Diagnosis and Effective Management of Preterm Labor

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ABSTRACT
Despite extensive research, we are still unable to diagnose, prevent and treat preterm labor. Monitoring efficacy of interventions that would allow this is largely biased by the inability to accurately identify true labor with the currently used crude technology. Progestin supplementation appears to be a promising approach to both preventing initiation of preterm labor and treating it once it is already established, given progesterone’s role in maintaining pregnancy as well as support from basic and clinical research. However, the questions on mechanisms of action, optimal progestin formulation, dose, route and timing of administration remain unanswered. We have established and reported noninvasive means to accurately monitor cervical ripening, by measuring collagen light-induced fluorescence (LIF) and myometrial contractility, by measuring uterine electromyography (EMG). By accurately assessing the two components of parturition, cervical LIF and uterine EMG can help to identify effective prevention strategies and treatment of preterm labor.

Keywords: Labor and delivery, Uterine electromyography, Cervical light-induced fluorescence, Progesterone, 17-alpha-hydroxyprogesterone acetate.


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INTRODUCTION
Preterm birth remains the biggest unsolved obstetrical problem. As much as 70% of perinatal mortality is attributed to prematurity, and many of the surviving preterm infants suffer serious lifelong morbidity, including cerebral palsy, blindness, hearing loss, learning disabilities and other chronic conditions. In spite of extensive research and a variety of interventions, the incidence of preterm birth has not declined. Presently, there are about 15 million babies born prematurely in the world among a total of about 150 million births per year. In Europe, the preterm birth rate varies from about 5 to 11%, with relatively lower preterm birth rates in Finland, the Baltic countries, France and Sweden, and higher rates in Austria and Germany. Births occurring before 32 weeks of gestational age, when the risk of death and handicap is especially increased, account for about 1% of all births. Even more disturbingly, in the United States the incidence of preterm birth has been consistently rising. A more than 20% increase in the preterm birth rate was observed between 1990 and 2006. Births after 32 weeks accounted for most of this increase, but births before 32 weeks also increased. There has been a slight decline in the incidence of prematurity recently, but this has been primarily among late preterm births.

Most common interventions recommended to prevent preterm birth, such as bed rest, tocolytics, antibiotics and cervical cerclage have been proven to have little or no benefit. Once preterm labor is established, the goal of treatment is merely to delay delivery in order to allow for the transfer of the pregnant patient to the most appropriate hospital and for administration of corticosteroids. None of the currently available treatments for preterm labor can prolong pregnancy sufficiently to allow further intrauterine growth and maturation of the fetus. There is experimental support from animal and in vitro studies, and also empirical evidence from large randomized placebo-controlled clinical trials, that treatment with progestins may reduce the risk of preterm birth in both high-risk asymptomatic patients and in those presenting with signs and symptoms of preterm labor. Progestins are a group of steroid hormones that include natural progesterone and its analogs, such as 17 alpha hydroxyprogesterone caproate (17P) and medroxyprogesterone acetate (MPA).

Research of progestin and other potential treatments for preterm labor is largely hindered by the inability to reliably distinguish patients who are going to deliver preterm from those who are not. The analyses of effects of treatments are largely confounded by inclusion of patients who would not deliver preterm regardless of intervention. The first step in finding an effective prevention and treatment for preterm labor is, therefore, finding a method that will allow targeting...
the treatment only to patients who would, if not treated, really deliver preterm.

**Model of Parturition**

Parturition, both at term and preterm, is a complex process involving ripening of the uterine cervix and activation of the myometrium. Understanding and accurate assessment of these two components is the key to reliably predict and effectively treat preterm labor.

**Cervix**

The term ‘cervical ripening’ summarizes many biochemical and functional changes that result in the softening, effacement, and dilatation of the cervix, eventually allowing the delivery of the fetus. During this progressive event, the connective tissue in the cervix, consisting predominantly of collagen, is degraded and rearranged.13 Cervical ripening does not depend on uterine contractions and is similar to an inflammatory reaction. It involves the infiltration of polymorphonuclear cells and a release of degradative enzymes—metalloproteinases, resulting in a decrease of collagen concentration in the tissue.14 The changes in collagen content in the cervix, and consequently the degree of cervical ripening, can be assessed noninvasively by measuring the light-induced fluorescence (LIF) of the non-soluble collagen (Graph 1).15

**Myometrium**

Several events in the myometrium precede labor. Excitability of cells increases due to changes in transduction mechanisms and synthesis of various proteins, including ion channels and receptors for uterotonins.16-18 At the same time, systems that inhibit myometrial activity, such as nitric oxide, are downregulated, leading to withdrawal of uterine relaxation.15 Electrical coupling between myometrial cells also increases, and an electrical syncytium allowing the propagation of action potentials from cell to cell is formed.19,20 These changes are required for effective contractions that result in the delivery (expulsion) of the fetus. The transition from the nonlabor to the labor state of the myometrium can be identified by monitoring the uterine electromyographic (EMG) activity from the abdominal surface noninvasively.15,21,22 An increase in uterine EMG activity corresponds to the increase of uterine contractility immediately preceding delivery in rats (Graph 2). Changes in certain EMG parameters, such as power spectrum (PS) peak frequency and amplitude, and propagation velocity of uterine electrical signals, also indicate the onset of true labor at term and preterm in humans (Graph 3B).21,23-25

**Timeline of Events**

The two components of parturition, i.e. cervical ripening and activation of the myometrium, take place in a different time frame. Studies of cervical LIF showed that the process of softening and shortening of the cervix starts in mid-pregnancy, or even sooner (Graph 3A).26 Although cervical LIF and cervical length have not been directly compared yet, this seems to be in accordance with transvaginal ultrasound studies which showed that the cervix gradually shortens throughout gestation.27 The myometrial activation, in contrast, is a more acute event, occurring relatively close to delivery. In rats, the uterine EMG activity increases not more than 24 hours before delivery (see Graph 2).28 Similarly, in humans, the increases of EMG PS peak frequency and propagation velocity, which accurately identify myometrial preparedness for labor, do not typically occur more than seven days from delivery preterm and generally even later at term (see Graph 3B).24,25

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**Graph 1:** Gradual changes in rat cervix during cervical ripening assessed by measuring the light-induced fluorescence (LIF) of collagen shown are LIF measurements as ratios of the collagen peak vs the reference peak values in nonpregnant rats, during different times of pregnancy (day 13 to day 22) and postpartum (day 1 to day 17)
In conclusion, parturition is composed of cervical ripening and myometrial contractility. The changes in the cervix and the muscle leading to delivery of the fetus take place in a different time frame. Cervical ripening is a slower process which is initiated already in mid pregnancy, whereas the myometrium becomes activated acutely, just prior to the delivery.

**Diagnosis of Preterm Labor**

The diagnosis of preterm labor today still often relies on presence of contractions assessed by tocodynamometry (TOCO) and cervical change assessed by digital cervical examination. However, contractions occur commonly in normal pregnancy, and their detection through maternal perception and/or TOCO has a low sensitivity and positive predictive value for preterm delivery. Moreover, digital cervical examination suffers from large variations among examiners, and its prognostic values have also been shown to be low.

There is substantial evidence that measuring the cervical length by transvaginal ultrasound and testing for fetal fibronectin in cervicovaginal fluid can help to identify patients at particularly high-risk for preterm delivery. Cervical length is inversely related to the rate of preterm delivery in both patients presenting with symptoms of preterm labor and in asymptomatic pregnant women. Fetal fibronectin is an extracellular matrix glycoprotein produced by amniocytes and by cytotrophoblast, that normally resides at the decidual-chorionic interface. Its presence in the cervicovaginal fluid indicates decidual activation. However, the value of these two tests lies mostly in their high negative predictive values (NPV), while their positive predictive values (PPV) are lower and they do not identify patients who are really going to deliver preterm reliably.

None of the currently used methods can, therefore, distinguish between true and false preterm labor reliably. This results in unnecessary treatments, missed opportunities to improve outcome of premature neonates, and also our inability to analyze the effects of treatments which largely hamper the development of more beneficial therapeutic approaches.

**Role of Light-induced Fluorescence of Collagen**

Disruption of collagen in the cervical extracellular matrix occurs prior to delivery at term and preterm. The methods currently available to clinicians to assess these changes in the cervix have several major drawbacks. Digital cervical examination is subjective, and not accurate in predicting preterm delivery. Measurement of cervical length by transvaginal ultrasound is a more reproducible method, and has a high NPV. It therefore reliably identifies patients in whom the probability of preterm delivery is very low.
but a short cervix does not necessarily mean that the patient is really going to deliver preterm.\textsuperscript{33,34} As mentioned previously, we have shown that cervical collagen content can be monitored noninvasively by measuring LIF of collagen.\textsuperscript{39} This methodology allows an objective assessment of the change in cervical structure, and can detect the change in the composition of the cervix, regardless of its length. It is, therefore, a more accurate method to diagnose cervical ripening. It can potentially detect pregnant women at risk of preterm birth well before changes in cervical length occur.

**Role of Uterine Electromyography**

Previous studies have established that the electrical activity of the myometrium is responsible for myometrial contractions (Fig. 1).\textsuperscript{40,41} Extensive studies have been done to monitor uterine contractility using the electrical activity measured from electrodes placed directly on the uterus.\textsuperscript{42,43} More recent studies published by our group and by others indicate that uterine EMG can be monitored non-invasively from the abdominal surface (Fig. 2).\textsuperscript{15,21,22,44,45}

Measuring uterine EMG activity has similar effectiveness of simple detection of uterine contractions as does TOCO, and even as compared to intrauterine pressure catheter.\textsuperscript{46-49} In addition, many studies have shown that different uterine EMG parameters can indicate myometrial properties that distinguish physiological preterm contractions from true preterm labor, which is something that the other contraction-monitoring devices cannot do.\textsuperscript{24,44,50}

Of all of the possible EMG diagnostic variables, ‘timing related’ EMG parameters seem to have the least predictive value. We recently analyzed the duration of uterine EMG ‘bursts’, the interburst interval duration (which is inversely proportional to the frequency of the bursts) and the standard deviation of burst and interburst interval duration in patients admitted with the diagnosis of preterm labor at <34 weeks. None of these parameters differed significantly between the group of patients who delivered within 7 days and those who did not.\textsuperscript{25} This is not in accordance with some studies, which found that the standard deviation of burst duration was smaller, and the frequency of burst was higher in labor patients.\textsuperscript{50,52} We did, however, confirm the findings of Leman et al and Buhimschi et al, who observed no differences in burst duration between preterm labor patients and women with preterm contractions that did not deliver preterm.\textsuperscript{44,50} Burst duration and frequency of bursts are the electrical equivalent of the duration and frequency of contractions, and these, not coincidentally, are the only properties of contractions that can be evaluated by TOCO. Thus, their poor predictive values are not surprising, since monitoring uterine activity with TOCO is not helpful in identifying patients in preterm labor.\textsuperscript{29,49}

Another type of EMG parameters can be categorized as ‘amplitude related’. Such parameters may represent the uterine EMG signal power or alternatively, the EMG signal energy. Buhimschi et al demonstrated that an increase in PS
peak amplitude precedes delivery. Other studies did not confirm these findings. In the previously mentioned study on preterm labor patients, neither power spectrum (PS) peak amplitude nor PS median amplitude were significantly higher in patients who delivered within 7 days compared to those who did not. It has been suggested that the major limitation of ‘amplitude related’ EMG parameters is the fact that attenuation of myometrial signals occurs more for some patients and less for others, depending on a variance in subcutaneous tissues, and a variance in conductivity at the skin-electrode interface. These limitations make the ‘amplitude related’ EMG parameters interesting, but perhaps less reliable, in the prediction of preterm labor.

The third group of EMG parameters can be defined as ‘frequency related’ parameters and it includes PS median and peak frequency. Median frequency, although usually the most important parameter in the analysis of striated muscle EMG, is rarely reported to be useful in the uterine EMG literature. The reason for that is probably the difference in the PS of the signals from the uterine and striated muscle cells. The PS of a striated muscle covers a broad frequency range (20-400 Hz), with a more or less bell-shaped distribution of signal energy. Thus, for striated muscle, the median frequency is a most useful parameter in the analysis of these signals. On the other hand, uterine EMG signals are filtered in order to exclude most components of motion, respiration and cardiac signals, which yields a narrow ‘uterine-specific’ band of 0.34 to 1.00 Hz. In this narrow frequency band produced by the uterus, the location of the power peak differs from one recording to another, and there are often competing ‘lesser’ power-spectral peaks, not generally of consequence in the broad power-spectra of striated muscle. This suggests that the type of narrow-band power distribution found in the uterine-specific range of frequencies may render using the median frequency a less useful parameter for characterizing the uterine electrical signals. Verdenik et al have, however, reported that as pregnancy approaches term, the median frequency of the uterine electrical activity becomes lower. It is not clear why this should be so, since other literature supports shifts to higher frequencies as a transition to labor occurs. Furthermore, shifts to lower median frequency in the electrical PS of muscle are generally attributed to muscle fatigue. A possible explanation for this is that the median PS frequency for the whole 30 minutes EMG recording, and not for each burst separately, was analyzed in that study. It may be that including non-uterine related electrical information (from the large portions of the recordings ‘in-between’ bursts) contributed somehow to this result.

In contrast, PS peak frequency has been one of the most predictive EMG parameters in both human and animal studies. Shifts to higher uterine electrical signal peak frequencies occur during transition from a nonlabor state to both term and preterm labor states, and can be reliably assessed by noninvasive transabdominal uterine EMG measurement. PS peak frequency also increases as the measurement-to-delivery interval decreases. The best predictive values of PS peak frequency have been identified at different measurement-to-delivery intervals by different authors. Generally, an increase in PS peak frequency occurs within approximately 24 hours from delivery at term, and before that (within several days from delivery) at preterm gestations.

We have recently explored a new EMG parameter: the propagation velocity (PV) of uterine EMG signals. It has been shown in vitro that the PV of electrical events in the myometrium is increased at delivery when gap junctions are increased. We demonstrated that PV can be assessed from the noninvasive uterine EMG recording in vivo by estimating the time interval between EMG signal arrivals at adjacent electrode pairs. We have also shown that PV may predict preterm delivery more reliably than any other EMG parameter investigated so far.

Both EMG PV and PS peak frequency more accurately identify true preterm labor than today’s clinical methods. By combining the PV and PS peak frequency, we constructed a model that predicted spontaneous preterm birth with and area under the receiver-operating-characteristics curve of 0.96 (Table 1). This makes this methodology extremely valuable in everyday clinical practice. When uterine EMG is measured in patients presenting with signs and symptoms of preterm labor and the combination (rescaled sum) of PV and PS peak frequency exceeds the cut-off value of 84.48, this predicts delivery within 7 days.
with a 100% certainty according to our data (PPV = 100% in 88 patients). EMG does, therefore, identify patients who really benefit from early institution of tocolytic therapy, transport to a hospital with facilities for neonatal intensive care, and administration of steroids. At the same time, this methodology also identifies patients in false preterm labor who are not going to deliver within the next 7 days. It can, therefore, help to avoid substantial economic costs associated with unnecessary hospitalization, the maternal risks associated with tocolytics, and the potential fetal risks associated with steroids. In the case of low PV + PS peak frequency values, it therefore stands to reason that it would be safe not to admit, treat, or transfer the patient, regardless of the presence of contractions on TOCO, and regardless of digital cervical examination and transvaginal cervical length results, since all of the changes in the myometrium required for labor are not yet fully established. Other than being extremely important clinically, a methodology to accurately diagnose preterm labor would also be important in the research of new and potentially better treatments for preterm labor.

Importance of Diagnostic Tests for Monitoring Efficacy of Treatment for Preterm Labor

The current standard treatment for preterm labor, i.e. tocolytics, was not shown to have any clear effect on perinatal mortality or on any measure of neonatal morbidity related to prematurity. Research of new and potentially more effective treatments for preterm labor is, therefore, extremely important. Development of such treatments, however, depends largely on the tests used to diagnose labor. Current methods do not allow to distinguish patients in true preterm labor, who would deliver preterm if not treated, from those in ‘false’ preterm labor, who present with signs and symptoms of labor, but would not deliver preterm regardless of treatment. The overall PPV of currently used methods to predict preterm delivery is only about 50%, as mentioned above. This invariably leads to inclusion of patients in ‘false’ labor into studies of effectiveness of treatments.

A method with higher diagnostic accuracy, such as uterine EMG, would allow the performance of clinical trials on just patients who are really in true preterm labor. It has been shown, that using the EMG of the uterus we are able to predict preterm delivery very accurately (with a PPV of 100% based on our data) (Graph 4 and Table 1).

The impact that this technology could have on investigation of new treatments can be illustrated by a hypothetical case of a 10% effective treatment, i.e. a treatment that would prolong pregnancy more effectively than tocolytics in 10% of patients. In order to test for the efficacy of such treatment one would need to compare a group of patients treated with the drug to a group of patients treated with a placebo. Consider the two studies presented in Flow Chart 1. In the first study preterm labor would be diagnosed with currently available methods. Fifty of 100 patients diagnosed as being in preterm labor will not deliver preterm regardless of whether they will be treated or not. On the other hand, 50 of these 100 patients will deliver preterm if not treated. With equal randomization to treatment groups, 50 patients will receive the 10% effective treatment. Twenty-five of these will not be in true preterm labor, and 25 will be in true preterm labor. Around 2.5 patients in true labor will therefore not deliver preterm due to treatment. Consequently, of the 50 treated patients diagnosed clinically as being in preterm labor, and 25 will be in true preterm labor. Around 2.5 patients in true labor will therefore not deliver preterm due to treatment. Consequently, of the 50 treated patients diagnosed clinically as being in preterm labor, 27.5 (25 who were not at risk in the first place and 2.5 at risk patients who responded to treatment) patients will not deliver preterm. The response rate will, therefore, be 55% (27.5/50 = 0.55). In the placebo group, 25 patients will not deliver preterm because they were not in true labor in the first place, thus 50% response rate (25/50 = 0.50). The difference in treated vs placebo group is only 0.05. Utilizing a calculator for sample size based on proportions, one would need 3129 total patients recruited to the study to find this small difference (with an alpha of 0.05 and 0.80 power).

In the second study, uterine EMG would be utilized to diagnose preterm labor. As a result, all 100 patients will theoretically be in true labor (PPV=100%). If patients are randomized equally and there is a 10% response rate, five patients (10%) in the treatment group will respond (not deliver preterm), and 0 in the placebo group. Utilizing sample size calculator based on proportions, one would need only 147 patients to find this large difference (again with an alpha of 0.05 and 0.80 power).
Increased effectiveness of treatment will lower the values in both studies. With a 30% effective treatment, e.g. one would need to include 339 patients in the first study, i.e. when currently available methods would be used to diagnose preterm labor, and only 42 patients when utilizing uterine EMG.\(^5\) This example emphasizes the importance of diagnostic accuracy of uterine EMG. It will not only allow physicians to make safer and more cost-effective clinical decisions but will also eventually lead to development of treatments for preterm labor that will improve neonatal outcome. Even if such treatment existed already, today one would need to include 3,129 patients in a study to demonstrate a 10% efficacy. On the other hand, the same rate of efficacy could be demonstrated on just 147 patients using uterine EMG to diagnose preterm labor.

**Management of Preterm Labor**

Prevention of preterm birth can be categorized as primary when aimed at prevention and reduction of risk factors in the general population, secondary when the aim is to identify and treat individuals with increased risk, and tertiary when treatment is initiated after preterm labor is already established. Although some interventions have been proven to be beneficial in selected population of pregnant women with certain risk factors, more than 50% of patients who deliver preterm have no apparent risk factors.\(^6\) Therefore, the tertiary prevention of preterm birth, i.e. treatment of preterm labor, is crucial in lowering the burden of prematurity.

**Current Treatment of Preterm Labor**

For several decades, stopping uterine contractions, i.e. tocolysis, has been the focus of treating preterm labor. The reason for this is the incorrect assumption that uterine contractions detected by the patient or TOCO indicate the changes in the myometrium responsible for initiation of labor. Inhibition of contractions should, therefore, prevent preterm delivery and reduce neonatal mortality and morbidity. Unfortunately, however, this is not the case. Neither do clinically used methods to assess uterine contractility detect the molecular changes characteristic of myometrial activation and true labor, nor have tocolytic agents available today been shown to improve neonatal outcome.\(^1\) Consequently, it is therefore only reasonable to use tocolytics in preterm labor patients who need to be transferred to a hospital with facilities for neonatal intensive care and in those who have not yet completed a full course of antenatal corticosteroids, since tocolytics reduce the proportion of births occurring within seven days from the beginning of treatment but do not improve outcomes per se.\(^12\)

**Mechanisms of Action of Tocolytics**

Several pharmacologic agents are currently used to achieve tocolysis: beta-adrenergic agonists (e.g. terbutaline); magnesium sulfate; nitric oxide donors (e.g. nitroglycerin), calcium channel blockers (e.g. nifedipine); cyclooxygenase inhibitors (e.g. indomethacin), and oxytocin receptor antagonists (e.g. atosiban). These agents cause uterine relaxation by several mechanisms: Beta-adrenergic agonists increase the levels of intracellular cyclic adenosine monophosphate (cAMP), which inactivates myosin light-chain kinase and consequently inhibit contractility.\(^6\) However, the ability to generate and react to cAMP decreases during labor. Calcium channel blockers inhibit the influx of calcium ions through the plasma membrane and the release of intracellular calcium from the sarcoplasmatic reticulum, leading to a decrease in calcium-mediated activity of myosin light-chain kinase.\(^mol\) Cyclooxygenase inhibitors achieve tocolysis by suppression of prostaglandin synthesis.
Prostaglandins increase the formation of myometrial gap junctions and increase the intracellular concentration of calcium by raising its transmembrane influx and its release from sarcoplasmatic reticulum.66 And finally, oxytocin receptor antagonists compete with oxytocin for binding to its receptors. They consequently reduce the oxytocin-mediated conversion of phosphatidylinositol triphosphate to inositol triphosphate which causes the release of calcium from the sarcoplasmatic reticulum.67

To summarize, currently used tocolytics inhibit myometrial contractility through altering the intracellular transduction pathways responsible for cell contraction, inhibiting the synthesis of myometrial stimulants, or blocking the actions of myometrial stimulants. None of them, however, can reverse the processes leading to activation of the myometrium during labor at term or preterm.

Use of Progestins to prevent Preterm Birth

Progesterone has been known to be important in maintaining pregnancy for more than 80 years, since the classic work of Corner, Allen and Csapo.68,69 A large body of experimental data available today demonstrates that progesterone exerts overall control on both cervical ripening and myometrial contractility. Supplementation of progesterone or its analogs seems, therefore, a very promising strategy for prevention of preterm birth.

Antiprogestins induce ripening of the uterine cervix.70 Therefore, the cascade of events leading to cervical ripening seems to be controlled at least in part by progesterone. In the cervix, progesterone modulates the expression of various genes, including those involved in regulation of epithelial and endothelial permeability and metabolism of components of the extracellular matrix.71 Progesterone also inhibits the ripening process by suppressing the production of proinflammatory cytokines and consequently reducing prostaglandins in the cervix.72 In a recent study, we used cervical LIF to study the effects of progesterone treatment on cervical ripening in rats. Subcutaneous (SC) and transdermal administration of progesterone significantly delayed cervical collagen degradation but did not completely suppress ripening.73 SC administration of 17P also delayed cervical ripening, although less effectively than did progesterone (Graphs 5A and B). This is in accordance with the results of some clinical trials (discussed below) that observed an attenuation of cervical shortening measured by ultrasound with intramuscular (IM) 17P and vaginal progesterone treatment.27,74,75

Progesterone also inhibits myometrial activity by several mechanisms. It suppresses a number of genes that are essential for effective uterine contractions, including genes for the gap junction protein connexin 43, calcium channels and oxytocin receptors, etc.15 It also upregulates the relaxation mechanisms, such as the generation and action of cAMP and cGMP.15 In addition, progesterone acts by functionally opposing estrogen, which increases myometrial contractility.15 Treatment with onapristone (ZK-98299), a pure antiprogestin, induces preterm delivery and increases the EMG activity in rats (Graph 6).28 Recently, we have examined whether progesterone inhibits birth at term in rats. We administered micronized progesterone topically and SC daily beginning from day 19, day 20, or day 21 of gestation and 8 hours before normal delivery on day 22. All topical or SC treatments prevented birth up to 80 hours beyond the normal time of delivery observed in control, vehicle treated, rats (Graph 7). The significance of these observations is that progesterone can prevent birth when given after the decline in progesterone levels in the blood and even after the cervix...
Graph 6: Treatment with onapristone (ZK-98299) on day 16 of pregnancy induces preterm delivery and an increase the EMG PS peak frequency in rats.

Graph 7: The delay in delivery in pregnant rats treated daily with parenteral (4 mg subcutaneous injection, SC, of micronized progesterone) or topical progesterone (2 times daily, 15 mg micronized progesterone in fish oil) for 3 days (days 19-21), 2 days (days 20-21), 1 day (day 21) or 8 hours before normal delivery (day 22) on day 22 at 8 AM of gestation compared to controls (CTR). Note that all progesterone treatments substantially delay delivery, even when progesterone is given 8 hours prior to normally delivery. All animals treated with progesterone were sacrificed at 80 hours following 8 AM on day 22 of gestation. This study illustrates that progesterone can prevent delivery if given prior to the end of gestation at times when the cervix is already soft and prepared for delivery.

is already ripened and prepared for delivery. To further examine, the effects of progesterone on uterine.

Electromyography (EMG) activity we used anesthetized rats at term and sometimes animals treated with antiprogestins which were delivering preterm. We placed electrodes directly on the uterus and recorded EMG activity by conventional recording equipment as we have done previously. We recorded EMG activity over several hours and remarkably these animals delivered fetuses, although sometimes the fetuses were not completely passed through the cervix, probably because the animals were under anesthesia and did not contract the abdominal muscles or push during delivery. We noted that the EMG activity was extremely high (Fig. 3) and very similar what we had observed previously in animals fitted with internal EMG devices to measure activity without anesthesia. Animals treated with progesterone 1 to 2 days prior to normal delivery had very low levels of EMG activity (Fig. 4). We repeated these experiments in many studies and noted the following: progesterone (SC) significantly reduces the EMG burst frequency (bursts/30 mins ± SEM: 1 day treatment = 11.1 ± 1.7 vs controls 23.2 ± 3.5, p < 0.01; 2 days treatment = 10.5 ± 1.3 vs controls 25.4 ± 4.4, p < 0.01). The EMG burst amplitudes are also significantly lower in the progesterone-treated animals (µV ± SEM: 1 day treatment = 74 ± 8 vs controls 280 ± 58, p < 0.008; 2 days treatment = 127 ± 31 vs controls 230 ± 14, p < 0.02). Thus, the mean burst integrals (V2) are suppressed at 1 (p < 0.001) and 2 (p < 0.002) days after P4 treatment vs controls, but not the burst duration (p > 0.05, cca. 30 seconds). This indicates that progesterone treatment suppresses uterine EMG activity and thereby inhibits birth. These studies demonstrate the crucial role of myometrial inhibition in prevention of preterm delivery. The initiation of human studies on effects of various progestins on uterine EMG activity, will further address the question of myometrial inhibition by progestin treatment.

In the last 40 years, progesterone and its analogs have been administered to pregnant women in attempts prevent preterm birth and miscarriage, but with variable success. Comparing these studies is extremely difficult because they differ in terms of formulation and dose of progestin used, route of administration and timing of progestin administration.

Formulations of Progestins

Progestins are available as natural (bioidentical, micronized) progesterone and its synthetic analogs. Of the various formulations, only two have generally been considered sufficiently safe and effective to be used for prevention of preterm birth: progesterone and 17P.

Natural (Bioidentical, Micronized) Progesterone

Micronized progesterone is manufactured in a laboratory from chemicals derived from plants (Mexican wild yams and soy). It has a molecular structure identical to that of the progesterone produced in humans by the corpus luteum in the luteal phase of the menstrual cycle and during the first
Fig. 3: Electromyography recordings from a normal pregnant rat on day 22 of gestation during delivery (arrows indicate delivery of a fetus). Recordings showing >60 minutes (top record) and for >7 minutes (bottom). Note delivery of fetuses as marked with arrows. This indicates that the animals deliver their fetuses while under anesthesia and the uterine contractions which accompany birth consists of large bursts of EMG activity. This is an example from studies using more than 20 rats.

Fig. 4: Examples of EMG recordings from pregnant rats on day 22 of gestation from control rat (vehicle treated during delivery, top tracing, arrows indicate delivery of a fetus) and rat after two days treatment with P4 (4 mg micronized progesterone, P4, given subcutaneously for 2 days on day 20 and day 21 of gestation (animals not delivering). Note the difference in the bursts (amplitude and frequency) of EMG activity in control rat recording compared to the rat treated with P4. Similar differences were observed following topical treatment of P4 but not after vaginal treatment.

trimester of pregnancy and (following the luteal-placental shift) in the largest quantity by the placenta. Micronized progesterone can consequently be referred to as the natural or the bioidentical, progesterone.

Safety of Progesterone in Pregnancy

In the randomized clinical trials comparing progesterone to placebo for the prevention of preterm birth, long-term infant outcomes were not evaluated.77-79 However, natural progesterone is FDA-approved to support embryo implantation and early pregnancy, and there have been no significant adverse effects from its use in pregnancy reported to date. It should be noted that progesterone production by the placenta during pregnancy can reach levels of about 500 mg/day at term.80

17 Alpha-hydroxyprogesterone Caproate (17P)

This chemical is an artificially made caproate ester of 17 hydroxyprogesterone, a natural progestin produced during pregnancy in much lower quantities than progesterone. It has been developed to produce longer-lasting effects than would be available from progesterone itself.80 The half-life of 17P is approximately 7.8 days, as compared to approximately 35 to 55 hours for progesterone.80,81

Physiologically, 17P is thought to have similar effects to that of progesterone. To a certain degree, this assumption is probably correct. For instance, both agents cause a secretory transformation of the endometrium.82,83 However, there are also important physiologic differences that should be considered when deciding which agent to use in the prevention of preterm birth. Our group and others have demonstrated that 17P does not suppress myometrial contractility, whereas progesterone does.84,85

Safety of 17P in Pregnancy

There is evidence suggesting that the use of 17P in pregnancy is safe. In the follow-up of a single randomized trial comparing 17P to placebo for preventing preterm birth, there were no statistically significant differences in the health and development of children at 2 years of age.86 However, there is also some data from animal and human studies suggesting that 17P may cause fetal harm by fetal toxicity (not teratogenicity). In mice, there was an increased fetal loss with 17P compared to placebo.87 In rhesus monkeys, total embryolethality resulted following the administration of 17P at both 1X and 10X the human equivalent dose.88 Moreover, although not statistically significant, there was an increase in intrauterine fetal death among women receiving 17P compared to placebo in the clinical trial, whose follow-up has been mentioned above.89 An earlier meta-analysis of 17P also showed a possible, again non-statistically significant, increase in miscarriage with an odds ratio of 1.3 (0.61-2.74).90 Further studies are needed in order to evaluate the potentially increased risk of miscarriage and stillbirth associated with the use of 17P. There are also some concerns regarding the vehicle used for 17P injections, namely castor oil. Castor oil was reported to induce labor through release of prostaglandins.91 17P is currently FDA pregnancy category D progestin, meaning that the FDA believes there is evidence of fetal harm.
Route of Administration

While 17P is given exclusively IM, progesterone has been administered by several routes in different studies: orally, IM and vaginally. Transdermal supplementation of progesterone to prevent preterm birth has not been studied in humans yet but it is common to use this route for application of steroids in humans.

The main advantage of oral administration of progesterone is its noninvasiveness and consequent acceptability. However, absorption of oral progesterone is quite variable, and it is rapidly metabolized by first-pass effect in the liver, which makes the oral administration essentially ineffective. Moreover, side effects, such as sleepiness, fatigue and headaches, are more common when progesterone is given orally. Three randomized trials to date compared oral progesterone to placebo for prevention of preterm birth. In the studies published in 1986 and 1991, oral progesterone did not prolong gestation in patients treated for preterm labor. In 2009, in contrast, Rai et al reported a reduction in preterm delivery in women with a history of preterm birth who received oral progesterone throughout pregnancy compared to placebo.

Effectiveness of IM injections of progesterone to prevent preterm birth has not been evaluated in clinical trials. The reason for this is that daily IM injections would be required to maintain therapeutic serum levels due to the relatively short half-life of progesterone. This would make this intervention very invasive, especially if progesterone was to be given by prolonged prophylactic administration to women at increased risk for preterm birth. 17P is a long-acting progestin, and can be administered once per week. Even with weekly IM injections, however, side effects, such as injection site pain, swelling, itching and bruising, have been reported in up to one-third of treated women, and were more common in the progestin group as compared to placebo.

The vaginal route of progesterone administration has been thought to be the preferred route when focused effects on the uterus are desired. It is noninvasive and the only side effect associated with vaginal progesterone reported in clinical studies was an increased vaginal discharge. Following the concept of the liver first-pass effect after administration of oral drugs, the term ‘uterine first-pass effect’ was established in order to point out the minimized systemic, but optimized uterine exposure after vaginal treatment with sex steroids. De Ziegler et al observed a 14-fold increase in the ratio of the endometrial-to-serum concentrations of progesterone after vaginal (compared to IM) administration. However, these studies were mostly done in postmenopausal women and not during pregnancy. Volume, viscosity and pH of vaginal fluid and physical properties of vaginal epithelium largely affect the absorption of vaginally administered drugs. All of these factors are significantly different in pregnant women as compared to those after menopause.

In addition, the effectiveness of vaginal progesterone seems to depend significantly on the vehicle utilized. Three randomized clinical trials in which progesterone was administered as vaginal suppositories or capsules showed a reduction in preterm delivery. The exact source of progesterone was specified only in one of these three publications. Fonseca et al used of 200 mg capsules of Utrogestan®, i.e. progesterone in arachis (peanut) oil and soy lecithin. On the other hand, vaginal gel (Crinone®) containing 90 mg of progesterone in a bioadhesive gel Replens®, was utilized in the two large studies which reported no benefit from vaginal progesterone. Vaginal gel is claimed by some to have practical advantages over the capsules or suppositories. It is thought to be easier to apply and it does not liquefy. It is suggested, therefore, that it could cause less vaginal discharge, irritation and infection. Replens®, in particular, is thought to release progesterone slowly, which potentially results in sustained levels of the hormone in the uterus. However, in addition to evidence from clinical studies, our results indicate that progesterone in Replens® may not be as effective as in other vehicles. For example, transdermaly administered progesterone in fish oil delayed delivery in rats, while topical application of progesterone in Replens® did not. This indicates that Replens® does not efficiently release progesterone. Furthermore, measurement of serum progesterone levels supports these conclusions (Graph 8).

More data is needed before any formulation and route of progesterone administration for prevention of preterm birth can be recommended over the others. Our study of various progestin treatments emphasizes this. Only subcutaneous injections of progesterone and transdermal administration of progesterone in fish oil (not in Replens®) delayed delivery (Graph 9). Notably, none of these routes has been used in clinical trials to date. On the other hand, oral progesterone and vaginal progesterone administration, studied in humans so far, did not have any effect on time of delivery.

These studies clearly show that in animal models progestins with different properties have varied effects and depend upon the route of administration and vehicle.

Timing of Administration

Another reason why clinical studies of efficacy of progestins in preventing preterm birth are difficult to compare is that participants included were significantly different. The majority of randomized trials evaluated the prophylactic
supplementation of progestins in asymptomatic pregnant women at high risk for preterm birth. Women were considered to be at high risk for several reasons, including past history of spontaneous preterm birth or miscarriages, multiple gestation, short cervical length, cerclage in place and uterine anomalies. Earlier small trials using 17P showed mixed results. Some reported benefit from prophyllactic treatment in high-risk singleton pregnancies, whereas 17P injections did not improve outcome in multiple gestations and lower-risk patients. In 2003, two studies reinvigorated the interest in progestin treatment for prevention of preterm birth. Meis et al reported results of a large multicenter trial of 17P involving 463 women with a history of spontaneous preterm delivery. Delivery at <37 weeks was reduced from 55% in the placebo group to 36% in the 17P group. Similar reduction was seen in delivery at <32 weeks, from 20 to 11%. Also in 2003, da Fonseca et al published a trial of vaginal progesterone vs placebo suppositories administered to 142 women found to be at high risk due to a history of previous preterm delivery, prophylactic cerclage placed or having a uterine anony.

As a result, further studies were performed to evaluate the use of progestins in pregnant women with other risk factors. Twin pregnancies were the obvious next subject of randomized trials, given the current epidemic of multiple gestations. In 2007 Rouse et al showed that treatment with 17P did not reduce the rate of preterm births among women with twins, which is in agreement with the results of an earlier study published in 1980. Recently, a large study of 500 women with twin gestation, randomized to receive either vaginal progesterone or placebo, also showed no benefit of vaginal progesterone treatment in twin pregnancies. Patients who were found to have a short cervix (<15 mm) measured by transvaginal ultrasound, however, benefited from vaginal progesterone administration. Da Fonseca et al reported a reduction of delivery at <34 weeks, from 34% in the placebo group, to 19% in the progesterone group. In 2009, the largest randomized clinical trial of progesterone in prevention of preterm birth to date was published. O’Brien et al evaluated the effect of vaginal progesterone in women with previous spontaneous preterm birth, and did not find any difference between vaginal progesterone and placebo groups. However, further subanalysis of the data from that same trial was performed by DeFranco et al, which demonstrated that there may be a subset of women with shortened cervical length (<28 mm) for whom progesterone may have a beneficial effect in prolonging pregnancy.

While many studies examined the effects of progestin prophylaxis in pregnancies considered at high risk, there is substantially less data on progestin treatment of patients following acute presentation with signs and symptoms of

Graph 9: The time of delivery (hours after 8 AM of day 22 of gestation) of pregnant rats treated with vehicles (controls) and progestins by different routes of administration – injections (SC; daily): vehicle: sesame oil; progesterone (P4) (4 mg); 17P (10 mg); vaginal (bid): vehicle: Replens®; P4 (15 mg, Crinone®); oral (bid): vehicle: sesame oil or H2O; P4 (15 mg); transdermal (bid): vehicle: Replens® or fish oil; P4 (15 mg). Rats with delayed parturition were sacrificed on day 25. Asterisks indicate p < 0.05 compared with controls.

Graph 8: Plasma progesterone (P4) levels in pregnant rats on day 18 and 21 without any treatment (controls) and on day 21 after treatment from day 18 until delivery with (a) vaginal P4 (15 mg, bid), (b) SC injections of P4 (4 mg), (c) topical P4 in fish oil (15 mg, bid). Asterisks indicate p < 0.05 compared with controls. Note the physiological P4 withdrawal from day 18 to day 21 in nontreated rats, that is prevented by SC and topical P4, but not by vaginal P4.
preterm labor. This is unfortunate, because the majority of patients who deliver preterm do not have any risk factors. There are, therefore, many apparently low-risk pregnant women who present acutely in preterm labor, and the use of progestins to prolong pregnancy and improve outcome in these patients has not been studied sufficiently. Between 1960 and 1991, four trials used progestins to stop preterm labor. None of these showed any benefit in prolonging pregnancy. Two studies used oral progesterone, one IM 17P, and one IM MPA. The only other reported use of MPA in humans for prevention of preterm birth was in the study from Hobel et al in which MPA was also ineffective as prophylactic oral supplementation. Thus, it is impossible to generalize the results of the early trials of progestin treatment for preterm labor because of their different designs. In 2007, Fachinetti et al reported a reduction of risk of preterm birth with the use of 17P as twice weekly IM injections in patients treated for preterm labor in which tocolysis was obtained with atosiban. Borna et al used large doses of vaginal progesterone and showed that progesterone may be beneficial as a maintenance tocolytic, since it prolonged the latency to delivery in the treatment group as compared to patients who received no treatment.

To summarize, most clinical studies on progestin treatment for prevention of preterm birth have been accomplished in patients with various risk factors who received prophylactic 17P IM or progesterone vaginally for several weeks. Since myometrial activation is an acute event, this chronic supplementation of progestins is most likely to affect cervical ripening alone. However, animal studies showed that only minor delay in the cervical changes were observed following 17P or progesterone application. However, progesterone (not 17P) injections completely blocked delivery even after the process of cervical ripening was already completed. The main action must, therefore, be on the myometrium to inhibit labor. The possible benefit of progesterone inhibition of myometrial activity has, however, not been studied sufficiently in humans yet.

CONCLUSION

Despite extensive research, we are still unable to accurately predict or effectively prevent preterm delivery. In fact, current approaches to prevention and treatment of preterm labor have been shown to be disappointingly unsuccessful. There is evidence from animal studies and in vitro studies on human tissues that supports the use of progestins for reducing the risk of preterm birth. Most of the randomized clinical trials conducted so far have evaluated prophylactic progestin supplementation in asymptomatic women throughout pregnancy. The rationale for this prolonged use is the inhibition of cervical ripening. In fact, the process of gradual remodeling of connective tissue in the cervix begins already in mid-pregnancy and is suppressed at least in part by progesterone and 17P. The use of these two compounds has been shown in some trials to be beneficial for preventing preterm birth in patients with certain risk factors, such as previous preterm birth and short cervix. Myometrial contractility is also suppressed by progesterone (but not 17P). The use of progesterone in patients presenting with signs and symptoms of preterm labor therefore seems promising due to its ability to control both cervical ripening and myometrial activity. On the other hand, the role of progesterone and other progestins in treatment of these patients has not been sufficiently studied in randomized trials yet. Additionally, effects of progestin treatments have been shown to vary extremely between different progestin formulations and different routes of administration. The optimal formulation of progestin and its vehicle, dose, and route of administration for prevention of preterm delivery remain to be determined. In our previous studies, we documented evidence that myometrial preparedness to labor and changes in cervical structure can be monitored non-invasively by measuring uterine EMG and cervical LIF. These two methods objectively assess the two components of parturition: myometrial contractility and cervical ripening. They provide a methodology to evaluate various therapeutic interventions for preterm labor. In the case of progestin treatment for prevention of preterm birth, uterine EMG and cervical LIF are essential tools to obtain the critically needed comparative data on effectiveness of various progestin formulations and their routes of administration in different patients at high risk for preterm delivery. Future studies on efficacy of these treatments should, therefore, utilize the uterine EMG and cervical LIF systems.

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