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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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Abstract

Objectives—To determine if optical methods can estimate cervix function during pregnancy and if progestins modify this process.

Study Design—Photos of the external cervix of timed-pregnant rats were taken every other day from day 13 (d13) until pp5 (postpartum day 5) following daily treatments with vehicle (controls) or progestin treatments (progesterone, P4 s.c., or vaginally; 17-alpha-hydroxyprogesterone caproate, 17P s.c. and RU-486 s.c., once on d16). Surface area (SA) of the cervix was estimated from photos.

Results—The SA of cervix increases throughout pregnancy and reverses pp in controls. In P4 s.c. or 17P s.c. groups, increases in SA are lower (17P group until day 19 only) ($P < .05$). Vaginal P4 does not prevent SA increases. Only the P4 s.c. blocked delivery. RU-486 increases SA of the cervix ($P < .05$) during preterm delivery.

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

Keywords

cervix; 17-alpha-hydroxyprogesterone caproate; preterm labor; progesterone; rats

Introduction

Preterm birth is a severe pregnancy complication that occurs in about 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 as well as successful treatment regimens 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

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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, which starts in the first trimester of pregnancy and progressively proceeds until term. It is usually described as a 3-step preparation process, which must occur in sequence and each step seems to be irreversible: First softening, then effacement and finally dilatation 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 (P4) and estrogen), as antiprogestins successfully induce cervical ripening.^{10–12} Other hormones and mediators shown to be involved in cervical ripening are dihydrotestosterone¹³, prostaglandins¹⁴, and local mediators such as platelet-activating factor¹⁵ and nitric oxide¹⁴

The consistent and precise identification of the changes that occur in the cervix is one of the challenges 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 (e.g. the Bishop Score) have been developed.^{16, 17} Other techniques to assess cervical changes are the measurement of the cervical length by transvaginal ultrasound and biochemical markers, like fetal fibronectin, a glycoprotein found at the chorionic-decidual interface, or Insulin-like growth factor binding protein-1, a protein 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 P4 are equally effective in inhibiting delivery in rats but 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), a synthetic caproate ester of the naturally occurring metabolite of P4, for the treatment or prevention of preterm labor.²⁷ Several recent randomized control trials have studied the effects of progestins on cervical length changes assessed by transvaginal ultrasound.^{24–26} Other possible treatments for woman at risk of cervical insufficiency include bed rest, cervical cerclage and antibiotics.^{28–30} None is 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, a well-known model to study pregnancy in animals, are exquisitely sensitive to changes in P4 with preterm delivery or prolonged gestation when P4 levels are manipulated or P4 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 consideration of routes of administration is important.

The objective of this study was to determine if optical methods can be used to estimate changes of the properties of cervical tissue during pregnancy and whether progestins given by various routes can alter these properties. We also used light-induced fluorescence to

assess cervical changes and compared them to an optical surface analysis of changes in area of the cervix during pregnancy and postpartum.

Study Design

Animals

Timed-pregnant Sprague-Dawley rats (200–250 g) from Charles-River Laboratories (Wilmington, MA, USA) 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 were spontaneously delivering on day 22 and 23 of gestation. For the measurements with the endoscopic camera and the collascope the animals were anaesthetized (i.p. injection) with a combination of xylazine (Gemini, Burns Veterinary Supply Inc, Rockville Center, NY, USA) and ketamine HCl (Ketaset; Fort Dodge Laboratories Inc, Fort Dodge, IO, USA). The animals were randomly allocated to one of the groups and sacrificed by carbon dioxide inhalation on day 5 postpartum 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.

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 a.m. and twice a day treatments at 8 a.m. and 8 p.m. All daily injections (4 mg P4 and 10 mg 17P) were by the subcutaneous route (s.c.) 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 P4 vaginal group (we used equivalent volumes of Crinone for 2–15 mg P4/ treatment, all data presented show the results of the highest dose (total daily dose of 30 mg P4 = 1/3 of an applicator of 8% Crinone that contains 90 mg P4). 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 s.c. once on day 16 of gestation.

Reagents

Crystalline P4 (used for subcutaneous P4), RU-486, sesame oil and ethanol were purchased from Sigma (St Louis, MO, USA). 17P was obtained from MP Biomedicals (Solon, OH, USA). P4, 17P, and RU-486 were dissolved in ethanol and then mixed with sesame oil. Crinone (micronized P4 in Replens, a bioadhesive gel, used for vaginal P4) and Replens from Columbia Laboratories (Livingston, NJ, USA).

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 (SA in mm²) of the cervix was calculated from digitized photographs by morphometric methods using the

software ImageJ 1.43, National Institute of Health, Bethesda, Maryland, USA (see at <http://rsbweb.nih.gov/ij/>).34- 35

Evaluation with the collascope using light-induced-fluorescence—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 previously 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 a.m., 12 a.m., 8 p.m.). The expulsion of one pup was defined as the start of delivery.

Analysis

The SA of the cervix and the LIF of control animals (Figure 2) obtained at different times of gestation were compared using one-way analysis of variance (ANOVA) and multiple pairwise comparison procedures (Dunn's Method for cervical SA and Holm-Sidak for cervical LIF). Student's t-test was used to compare the SA of a treatment group to its specific control group at any time in gestation and postpartum (Figure 3) and also to determine the differences in delivery times (Figure 4). A two-tailed probability value of $P < 0.05$ was considered statistically significant.

Results

The SA of the cervix in normal pregnancy continuously increases throughout gestation (d13: 12.6 ± 1.6 to 34.6 ± 3.7 at d21) and reverses pp (pp5: 12.2 ± 0.7) (Figure 2A). Measurements of cervical light-induced-fluorescence (LIF) in pregnant, non-treated animals show (Figure 2B) a continuously decreasing photon count throughout pregnancy and reversal postpartum. After significant ($P < .05$) changes from day 13 to day 15 the cervical SA and the LIF reaches a wider plateau of non-significant ($P > .05$) changes prior to delivery. LIF values progressively increase postpartum ($P < .05$), whereas cervical SA decreases respectively ($P < .05$). In parenterally treated P4 (Figure 3A) or 17P (Figure 3B) groups increases in SA are lower ($P < .05$) (d15/d19: P4-group: $15.3 \pm 1.7/23.5 \pm 3.4$; 17P-group: $16.0 \pm 2.8/24.2 \pm 2.1$; controls: $21.2 \pm 3.9/28.9 \pm 4.2$). Only for the P4-injection group is a lower ($P < .05$) SA noted on d21 (P4 vs. control: 25.8 ± 4.6 vs. 34.6 ± 3.7). Vaginal P4 (Figure 3C) does not prevent SA increases. RU-486 treatment increases the SA ($P < .05$) during preterm delivery (Figure 3D). Only parenteral P4 treatment blocked delivery (Figure 4). Neither vaginal P4 nor parenteral 17P delayed delivery significantly (Figure 4).

Comment

This study demonstrates that an optical evaluation can be useful to assess quantitative changes in cervical ripening in vivo. This method has never been described previously and may reveal a huge potential for the assessment of cervical changes in pregnancy as well as

for other gynecological conditions. The hereby introduced method is not only helpful to observe the regular changes throughout pregnancy and postpartum, which have been investigated in other studies using 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 SA of the external cervix increases almost 300% from day 13 ($12.6 \pm 1.6 \text{ mm}^2$) of pregnancy to term (day 21: $34.6 \pm 3.7 \text{ mm}^2$) reflecting ripening (Figure 2A). Since we measure the overall surface area in photographs, not regarding folds, furrows etc. in the exterior of the cervix, it is undoubted that the absolute surface area is still underestimated and thus the method might be considered semiquantitative. Another method to assess early changes besides the optical evaluation is the use of light-induced fluorescence (LIF) of cross-linked collagen with an instrument called collascope.³⁶ One of our previous studies used LIF to assess cervical changes during pregnancy and the influence of progestins and the results and the conclusions support the present study.¹⁹ Throughout gestation the collascope detects a decreasing photon count which describes the remodeling of the extracellular matrix including a decrease in collagen concentration and switch from insoluble to more soluble collagen (Figure 2B).^{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 SA of the cervix in consequence. As anticipated in the postpartal period the LIF increases progressively whereas the SA of the cervix decreases.

As described previously the tremendous changes in the cervix occur early in pregnancy, in midgestation. Following the concept of the 3-step process cervical ripening starts with a process called softening.³ This is a vital process and can not be assessed with the tools used at present in clinics to determine cervical problems in pregnancy. This optical evaluation reveals changes in the cervix earlier than many other techniques 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 tissue⁴¹ that may contribute to the increase in cervical SA.

The increase in SA of the cervix is decelerated in the parenteral P4 (Figure 3A) and 17P (Figure 3B) groups and thus we conclude that these treatments delay cervical ripening but do not entirely prevent it, indicating the involvement of other control pathways. In analogy to 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 P4, even at $7.5 \times$ the parenteral dose, does not inhibit ripening, as indicated in this study by changes in the surface area of the cervix, or delivery possibly because of reduced P4 uptake (Figure 3C). Parenteral P4, but not 17P, inhibits delivery and may be more effective for treatment of preterm labor (Figure 4). As the cervix manages to ripen also in the parenteral P4 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 an 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 P4.¹⁹

Softening of the cervix is a chronic process, whereas effacement and dilatation are acute events.³ Techniques that measure early pathological 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 that are in high-risk for prematurity. This may be the key why pharmacological interventions in clinical studies with P4 or 17P are not successful or with contrary results: Women 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, where 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 et al. developed the concept of a P4 withdrawal as a key step for parturition.⁴² Several groups have recently investigated the effects of vaginal P4 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 P4 and 17P to prevent preterm labor and cervical shortening. Different study populations complicate the comparison of the studies and it is not established which of the progestins and which route of administration is superior and there is controversy in the findings.

Still, the ideal P4 formulation is unidentified, as well as the dosage and route of administration. The half-life of P4 is approximately 35 to 55 hours.⁴³ Therefore P4 needs to be administered daily which clearly demonstrates the importance to find 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 P4 was ineffective to inhibit delivery in this study and this questions the advantages of this common route for application of P4. In addition treatment of acute preterm labor by progestins has yet to be extensively studied 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 greatly improved 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 so far used colposcope could be of value for assessment of other obstetrical problems and diseases like infections, dysplasias and cancers, especially in the context of follow-up examinations. The optical evaluation and the assessment of the SA of the cervix is a new, effective, non-invasive, objective, low-cost method to assess cervical changes during pregnancy as well as the success of pharmacological interventions.

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Figure 1.
Photos of the external cervix throughout pregnancy (D13 = day 13 of gestation to D21) and postpartum (day 5 postpartum).

Figure 2.

Figure 2A: Figure showing surface area of cervix (means $\pm$ SD of the surface area of the cervix) obtained in vivo from pregnant rats ($N = 6$) at different days of pregnancy and postpartum treated daily with vehicle (controls). Different letters indicate significant differences between mean values ($P < .05$). Figure 2B: Bar graphs showing means $\pm$ SD of cervical light-induced fluorescence (LIF) obtained in vivo from pregnant rats ($N = 6$) at different days of pregnancy and postpartum treated daily with vehicle (controls). Significant differences ($P < 0.05$) between groups are marked with different letters.

Figure 3.

Figure showing surface area of cervix (means $\pm$ SD of the surface area of the cervix) obtained in vivo from pregnant rats ($N = 6/\text{group}$) at different days of pregnancy and postpartum treated with various progestins or vehicle. Figure 3A: Daily treatment with vehicle (controls) or P4 (4 mg, s.c.). Note that delivery is inhibited in the treatment group. Figure 3B: Daily treatment with vehicle (controls) or 17P (10 mg, s.c.). Note that significant differences are only observed until day 19 of gestation. Figure 3C: Twice a day treatment with vehicle (controls) or vaginal P4 (15 mg bid). Note that no significant differences are observed at any time between controls vs. treated animals. Figure 3D: Treatment once on day 16 with vehicle (controls) or RU-486 (3 mg s.c.). Note that animals treated with RU-486 delivered prematurely. Asterisks indicate $P < 0.05$ compared with controls.

Figure 4.

Shown is the percent of animals delivering versus day of pregnancy after various treatments: RU-486 (single treatment, 3mg s.c., on day 16), daily treatments from day 13 until delivery for P4 (4 mg s.c. or 15 mg vaginal bid), 17P (10 mg s.c.) and controls (0.2 ml sesame oil (vehicle) s.c. or 0.18 ml vaginal gel bid). Note that injections of P4 completely blocked delivery, whereas 17P or vaginal P4 had no effect on delaying term delivery. RU-486 induced preterm delivery.