC exist

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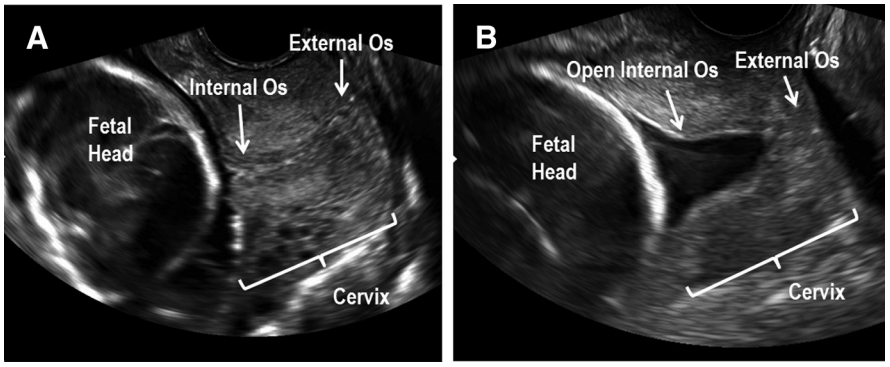
FIGURE 1
Transvaginal sonogram images



Transvaginal sonogram images of **A**, normal pregnant cervix and **B**, prematurely remodeled short cervix with funneling at internal os.

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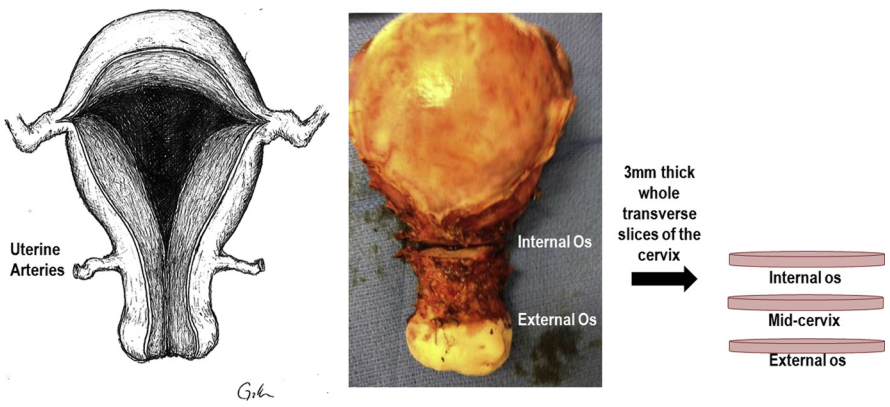
in the cervix. Interestingly, Bryman et al[9-11] found that adrenoreceptor agonists and oxytocin stimulate human tissue from the external os (lowermost aspect of the cervix closest to the vagina) to contract, suggesting SMC play a role in cervical tissue contractility.

Since the turn of this century, however, cervical SMC (CSMC) have been largely ignored since CSMC content was thought to be minimal. Instead, researchers focused on identifying alterations in the human cervical collagen network to explain premature cervical failure.[12-17] Since clinical observation demonstrates the area of the internal os can funnel first and/or is dynamic in some cases of premature cervical remodeling, we believe that the working paradigm of cervical tissue architecture needs to be reevaluated. Here, we use improved immunohistochemical techniques and functional studies to determine if regional differences in CSMC content and distribution influence cervical tissue function. The knowledge from this study will expand our understanding of cervical tissue characteristics that may contribute to normal and abnormal cervical function in pregnancy.

FIGURE 2
Tissue collection protocol



Tissue collection protocol: 3-mm thick whole transverse slices of cervix were obtained at level of internal and external os. Internal os was defined as top of cervix where uterine arteries meet uterus/cervix. Midcervix was defined as midway between internal and external os (which was defined as outermost portion of cervix).

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Materials and Methods

Tissue collection

This study was approved by the institutional review board at Columbia University Medical Center and Intermountain Healthcare. Nonpregnant, premenopausal women (<50 years old) undergoing a total hysterectomy for benign indications were consented. Women with an abnormal pap smear or prior cervical surgery were excluded. Demographic data (age, parity, obstetric history, menstrual phase, body mass index, and race) were collected.

Evaluation of CSMC at the internal and external os

Immediately following hysterectomy, whole transverse slices (3-mm thick) of the cervix were obtained at the internal os and the external os (Figure 2). The slices were fixed in 10% formalin for 24 hours, transferred to 70% ethanol, and paraffin embedded. After normal pathology was confirmed, 5-μm sections were stained with Movat pentachrome to appreciate general tissue structure (collagen, mucin, muscle distribution).[18,19] To visualize the entire cervical slice, ×10 images from the Movat Pentachrome–stained sections were collected and digitally reconstructed with ZEN software (Zeiss, Thornwood, NY).

Since previous studies that established cervical tissue morphology were technically limited to immunohistochemical methods that could not identify mature, contractile SMC,[6-8] we initially sought to determine if CSMC at the internal and external os expressed both mature SMC markers and contraction-associated proteins (CAPs). Paraffin-embedded human myometrial tissue obtained from a hysterectomy specimen was used as a positive control. The 5-μm sections of myometrial and cervical tissue (from the internal and external os) were incubated at 40°C overnight, paraffin melted at 60°C for 20 minutes, and then rehydrated using descending ethanol dilutions. Heat-mediated antigen retrieval was performed for 20 minutes using Dako target retrieval solution

(Dako, Carpinteria, CA) and colorimetric or immunofluorescent immunohistochemistry (IHC) was performed.

Colorimetric IHC

Tissue sections were incubated in 0.3% hydrogen peroxide for 20 minutes at room temperature to block endogenous peroxidase. Endogenous avidin and biotin were blocked using an avidin/biotin blocking kit (Vector Laboratories, Burlingame, CA). Slides were incubated in blocking solution (phosphate-buffered saline containing 3% bovine serum albumin and 2% goat serum; both Sigma, St Louis, MO) for 1 hour at room temperature. Primary antibodies for commonly used mature SMC markers (α-smooth muscle actin [SMA], SM22, calponin)[20] and CAPs (oxytocin receptor, cyclooxygenase-2) were incubated overnight at 4°C. Primary antibody information is listed in Table 1. Incubation with biotinylated goat antirabbit secondary antibody (1:500; Vector Laboratories) was performed for 30 minutes at room temperature followed by incubation with avidin and horseradish-peroxidase conjugated biotin in phosphate-buffered saline (Vector Laboratories). Color reaction was performed using diaminobenzidine tetrahydrochloride and the peroxidase substrate (Vector Laboratories). Tissues were counterstained with hematoxylin (Fisher Scientific, Pittsburgh, PA). Imaging was performed on an AxioObserver Z.1 microscope (Zeiss) using an Axiocam ICc camera (Zeiss).

Immunofluorescent IHC

Immunofluorescent IHC was used to assess connexin 43 (gap junction protein) expression as our experience with the antibody for connexin 43 showed optimal staining with this technique. After heat-mediated antigen retrieval (Dako target retrieval solution, Dako), enzyme-mediated antigen retrieval (pepsin 1 mg/mL, [Sigma] in 0.01 N hydrogen chloride) was performed for 30 minutes at 37°C. Sections were incubated in blocking solution (detailed above) for 1 hour at room temperature. Primary connexin 43 antibody (detailed information listed in Table 1) was incubated

TABLE 1
Primary antibodies for immunohistochemistry

Antibody	Vendor/catalog no.	Dilution	Description
Smooth muscle cell markers			
α-Smooth muscle actin	Abcam/5694	1:100	Rabbit polyclonal
SM22	Abcam/14106	1:200	Rabbit polyclonal
Calponin	Thermo Scientific/EP798Y	1:200	Rabbit monoclonal
Contraction-associated proteins			
Oxytocin receptor	Sigma/04389	1:25	Rabbit polyclonal
Cyclooxygenase-2	Abcam/52237	20 μg/mL	Rabbit polyclonal
Connexin 43	Abcam/11370	1:100	Rabbit polyclonal

Abcam, Cambridge, MA; Sigma, St. Louis, MO; Thermo Scientific, Waltham, MA.
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TABLE 2
Patient demographics for cervical tissue

Patient no.	Age, y	Gravidity/parity	Obstetric history	Menstrual cycle phase	BMI, kg/m^2	Race
1	36	0		Proliferative	28	White
2	49	0		Proliferative	26	Hispanic
3	44	0		Proliferative	32	Hispanic
4	49	0		Proliferative	23	Hispanic
5	40	0		Proliferative	37	Black
6	46	3/2002	VD × 2	Proliferative	20	White
7	42	5/3023	VD × 3	Secretory	31	White
8	49	3/2012	VD × 2	Proliferative	25	Hispanic
9	43	5/4014	VD × 4	Proliferative	36	Hispanic
10	45	2/2002	VD × 2	Proliferative	40	Hispanic
11	49	1/0101	VD × 1[a]	Proliferative	28	White
12	42	2/1001	VD × 1	Secretory	25	White
13	46	5/4004	VD × 3, CD × 1	Secretory	26	Black
14	49	0		Proliferative	24	White
15	46	0		Secretory	31	Black
16	45	0		Proliferative	24	Hispanic
17	49	0		Secretory	27	Hispanic
18	45	3/3003	VD × 3	Secretory	29	White
19	43	1/1001	VD × 1	Secretory	28	Hispanic
20	49	1/1001	VD × 1	Proliferative	29	Hispanic
21	46	4/1031	VD × 1	Secretory	38	Black
22	46	3/3003	VD × 2, CD × 1	Proliferative	18	Asian
23	40	3/3003	VD × 3	Secretory	29	Hispanic
24	46	2/2002	VD × 2	Proliferative	31	Asian

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TABLE 2
Patient demographics for cervical tissue *(continued)*

Patient no.	Age, y	Gravidity/ parity	Obstetric history	Menstrual cycle phase	BMI, kg/m^2	Race
25	45	2/2002	CD × 2	Proliferative	31	Hispanic
26	36	4/4004	CD × 4	Secretory	24	Hispanic
27	44	3/2012	CD × 2	Secretory	28	White
28	46	3/1021	VD × 1	Secretory	29	Hispanic
29	47	3/2012	VD × 2	Secretory	21	White
30	43	5/4014	CD × 4	Proliferative	24	Hispanic
31	48	5/4014	CD × 4	Proliferative	23	White
32	49	4/4004	CD × 4	Secretory	26	Black
33	44	4/2022	VD × 2	Proliferative	30	White
34	46	3/3003	VD × 3	Proliferative	33	Black
35	42	1/1001	CD × 1	Secretory	27	White
36	47	2/2002	CD × 2	Proliferative	27	Black
37	48	2/1001	VD × 1	Secretory	24	White
38	48	3/3003	VD × 3	Proliferative	19	Black
39	45	4/4004	VD × 4	Proliferative	24	Black
40	44	2/2002	VD × 2	Proliferative	23	Hispanic
41	45	2/2002	VD × 1, CD × 1	Proliferative	24	Black

BMI, body mass index; *CD*, cesarean delivery; *VD*, vaginal delivery.

[a] 35-wk Induction of labor for preeclampsia.

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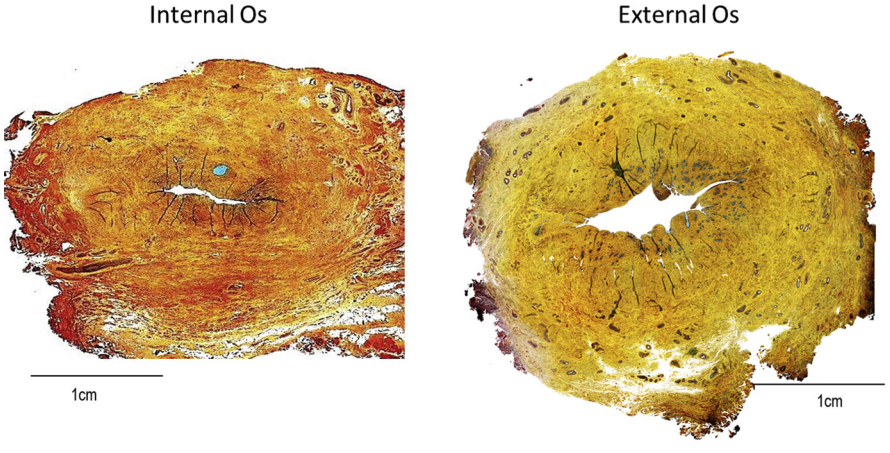
FIGURE 3
Comparison of internal and external os



Tiled images, 10×, of whole mounted transverse cervical tissue slices obtained from internal and external os and stained with Movat pentachrome. Internal os exhibits abundant red staining (muscle) while external os is mostly collagen (yellow).

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overnight at 4°C and then incubated with goat antirabbit Alexa Fluor 594 secondary antibody for 30 minutes at room temperature (1:200; Invitrogen, Carlsbad, CA). 4′,6-Diamidino-2-phenylindole dihydrochloride (Sigma) was used to stain nuclei. Images were obtained on Eclipse E 800 microscope (Nikon Inc, Melville, NY). For negative controls (colorimetric and immunofluorescent IHC studies) primary antibody was omitted and tissue was incubated in blocking solution overnight.

Evaluation of CSMC architecture in the entire cervix

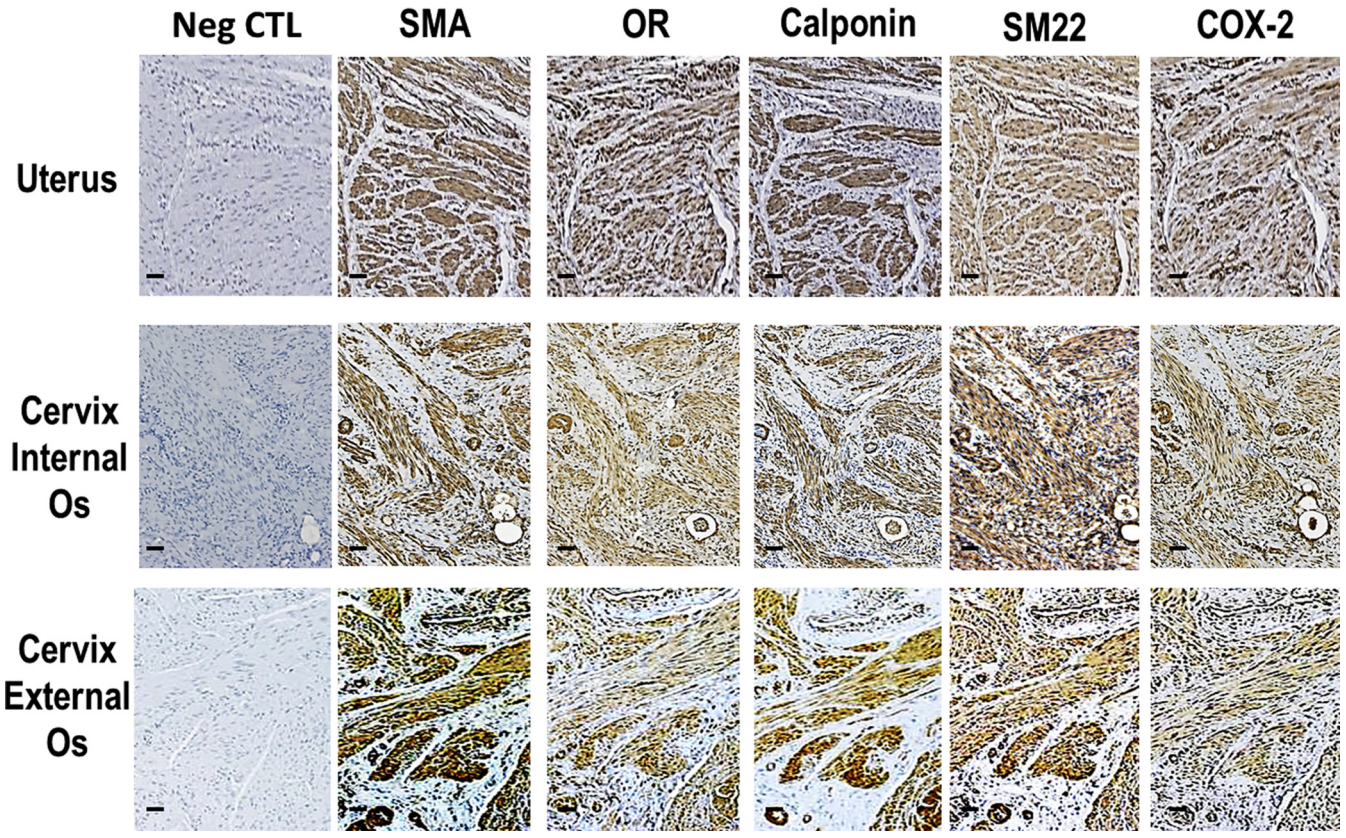
To investigate CSMC if morphology changed from the proximal to distal end of the cervix, 3-mm whole transverse slices were obtained from the internal os, midcervix (the midpoint between the internal and external os), and external os immediately after hysterectomy (Figure 2). Slices were fixed in 10% formalin for 24 hours, transferred to 70% ethanol, and paraffin embedded. After normal pathology was confirmed, 5-μm sections of each slice were immunostained with SMA (outlined above). To appreciate CSMC organization in each slice, ×10 images were obtained on an AxioObserver Z.1 microscope (Zeiss) and Axiocam ICc camera. Images were then digitally reconstructed with ZEN software (Zeiss). To approximate CSMC content at the internal os, midcervix, and external os, Photoshop CS5 (Adobe Systems Inc., San Jose, CA). 1 was used to count the number of SMA-positive pixels in the tiled images at the internal and external os. Specifically, the number of SMA-positive pixels was divided by the total number of pixels in the cervical slice to obtain the percent of positive SMA pixels in the entire cervical slice.

Organ bath studies

Evaluating regional cervical tissue contractility in response to oxytocin

To evaluate how CSMC content and distribution influences regional cervical tissue contractility, organ bath studies were performed using an additional set of cervical tissue slices collected immediately following hysterectomy. The

FIGURE 4
Smooth muscle cell markers at internal and external os



Immunohistochemistry of human myometrium (positive control) and human cervical tissue from internal and external os. This figure shows that similar to human uterine smooth muscle cells (SMC), cervical SMC at internal and external os that express α-smooth muscle actin (SMA) also express mature SMC markers (SM22, calponin) and contraction-associated proteins (oxytocin receptor and cyclooxygenase [COX]-2). Scale = 200 μm.

Ned CTL, negative control; *OR*, oxytocin receptor.

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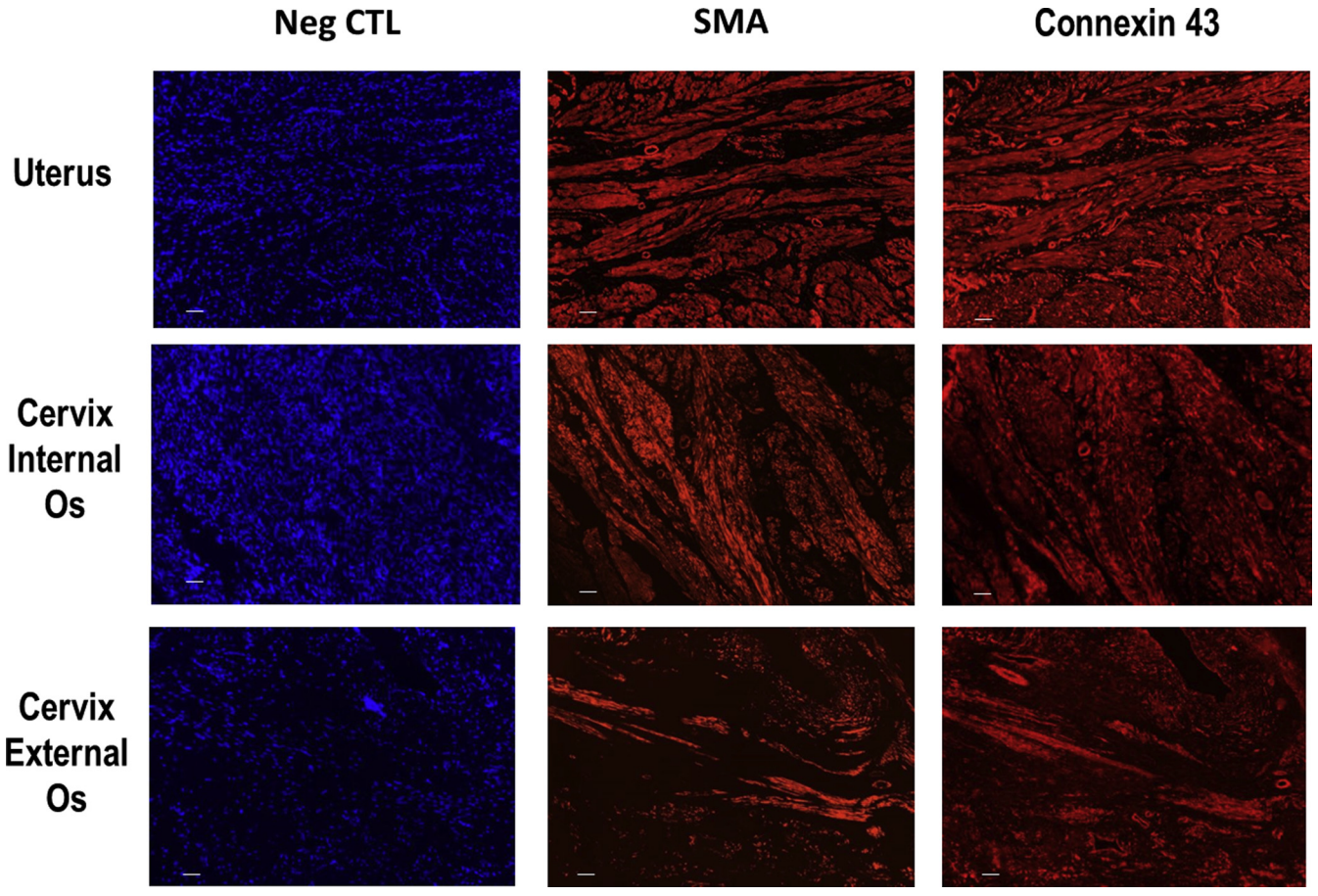
3-mm whole transverse cervical tissue slices (from the internal and external os) were immediately placed in ice-cold SmBMII media with the recommended additives (Lonza, Walkersville, MD). For each slice, one end of the tissue slice was fixed to the bottom of 16 mL water-jacketed (37°C) organ bath (Radnoti Glass Technology, Monrovia, CA) and the opposite end was attached to a Grass force transducer (Grass-Telefactor, West Warwick, RI) coupled to Biopac hardware (Biopac Systems Inc, Goleta, CA). Acknowledge 7.3.3 software (Biopac Systems Inc) was used for continuous digital recording of muscle force. Baths contained a modified Krebs–Henseleit buffer (115.0 mmol/L sodium chloride, 2.5 mmol/L potassium chloride, 1.9 mmol/L calcium chloride, 2.5 mmol/L magnesium sulfate, 25 mmol/L sodium bicarbonate, 1.4 mmol/L sodium dihydrogen phosphate, 5.6 mmol/L D-glucose) that was continuously bubbled with 95% oxygen/5% carbon dioxide. Tissues were equilibrated to 1.0 g of isometric tension for 1 hour (with buffer replacement every 20 minutes) and then stimulated to contract using 1 μmol/L oxytocin (Sigma).[9,11] Muscle force was analyzed for 20 minutes. Prizm GraphPad software (GraphPad Software Inc, La Jolla, CA) and Student *t* test were used to compare mean amplitude of force between the internal and external os slices.

Evaluating if nifedipine tocolyzes the internal os

In a subset of the women enrolled for the oxytocin studies detailed above, an additional 3-mm whole transverse cervical tissue slice was obtained from the internal os. After similar preparation and equilibration, 1 internal os slice from each patient was stimulated to contract using 1 μmol/L oxytocin alone (as outlined above) to assess for expected time-dependent reductions in contractility after oxytocin exposure. The adjacent slice from the internal os was treated with 1 μmol/L oxytocin followed by increasing doses of nifedipine (10^{-9} to 10^{-3} mol/L, Sigma) every 15 minutes to generate a dose response and IC_{50}, which was calculated using Prizm GraphPad software (GraphPad Software Inc).[21] Nifedipine-mediated effects on oxytocin-induced contractility were expressed as a percentage change from the initial contraction before nifedipine was added.

FIGURE 5
Immunofluorescent immunohistochemistry of human myometrium (positive control) and human cervical tissue from internal and external os



Immunofluorescent immunohistochemistry of human myometrium (positive control) and human cervical tissue from internal and external os. Similar to human uterine smooth muscle cells (SMC), cervical SMC at internal and external os that express α-smooth muscle actin (SMA) also express contraction-associated protein, connexin 43. Scale bars = 200 μm.

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To calculate the effect of nifedipine at each dose/time point, the percent remaining force contraction in the nifedipine samples were normalized to the appropriately timed percentage of the oxytocin-induced contraction in our control (oxytocin only) samples. Student *t* test was used to compare the percent remaining cervical tissue muscle force at each time point in the oxytocin + nifedipine vs timed vehicle control (oxytocin alone) samples.

Results

Evaluation of CSMC at the internal and external os

In some women with premature cervical failure, the internal os dilates first while the external os remains closed. To understand how cervical tissue structure influences its function, our initial goal was to understand general cervical tissue composition at the internal and external os using a Movat pentachrome stain, which allows for simultaneous visualization of collagen, mucin, and muscle. Whole transverse slices from the internal and external os were obtained from 13 nonpregnant women (5 nulliparous, 8 multiparous; patients no. 1-13 in Table 2) undergoing hysterectomy and tissue sections were stained with Movat pentachrome. The external os is predominantly collagen (yellow). Contrary to the prevailing paradigm, which states the cervix is mostly collagen, the area of the internal os exhibits substantial muscle staining (red). Mucin (green) is observed in mucinous glands located near the endocervical canal (Figure 3). No identifiable architectural differences are noted between the nulliparous and multiparous women (data not shown).

Since the previous studies that established cervical tissue structure were technically limited to Masson trichrome staining and subjective evaluation of CSMC morphology,[6-8] we evaluated if CSMC at the internal and external os were mature, contractile SMC that express CAPs. Cervical tissue sections from patients 1-13 listed in Table 2 showed that SMA-positive cells at the internal and external os do express mature SMC

markers (SM22, calponin) and CAPs (oxytocin receptor, cyclooxygenase-2, connexin 43) (Figures 4 and 5). No identifiable architectural differences were noted between the nulliparous and multiparous women.

Evaluation of CSMC architecture in the entire cervix

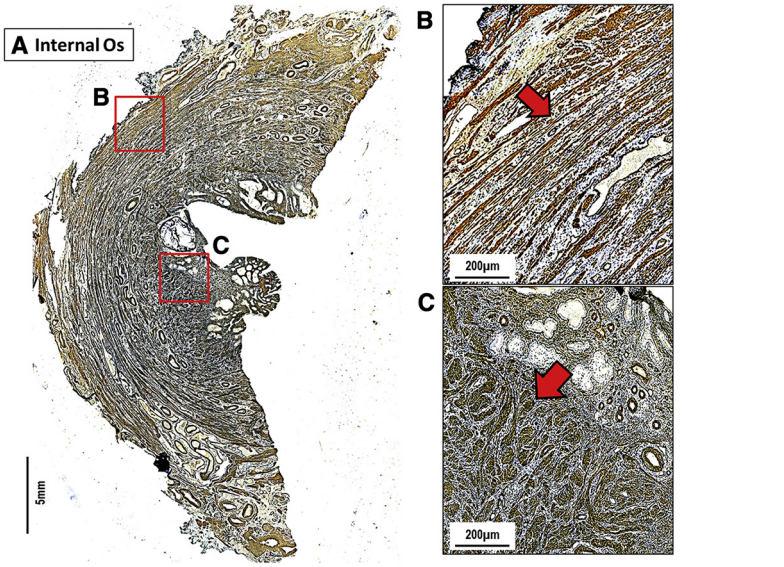
Whole transverse cervical tissue slices obtained from the internal os, mid-cervix, and external os from a separate cohort of 14 nonpregnant women (4 nulliparous, 10 multiparous; patients no. 14-27 in Table 2) reveal regional differences in CSMC content and organization. At the level of the internal os, SMA-positive cells comprise 50-60% of the tissue and bundles of CSMC can be found *circumferentially* oriented around the periphery of the cervix (Figure 6, A and B). SMA-positive cells located closer to the endocervical canal are oriented parallel to the canal (Figure 6, C). This orientation persisted to the mid-cervix, which contained approximately 40% of SMA-positive cells (Figure 7). From the midcervix to the external os, SMA-positive cell content gradually decreases while maintaining the circumferential orientation around the endocervical canal. The external os exhibited randomly scattered SMA-positive cells that accounted for about 10% of the tissue (Figure 8). Although variability exists between patients, overall CSMC morphology remained consistent in the 14 cervices that were analyzed. No identifiable CSMC architectural differences were noted between the nulliparous and multiparous women.

Organ bath studies

Evaluating regional cervical tissue contractility in response to oxytocin

To correlate how CSMC content and distribution influences cervical tissue function, 3-mm whole transverse cervical tissue slices from the internal and external os were obtained from an additional set of 14 multiparous women (patients no. 28-41 in Table 2). Tissue slices were subjected to organ bath studies to determine regional

FIGURE 6
Evaluation of smooth muscle at internal os



A, α-Smooth muscle actin (SMA) staining of transverse slice of cervix obtained from level of internal os. SMA-positive cells: **B**, are circumferentially oriented around periphery of cervix; and **C**, near endocervical canal are oriented parallel to canal (thus seen as round muscle bundles that are transected in this transverse slice). **B** and **C**, ×10 Close-up images of red boxes.

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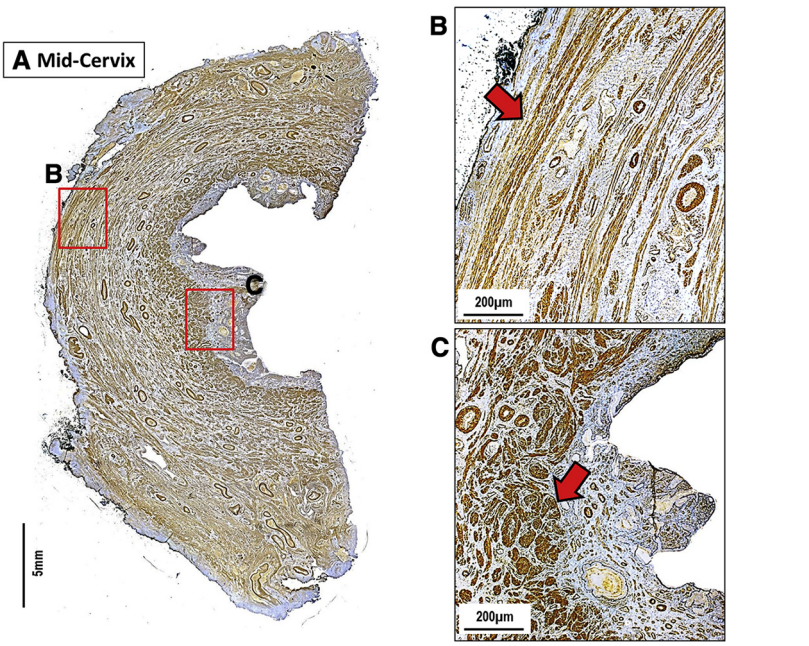
FIGURE 7
Evaluation of smooth muscle at the mid-cervix level



A, α-Smooth muscle actin (SMA) staining of transverse slice of cervix obtained from midcervix. SMA-positive cells: **B**, are still circumferentially oriented around periphery of cervix; and **C**, near endocervical canal are still oriented parallel to canal. **B** and **C**, ×10 Close-up images of red boxes.

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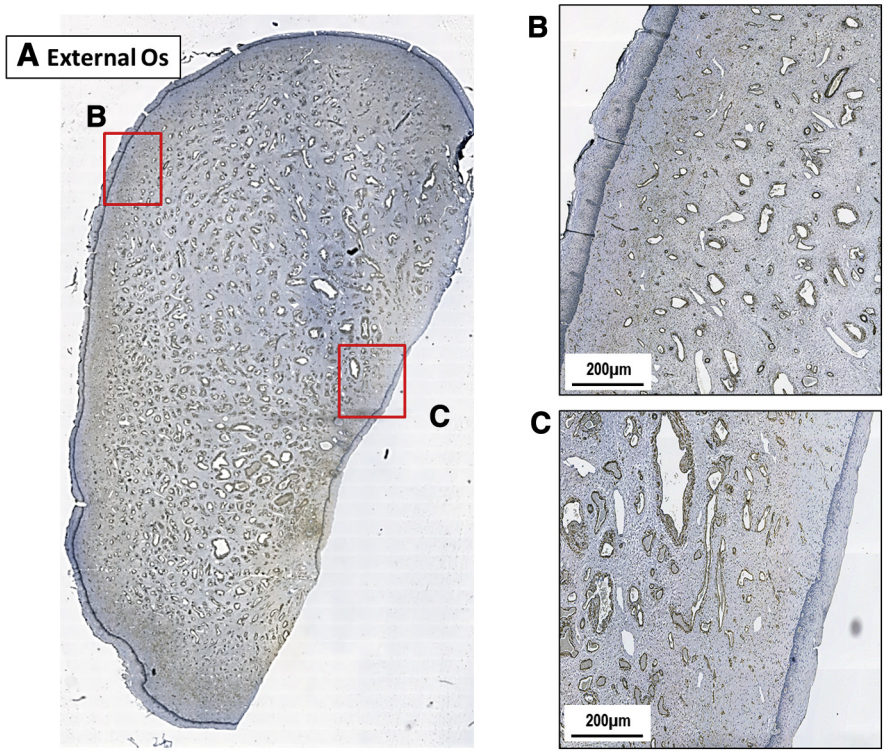
FIGURE 8
Evaluation of smooth muscle at the external os



A, α-Smooth muscle actin (SMA) staining of transverse slice of cervix obtained from external os showing SMA-positive cells are rare and scattered throughout tissue. **B** and **C**, ×10 Close-up images of red boxes.

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tissue contractility differences in response to 1 μmol/L oxytocin. Nonpregnant cervical tissue from the internal and external os contracts response to oxytocin (Figure 9, A). Regional differences in contractility were noted with the internal os contracting with more than double the force of the external os (1341 ± 693 vs 523 ± 536 integrated grams × seconds, respectively, $P = .009$) (Figure 9, B).

Evaluating if nifedipine tocolyzes the internal os

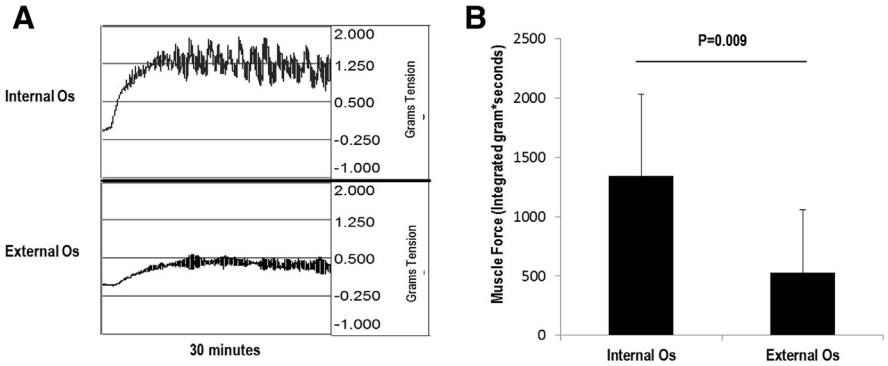
Since the effect of nifedipine (a commonly used tocolytic) on the human cervix has not been studied, we subsequently evaluated if nifedipine inhibits oxytocin-induced cervical tissue contractility. In 8 of the 14 nonpregnant women enrolled for oxytocin studies above (patients no. 33-36 and 38-41 in Table 2), two 3-mm whole transverse slices of cervical tissue from the internal os were obtained. We focused on the internal os for these experiments because the external os is mostly devoid of CSMC and exhibited decreased contractility (Figures 8 and 9). One slice was given oxytocin alone and the other oxytocin + nifedipine in increasing doses. In the slices treated with oxytocin alone, the contractile effect of oxytocin decreased over time (Figure 10, A). After 2 hours, the percent remaining muscle force was 42% ± 14% of the original muscle force. In slices treated with oxytocin + nifedipine, nifedipine significantly decreased cervical tissue muscle force compared to timed vehicle control at doses of 10^{-5} mol/L (vehicle 47% ± 10% vs oxytocin + nifedipine 24% ± 12%, $P = .007$), 10^{-4} mol/L (vehicle 46% ± 16% vs oxytocin + nifedipine −4% ± 20%, $P = .003$), and 10^{-3} mol/L (vehicle 42% ± 14% vs oxytocin + nifedipine −15% ± 18%, $P = .0006$) (Figure 10, B). Analysis of the nifedipine dose response generated an IC_{50} of 1.35×10^{-5} mol/L (Figure 10, C).

Comment

The findings from this study suggest a revised paradigm of SMC organization and function in the human cervix (Figure 11). Unlike the prevailing paradigm that states the cervix is mainly collagen/ECM with minimal cellular content, our revised paradigm shows that the area of the internal os, the area that "fails" or "funnels" first in some cases of premature cervical remodeling, contains 50-60% CSMC and bundles of CSMC can be found *circumferentially* oriented around the periphery of the cervix. From the midcervix to the external os, CSMC content gradually decreases with the external os exhibiting randomly scattered CSMC that consist of about 10% of the tissue (Figure 11). Our organ bath experiments correlate our new paradigm of CSMC architecture with cervical tissue function. We found that nonpregnant cervical tissue contracts in response to oxytocin and cervical contractility exhibits regional dependency with the area of the internal os of the cervix contracting with more force than the external os.

The fact that the upper aspect of the cervix (1) contains a significant amount of CSMC that are circumferentially oriented around the periphery of the stroma, and (2) is significantly more contractile than the external os, raises the intriguing question "do CSMC at the internal os form a sphincter"? Consider the pelvic organs adjacent to the uterus; both the bladder and rectum have sphincters that respond to pressure. Interestingly, the uterus also retains and then releases its "contents" after a certain amount of time. While the cervix may not share all of the characteristics of these sphincters, it is intriguing to consider that a specialized sphincter may exist at the internal os. Teleologically, it is curious that macaques, which share significant homology with human beings, develop a strong functional sphincter at the internal os during pregnancy.[22]

FIGURE 9
Organ bath experiments—effect of oxytocin



A, Representative data from 1 organ bath experiment showing that when whole transverse slices from internal and external os are stimulated with 1 μmol/L oxytocin, regional functional differences exist in cervix. Slices from internal os contract more forcefully compared to external os, which shows minimal contractility. **B**, When comparing internal os to external os, we found that internal os contracts with more than double force of external os (1341 $\pm$ 693 vs 523 $\pm$ 536 integrated grams $\times$ seconds, respectively, $P = .009$).

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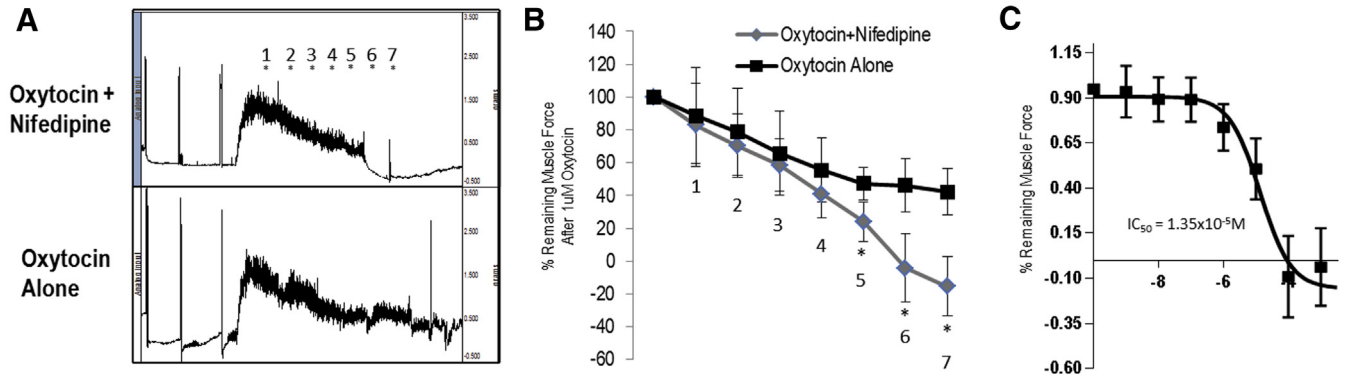
Further evidence for the possibility of a sphincter at the internal os is provided by a study in the 1950s that showed that the pregnant cervix can contract independently from the uterus in response to contractile agonists.[23] Studies have also found that electromyographic activity is present in the cervix during labor and is correlated to the duration of the latent phase. The authors concluded that the cervix contracts during the latent phase of labor, providing resistance and potentially prolongation of the latent phase.[24-30] Clearly, further studies are needed to evaluate human cervical tissue structure and function at the internal os *during* pregnancy. However, if there is a specialized sphincter at the internal os in the human cervix, the next tantalizing question is: "are we actually visualizing 'sphincter failure' sonographically in women with premature cervical remodeling who possess a dynamic and/or a funneled cervix?"

A further challenge to the prevailing paradigm is that we show CSMC express CAPs. Connexin 43 is of particular interest because if CSMC express gap junctions, then they possess direct channels to communicate with neighboring uterine SMC. Contrary to the prevailing thought that labor contractions *start* in the uterus and the cervix is a *passive* bystander that remodels/softens in response to uterine contractions, our findings have led us to question whether CSMC may possibly play a role in propagating or activating uterine contractility. In addition, CSMC are known to produce enzymes and inflammatory chemokines/cytokines

FIGURE 10
Organ bath experiments—effect of nifedipine



Organ bath experiments. **A**, Representative sample of experiments where we tested 2 whole cervical slices from level of internal os with either 1 μmol/L oxytocin alone or 1 μmol/L oxytocin and then increasing doses of nifedipine ($1 = 10^{-9}$ mol/L, $2 = 10^{-8}$ mol/L, $3 = 10^{-7}$ mol/L, $4 = 10^{-6}$ mol/L, $5 = 10^{-5}$ mol/L, $6 = 10^{-4}$ mol/L, $7 = 10^{-3}$ mol/L) every 15 minutes. This graph shows inhibitory effect of nifedipine compared to oxytocin alone, particularly starting at dose 5 (10^{-5} mol/L). **B**, Average results for percent remaining muscle force over time for internal os slices treated with 1 μmol/L oxytocin alone or 1 μmol/L oxytocin and increasing doses of nifedipine. After 2 hours, percent remaining muscle force in slice with vehicle alone 42% $\pm$ 14% of original muscle force. In slices treated with oxytocin and nifedipine, nifedipine significantly decreased cervical tissue muscle force compared to timed vehicle control at doses of 10^{-5} mol/L (vehicle 47% $\pm$ 10% vs oxytocin + nifedipine 24% $\pm$ 12%, $P = .007$), 10^{-4} mol/L (vehicle 46% $\pm$ 16% vs oxytocin + nifedipine $-4\% \pm 20\%$, $P = .003$), and 10^{-3} mol/L (vehicle 42% $\pm$ 14% vs oxytocin + nifedipine $-15\% \pm 18\%$, $P = .0006$). *$P < .05$. **C**, Nifedipine dose response curve showing IC_{50} of 1.35×10^{-5} mol/L.

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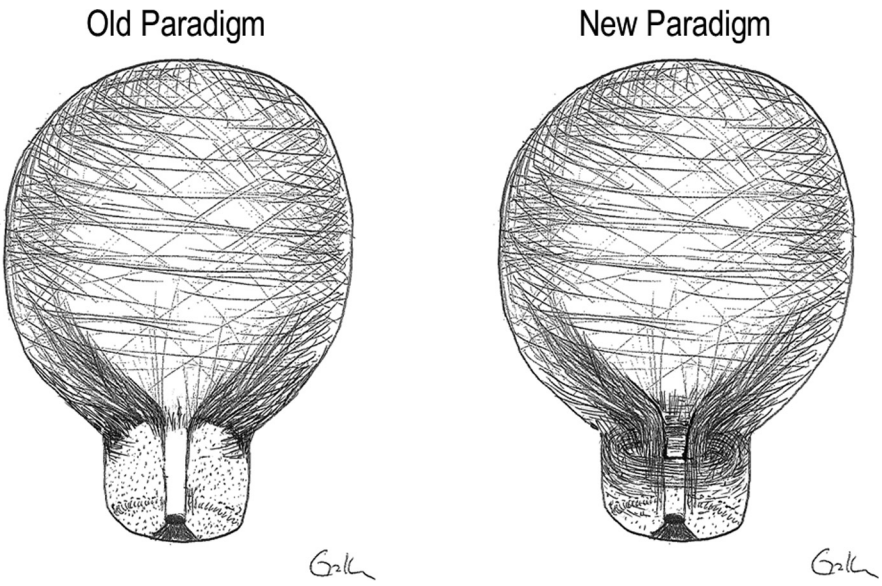
FIGURE 11
New paradigm of cervical smooth muscle cell architecture in human cervix



New paradigm of cervical smooth muscle cell (CSMC) architecture in human cervix—unlike prior studies that indicated distinct transition between muscular uterus and mainly collagenous cervix, our new paradigm shows that: (1) CSMC content in upper half of cervix is more abundant than lower half of cervix; and (2) in upper half of cervix, smooth muscle cell are circumferentially oriented around endocervical canal possibly similar to sphincter.

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that are involved in ECM remodeling.[31-34] Thus, we further question if CSMC could potentially be a key resident cell *in the cervical stroma* that orchestrates ECM remodeling *and/or* participates in uterine contractions. Clearly, further studies that evaluate if CSMC initiate or modulate uterine contractions are needed to test these hypotheses.

Previous studies have shown cervical tissue contractility can be inhibited by various tocolytics, but none had tested nifedipine.[10,11,35-37] Our studies show that oxytocin-induced cervical tissue contractility is inhibited by nifedipine. In addition, the IC_{50} for nifedipine is orders of magnitude more than the known IC_{50} for nifedipine in the pregnant uterus (cervix 1.35×10^{-5} vs uterus 1×10^{-8} mol/L).[21] Although no studies have generated IC_{50} values for nifedipine in the nonpregnant uterus, it would be interesting to see if the cervix is more resistant to nifedipine than the uterus during pregnancy. If CSMC can propagate contractile signals to the uterus, and if CSMC are more resistant to tocolytics, this may in part explain why tocolytics ultimately fail to effectively stop preterm labor. These hypotheses highlight the limitations of our current study as we have only tested nonpregnant tissue. Further studies evaluating pregnant human cervical tissue are needed. Other limitations include the small sample size for each experiment, a limited number of white women, and the functional organ bath studies included only tissue from parous women.

In summary, our findings suggest a new paradigm for cervical tissue morphology—one that includes the possibility of a specialized sphincter at the internal os (Figure 11) and introduces CSMC as possible players in: (1) propagating uterine contractions, and (2) cervical remodeling. Such a paradigm provides exciting new frontiers to further investigate the pathophysiology of premature cervical remodeling and possible new avenues to discover novel, effective therapies to prevent sPTB. ■

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