BASIC SCIENCE: OBSTETRICS

A novel optical method to assess cervical changes during pregnancy and use to evaluate the effects of progestins on term and preterm labor

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OBJECTIVE: The purpose of this study was to determine whether optical methods can estimate cervix function during pregnancy and whether progestins modify this process.

STUDY DESIGN: Photos of the external cervix of timed-pregnant rats were taken every other day from day 13 until postpartum day 5 after daily treatments with vehicle (controls) or progestin treatments (progesterone, subcutaneously or vaginally; 17-alpha-hydroxyprogesterone caproate [17P] and RU-486 subcutaneously, once on day 16). The surface area of the cervix was estimated from photos.

RESULTS: The surface area of cervix increases throughout pregnancy and reverses after delivery in controls. In the progesterone subcutaneously or 17P subcutaneously groups, increases in surface area are lower (17P group until day 19 only; $P < .05$). Vaginal progesterone does not prevent surface area increases. Only the progesterone subcutaneously blocked delivery. RU-486 increases the surface area of the cervix ($P < .05$) during preterm delivery.

CONCLUSION: An optical method is useful for quantitative assessment of the cervix and evaluation of agents that modify cervical function.

Key words: cervix, 17-alpha-hydroxyprogesterone caproate, preterm labor, progesterone, rat

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Preterm birth is a severe pregnancy complication that occurs in approximately 10% of all pregnancies in the developed countries and even more often in developing countries.[1,2] Despite all efforts, at this date reliable tools that predict and diagnose preterm birth and successful treatment regiments for a better outcome of the pregnant woman and the baby are still missing. The development of effective therapies to prevent or reduce the occurrence of this difficult medical condition depends on the understanding of the circumstances that initiate labor.

After staying rigid and closed throughout most of pregnancy, to protect and secure the special environment created inside the uterus, the cervix switches to a soft and easy-to-open state that is essential for successful vaginal delivery. Many biochemical and functional changes occur in the cervical connective tissue during gestation, which are summarized in the term *cervical ripening*. It is a chronic process that starts in the first trimester of pregnancy and progressively proceeds until term. It is usually described as a 3-step preparation process that must occur in sequence, and each step seems to be irreversible: softening, effacement, and dilation of the cervix.[3] This sequence is associated with a dramatic reorganization of the extracellular matrix, which consists of elastin, proteoglycans, and especially collagen, that decreases by 30-70% and is accountable by a change from insoluble to more soluble collagen.[4,5] Cervical ripening is an active biochemical process with similarities to an inflammatory-like reaction (infiltration of leukocytes, increase of cytokines and metalloproteinases) and occurs independent of uterine contractions.[6-9] This process also appears to be at least partially regulated by steroid hormones (in particular progesterone and estrogen), because antiprogestins successfully induce cervical ripening.[10-12] Other hormones and mediators that have been shown to be involved in cervical ripening are dihydrotestosterone,[13] prostaglandins,[14] and local mediators, such as platelet-activating factor[15] and nitric oxide.[14]

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The consistent and precise identification of the changes that occur in the cervix is one of the challenges that obstetricians face today. Various methods have been used to identify this condition. Physical examination is one of the oldest techniques, and a number of scoring systems to characterize the cervix (eg, the Bishop Score) have been developed.[16,17] Other techniques to assess cervical changes are the measurement of the cervical length by transvaginal ultrasound scans and biochemical markers (such as fetal fibronectin, which is a glycoprotein that is found at the chorionic-decidual interface, or insulin-like growth factor binding protein-1, which is a protein that is synthesized by the maternal decidua).[17] Our group has used light-induced fluorescence (LIF) of the cervix to estimate changes in cervical collagen and effects of treatments.[18,19] These studies show that parenteral or topical progesterones are equally effective in inhibiting delivery in rats but that these treatments only partially prevent cervical ripening.[19]

Recent studies have investigated the use of progestins as treatment for preterm delivery.[20-26] Early studies also discussed the potential benefit of 17-α hydroxyprogesterone caproate (17P), which is a synthetic caproate ester of the naturally occurring metabolite of progesterone, for the treatment or prevention of preterm labor.[27] Several recent randomized controlled trials have studied the effects of progestins on cervical length changes that were assessed by transvaginal ultrasound scanning.[24-26] Other possible treatments for woman who are at risk of cervical insufficiency include bed rest, cervical cerclage, and antibiotics.[28-30] None of these reports are evidence-based, and there is a controversy in the findings. Uterine contractions can be suppressed by tocolytic drugs, but only for a very limited time and all compounds have considerable side-effects.[31,32]

Pregnant rats, which are a well-known model to study pregnancy in animals, are exquisitely sensitive to changes in progesterone with preterm delivery or prolonged gestation when progesterone levels are manipulated or when progesterone receptor antagonists are used.[33] Our hypothesis is that exogenous progestins inhibit cervical ripening and prevent term delivery, that there are differences in abilities of the progestins, and that the consideration of routes of administration is important.

The objective of this study was to determine whether optical methods can be used to estimate changes of the properties of cervical tissue during pregnancy and whether progestins that are given by various routes can alter these properties. We also used LIF to assess cervical changes and compared them with an optical surface analysis of changes in the area of the cervix during pregnancy and after delivery.

Materials and Methods

Animals

Timed-pregnant Sprague-Dawley rats (200-250 g) from Charles-River Laboratories (Wilmington, MA) were transferred to our animal care facilities on day 12 of gestation (day 1 being the day when a sperm plug was observed). The animals were housed separately with free access to food and water and maintained on a constant 12-hour light-dark cycle. Control pregnant rats spontaneously delivered on days 22 and 23 of gestation. For the measurements with the endoscopic camera and the collascope, the animals were anesthetized (intraperitoneal injection) with a combination of xylazine (Gemini; Burns Veterinary Supply Inc, Rockville Center, NY) and ketamine HCl (Ketaset; Fort Dodge Laboratories Inc, Fort Dodge, IA). The animals were allocated randomly to 1 of the groups and killed by carbon dioxide inhalation on postpartum day 5 or on day 25 of pregnancy in the groups with delayed delivery. All procedures were approved by the Animal Care and Use Committee of the St. Joseph's Hospital and Medical Center in Phoenix, AZ.

Treatments

Pregnant rats (n = 6/group) were treated (when not otherwise mentioned) from day 13 of pregnancy until delivery. Single daily treatments were performed at 8 AM, and twice daily treatments were performed at 8 AM and 8 PM. All daily injections (4 mg progesterone and 10 mg 17P) were by the subcutaneous route in sesame oil (0.2 mL), which was also used for the controls of the injection groups. Vaginal gels were applied twice a day with a blunt ball-top needle deep into the vagina. Crinone was used for the progesterone vaginal group (we used equivalent volumes of Crinone for 2-15 mg progesterone/treatment); all data presented show the results of the highest dose (total daily dose of 30 mg progesterone = one-third of an applicator of 8% Crinone that contained 90 mg progesterone). The control rats for the vaginal groups were treated with Replens (0.18 mL/treatment). RU-486 (3 mg in 0.2 mL sesame oil) was injected subcutaneously once on day 16 of gestation.

Reagents

Crystalline progesterone (used for subcutaneous progesterone), RU-486, sesame oil, and ethanol were purchased from Sigma Chemical Company (St. Louis, MO). 17P was obtained from MP Biomedicals (Solon, OH). Progesterone, 17P, and RU-486 were dissolved in ethanol and then mixed with sesame oil. Crinone (micronized progesterone in Replens, which is a bioadhesive gel, was used for vaginal progesterone) and Replens were gifts from Columbia Laboratories (Livingston, NJ).

Assessment of cervical changes

Measurements with the endoscopic camera and the collascope were performed on every other day starting at day 13 until day 21 of gestation and on postpartum day 3 and/or postpartum day 5 and for some animals also on postpartum days 4, 8, and 10.

Optical evaluation with an endoscopic camera. A small pediatric speculum was inserted into the vagina of the anesthetized animal and always opened to a certain level (distance between the top and the bottom part of the end of the speculum was set to standard width of 9 mm, which was used for calibration of the measurements of the surface area). An endoscopic camera was placed in front of the cervix at approximately 10 mm, and photos were taken of the cervix and ends of the speculum (Figure 1). The surface area (in square millimeters) of the cervix was calculated from digitized photographs by morphometric methods with

the use of ImageJ software (version 1.43; National Institutes of Health, Bethesda, MD).[34,35]

Evaluation with the collascope with LIF. The amount of cervical collagen was evaluated by measurement of the autofluorescent properties of cross-linked collagen with a new prototype of an instrument, termed *collascope* (Reproductive Research Technologies, Houston, TX), as used with an earlier prototype.[15,18] After insertion of a small speculum into the vagina of the anesthetized animal, the optical probe of the collascope was placed on the surface of the exocervix. The probe, which is connected to the main unit of the instrument by a fiber optic cable, delivers excitation light (wavelength, 339 nm) onto the cervix and also carries the fluorescent light (mainly caused by pyridinoline cross-links of collagen with a maximum peak at 390 nm) back to the instrument to a charge-coupled device camera to display the full spectrum of fluorescence and analysis of the photons that are emitted by the cervix. The exposure time for excitation was 100 msec. The average of 20 measurements of the detected fluorescent intensity (photon count) at 390 nm was used for each animal at any given time.

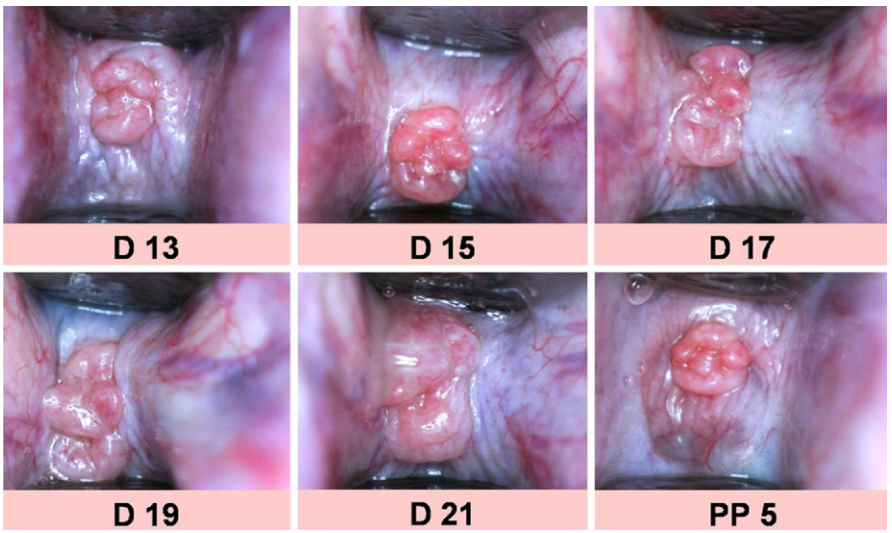
Determining the changes in delivery time

Pregnant animals were checked for delivery 3 times per day (8 AM, 12 AM, 8 PM). The expulsion of 1 pup was considered to be the start of delivery.

Analysis

The surface area of the cervix and the LIF of control animals (Figure 2) that were obtained at different times of gestation were compared by 1-way analysis of variance and multiple pairwise comparison procedures (Dunn's Method for cervical surface area and Holm-Sidak for cervical LIF). The Student *t* test was used to compare the surface area of a treatment group to its specific control group at any time in gestation and after delivery (Figure 3) and to determine the differences in delivery times (Figure 4). A 2-tailed probability value of $< .05$ was considered statistically significant.

FIGURE 1
Photos of the external cervix throughout pregnancy



D, day; *PP*, postpartum.

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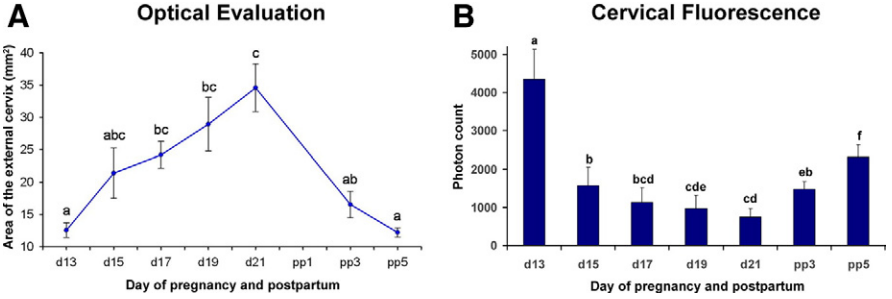
RESULTS

The surface area of the cervix in normal pregnancy continuously increases throughout gestation (day 13, 12.6 ± 1.6 mm^2; day 21, 34.6 ± 3.7 mm^2) and reverses after delivery (postpartum day 5, 12.2 ± 0.7 mm^2; Figure 2, A). Measurements of cervical LIF in pregnant, nontreated animals show a continuously decreasing photon count throughout pregnancy and reversal after delivery (Figure 2, B). After significant ($P < .05$) changes from days 13-15, the cervical surface area and the LIF reaches a wider plateau of nonsignificant ($P > .05$) changes before delivery. LIF values progressively increase after delivery ($P < .05$), whereas cervical surface area decreases respectively ($P < .05$).

In parenterally treated progesterone (Figure 3, A) or 17P (Figure 3, B) groups, the increases in surface area are lower ($P < .05$; day 15/day 19: progesterone group,

FIGURE 2
Cervical changes in pregnant and postpartum rats



A, Surface area of cervix (means ± SD of the surface area of the cervix) that were obtained in vivo from pregnant rats (n = 6) at different days of pregnancy and after delivery that had been treated daily with vehicle (controls). Letters *a-c* indicate significant differences between mean values ($P < .05$). **B,** Bar graphs show the mean ± SD of cervical light-induced fluorescence that were obtained in vivo from pregnant rats (n = 6) at different days of pregnancy and after delivery that were treated daily with vehicle (controls). Significant differences ($P < .05$) between groups are marked with different letters (*a-f*).

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FIGURE 3
Effects of various treatments on cervical ripening

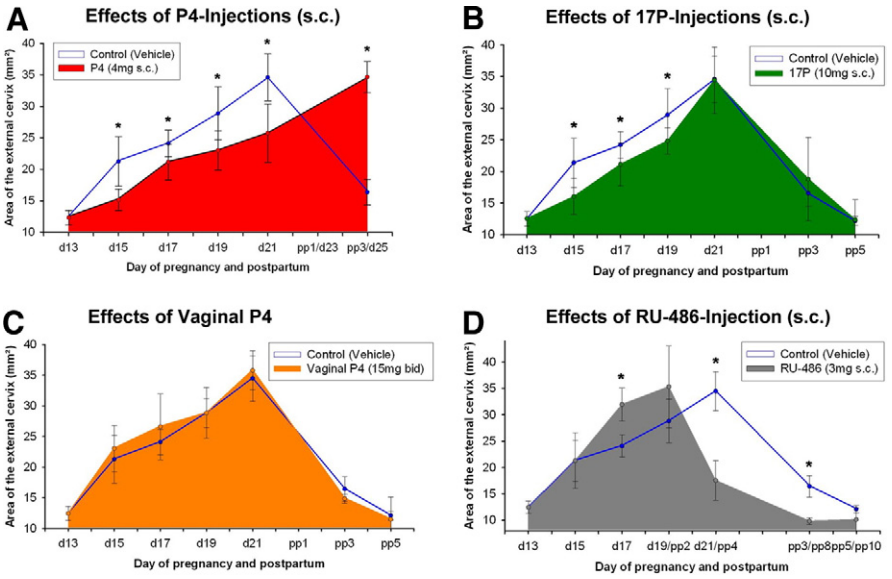

Figure shows surface area of cervix (mean ± SD of the surface area of the cervix) that was obtained in vivo from pregnant rats (n = 6/group) at different days of pregnancy and after delivery that were treated with various progestins or vehicle. **A,** Daily treatment with vehicle (controls) or progesterone (*P4*; 4 mg, subcutaneously [*s.c.*]). Note that delivery is inhibited in the treatment group. **B,** Daily treatment with vehicle (controls) or 17-alpha-hydroxyprogesterone caproate (*17P*; 10 mg, subcutaneously [*s.c.*]). Note that significant differences are observed only until day 19 of gestation. C, Twice a day treatment with vehicle (controls) or vaginal progesterone (*P4*; 15 mg, twice daily). Note that no significant differences are observed at any time between controls and treated animals. **D,** Treatment once on day 16 with vehicle (controls) or RU-486 (3 mg, subcutaneously [*s.c.*]). Note that animals that were treated with RU-486 delivered prematurely. The *asterisks* indicate a probability value of $< .05$ compared with controls.

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15.3 ± 1.7/23.5 ± 3.4 mm^2; 17P-group, 16.0 ± 2.8/24.2 ± 2.1 mm^2; control group, 21.2 ± 3.9/28.9 ± 4.2 mm^2). Only for the progesterone-injection group is a lower ($P < .05$) surface area noted on day 21 (progesterone vs control, 25.8 ± 4.6 mm^2 vs 34.6 ± 3.7 mm^2). Vaginal progesterone (Figure 3, C) does not prevent surface area increases. RU-486 treatment increases the surface area ($P < .05$) during preterm delivery (Figure 3, D). Only parenteral progesterone treatment blocked delivery (Figure 4). Neither vaginal progesterone nor parenteral 17P delayed delivery significantly (Figure 4).

Comment

This study demonstrates that an optical evaluation can be useful for the assessment of quantitative changes in cervical ripening in vivo. This method has never been described and may reveal a huge potential for the assessment of cervical changes in pregnancy and for other gynecologic conditions. The hereby introduced method is not only helpful for the observation of the regular changes throughout pregnancy and after delivery, which have been investigated in other studies that used different techniques, but also indicates preterm cervical changes and the success of pharmacotherapy and interventions. As shown in this study, progestins have the ability to delay cervical ripening and delivery in term pregnant rats. These effects depend critically on the choice of the progestin and the route of administration. Cervical ripening can be assessed by an optical examination of the exterior of the cervix. The surface area of the external cervix increases almost 300% from day 13 (12.6 ± 1.6 mm^2) of pregnancy to term (day 21, 34.6 ± 3.7 mm^2), which reflects ripening (Figure 2, A). Because we measure the overall surface area in photographs, not regarding folds and furrows in the exterior of the cervix, it is undoubted that the absolute surface area is still underestimated; therefore, the method might be considered semiquantitative. Another method to assess early changes besides the optical evaluation is the use of LIF of cross-linked collagen with an instrument called a collascope.[36] One of our previous studies used LIF to assess cervical changes during pregnancy and the influence of progestins, the results and the conclusions of which support the present study.[19] Throughout gestation, the collascope detects a decreasing photon count that describes the remodeling of the extracellular matrix that includes a decrease in collagen concentration and switch from insoluble to more soluble collagen (Figure 2, B).[18,19,37] This decrease of collagen could explain the softening of the cervix, which is assessed by the endoscopic camera as an increase in surface area of the cervix in consequence. As anticipated in the postpartum period, the LIF increases progressively, whereas the surface area of the cervix decreases.

As described previously, the tremendous changes in the cervix occur early in pregnancy in mid gestation. According to the concept of the 3-step process, cervical ripening starts with a process called *softening*.[3] This is a vital process and cannot be assessed with the tools that are used at present in clinics to determine cervical problems in pregnancy. This optical evaluation reveals changes in the cervix earlier than many other techniques that have been used to assess the cervix. Ripening is associated with a strong reorganization of the extracellular matrix and an increase of proteoglycans.[7,38-40] Related with this, there is an influx of water into the tissue[41] that may contribute to the increase in cervical surface area.

The increase in surface area of the cervix is decelerated in the parenteral progesterone (Figure 3, A) and 17P (Figure 3, B) groups; thus, we conclude that

these treatments delay cervical ripening but do not entirely prevent it, which indicates the involvement of other control pathways. In the comparison of the control groups, the collascope again supports the results of the endoscopic camera in the same way for the treatment groups (results not shown).[19] Vaginal progesterone (even at 7.5 times the parenteral dose) does not inhibit ripening, which is indicated in this study by changes in the surface area of the cervix or delivery possibly because of reduced progesterone uptake (Figure 3, C). Parenteral progesterone, but not 17P, inhibits delivery and may be more effective for treatment of preterm labor (Figure 4). Because the cervix managed to ripen also in the parenteral progesterone-treated group at the end of gestation, we conclude that the inhibition of delivery is not due to an unripe cervix but must be due to a suppression of uterine contractions. Similar to the parenteral route, we demonstrated in a previous study the block of delivery also for a topical (transdermal) route of administration of progesterone.[19]

Softening of the cervix is a chronic process, whereas effacement and dilation are acute events.[3] Techniques that measure early pathologic changes of the cervix before the irreversible steps of reorganization of the extracellular matrix are accomplished could be of great value in the identification of patients who are at high risk for prematurity. This may be the reason that pharmacologic interventions in clinical studies with progesterone or 17P are not successful or with contrary results. Women who are in danger of prematurity might be treated too late when the drugs lose their influence to exert beneficial effects. Consequently, many women could be exposed to steroids whether they needed them or not. One of our previous studies, in which we used LIF to assess cervical changes during pregnancy and the influence of progestins, supports the results and the conclusions of this study.[19]

In 1956, Csapo[42] developed the concept of a progesterone withdrawal as a key step for parturition. Several groups recently have investigated the effects of vaginal progesterone and intramuscular 17P to prevent preterm birth.[20-26] Some studies showed a lower rate of preterm birth[20,21,23] or attenuated cervical shortening[24,25] in the treatment groups. However, other studies seemed to contradict the results of those trials.[22,26] The studies raise questions about the ability of progesterone and 17P to prevent preterm labor and cervical shortening. Different study populations complicate the comparison of the studies; which of the progestins and which route of administration is superior has not been established, and there is controversy in the findings.

Still, the ideal progesterone formulation, the dosage, and the route of administration are still unidentified. The half-life of progesterone is approximately 35-55 hours.[43] Therefore, progesterone needs to be administered daily, which clearly demonstrates the importance of finding the least invasive route of administration to minimize side-effects and maximize safety and compliance of the patients without compromising the effectiveness of the treatment. Vaginal progesterone was ineffective to inhibit delivery in this study; this questions the advantages of this common route for the application of progesterone. In addition, treatment of acute preterm labor by progestins has yet to be studied extensively, and management is limited to the use of tocolytics, which suppress uterine contractility but do not reverse the labor process. Treatment of preterm labor might be improved greatly if methods of administration, compounds, and vehicles were optimized and if we had a better understanding of how the progestins affect the uterus and cervix.

The optical evaluation of the cervix with an endoscopic camera instead of the colposcope could be of value for the assessment of other obstetric problems and diseases such as infections, dysplasias, and cancers, especially in the context of follow-up examinations. The optical evaluation and the assessment of the surface area of the cervix is a new, effective, noninvasive, objective, low-cost method to assess cervical changes during pregnancy and the success of pharmacologic interventions. ■

FIGURE 4
Effects of antiprogestin and various progestin treatments on time of delivery

The percentage of animals that delivered vs the day of pregnancy after various treatments are shown: RU-486 (single treatment, 3 mg, subcutaneously [*s.c.*], on day 16), daily treatments from day 13 until delivery for progesterone (*P4*; 4 mg, subcutaneously, or 15 mg, vaginally twice a day), 17-alpha-hydroxyprogesterone caproate (*17P*; 10 mg, subcutaneously) and controls (0.2 mL sesame oil [vehicle], subcutaneously, or 0.18 mL vaginal gel, twice daily). Note that injections of progesterone completely blocked delivery, whereas 17P or vaginal progesterone had no effect on delaying term delivery. RU-486 induced preterm delivery.

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