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Issues in Obstetrics, Gynaecology and Reproductive Health
in ObGyn



PRETERM BIRTH

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Robert E. Garfield

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Editorial

It is a pleasure for me to organize a monograph on the diagnosis and treatment of preterm labor/birth for Facts, Views & Visions in ObGyn. In doing so, I sought to obtain papers from around the globe from some of the leading authors of papers on preterm labor and birth, and on function of the uterus and cervix during pregnancy. I thank all the authors for their manuscripts and I am particularly grateful to Professor Ombelet for his patience and guidance in preparation of this issue. Preterm birth remains one of the most important problems in medicine today. The subject of preterm birth was recently reviewed in Born Too Soon: The Global Action Report on Preterm Birth (see and download at http://www.who.int/pmnch/media/news/2012/preterm_birth_report/) edited by CP Howson, MV Kinney, JE Lawn of the World Heath Organization, Geneva, 2012. How to diagnosis and treat preterm labor and imminent preterm birth are the most challenging questions facing physicians caring for pregnant patients. In the past 10 years we have come full circle back to considering treatment of preterm birth with progestins. Thus, in this review we obtained facts, views and visions from investigators that have concentrated their studies on the mechanisms for progestin action on the uterus and cervix. We also acquired opinions from outstanding scientists and clinicians on function of the myometrium during pregnancy, and also considered postterm pregnancy. It is hoped by all the contributors that someday our studies and others will lead to better care of pregnant patients faced with the prospects of preterm birth.

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An update on prevention and management of preterm birth

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Abstract

Preterm birth is a pregnancy complication that is associated with neonatal adverse outcomes. It is considered an obstetric syndrome and several factors contribute to its pathogenesis, both of maternal and fetal origin. The knowledge of the pathogenetic mechanisms leading to preterm birth is very important in order to recognize women at risk and to perform the strategies for reducing the morbidity and mortality. Nowadays, the interventions to prevent preterm birth are represented by the administration of tocolytic and steroids drugs to mother and fetus, respectively. The development of new and safe drugs decreasing uterine contractility and infection will improve the prognosis of preterm birth.

Key words: **Preterm birth, stress, inflammation, CRH, prevention, risk factors, tocolytics.**

1. Inflammation and stress: impact on preterm birth

Preterm birth (PTB) is defined as birth before 37 weeks gestational age, occurs with an incidence of 7-11% and represents one of the most important causes of perinatal mortality and morbidity (Lockwood, 2002).

PTB may be considered a clinical syndrome that arises from different pathological processes that activate before 37 weeks of gestation one or more of the components of the pathway of parturition: cervix, fetus, fetal membranes, placenta and myometrium. This premature activation may be caused by multiple conditions, as inflammation/infection, uteroplacental ischaemia or haemorrhage, uteroplacental insufficiency (hypertension, insulin-dependent diabetes, drug abuse, smoking, alcohol consumption), uterine overdistension, cervical disease, stress and endocrine disorders, and/or other immunologically mediated processes (Romero et al., 2006) (Fig. 1).

Among all causes of PTB, inflammation, with or without infection, is a common condition leading to PTB and its pathogenetic mechanisms are related to the activation of the innate immune system (Romero et al., 2006; Romero et al., 2007; Challis et al., 2009). In this context, placenta, fetal membranes and the other key tissues activate the inflammatory pathway leading to PTB throughout the up-regulation of several molecules such as proinflammatory interleukin-1 beta (IL-1β), tumor necrosis factor alpha (TNF-α) and interleukin-8 (IL-8), chemokines and prostaglandins (PG) (Romero et al., 2002).

Therefore, the various causes of PTB act through some common mechanisms (Fig. 2):

a) ripening of the cervix: cytokines increases the production of matrix metalloproteinases (MMP-8, MMP-9), cyclooxygenase (COX)-2, PGE2, which are involved in the softening and dilation of the cervix (Sennström et al., 2000).
b) rupture of the membranes: proinflammatory cytokines increase MMP-9, collagenases and PG and decrease levels of tissue inhibitor of MMP-2, resulting in the rupture of the membranes (Xu et al., 2002).
c) myometrial contractility: TNF-α and IL-1β increase uterine contractility, the local expression

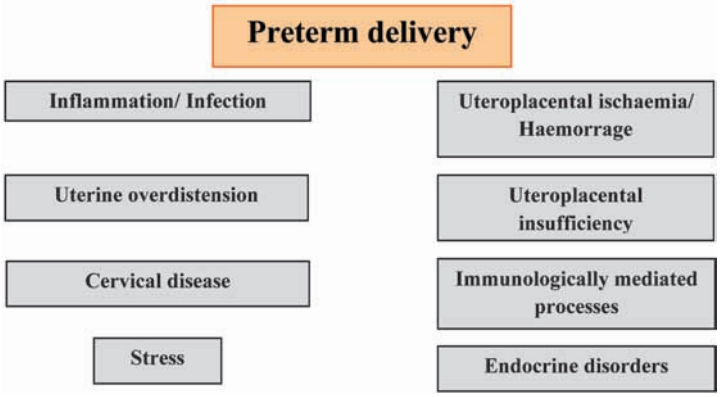

Fig. 1. — Pathogenetic mechanisms of preterm delivery

of COX-2 and the production of PGE2 (Slater et al., 1999). IL-6 up-regulates the expression of oxytocin receptors on myometrial cells (Rauk et al., 2001).

Beside inflammation, stress represent another key event for PTB (Copper et al., 1996), involving both neuroendocrine and immune functions. In this context, an increased secretion of several neuropeptides, such as corticotropin releasing hormone (CRH), occurs. It represents a key pivotal brain factor activated by all stressful conditions and modulating the endocrine (ACTH release from pituitary), the metabolic (adrenaline release from the adrenal medulla), the immune (cortisol release from the adrenal cortex) and the behavioural responses.

During pregnancy, CRH is produced by placenta and gestational tissues (Petraglia et al, 1987) and it is involved in different processes of pregnancy, as uteroplacental blood flow regulation, myometrial contractility, regulation of maternal-fetal HPA axis and feto-placental inflammatory/immune response (Petraglia et al., 2010). Concerning PTB, serum concentration and placental mRNA expression of CRH are higher than in control group (Wolfe et al., 1988; Torricelli et al, 2007) and in particular when associated with vaginal infection (Petraglia et al., 1995). Indeed, microbial invasion of the amniotic cavity represent a stress condition associated with significant CRH elevation in placenta extracts, both in maternal plasma and in amniotic fluid (Petraglia et al., 1995). To corroborate the important role of CRH in inflammatory events occurring in a recent study it was demonstrated that the expression of CRH mRNA was higher in placental tissues collected from women delivered preterm with histological chorioamnionitis (Torricelli et al., 2011). A feed-forward loop between proinflammatory cytokines and CRH exists: CRH enhances the LPS induced IL-1β secretion, and in turn IL-1β increases CRH production from cultured human placental cells (Petraglia et al., 1990). All together, this findings suggest that

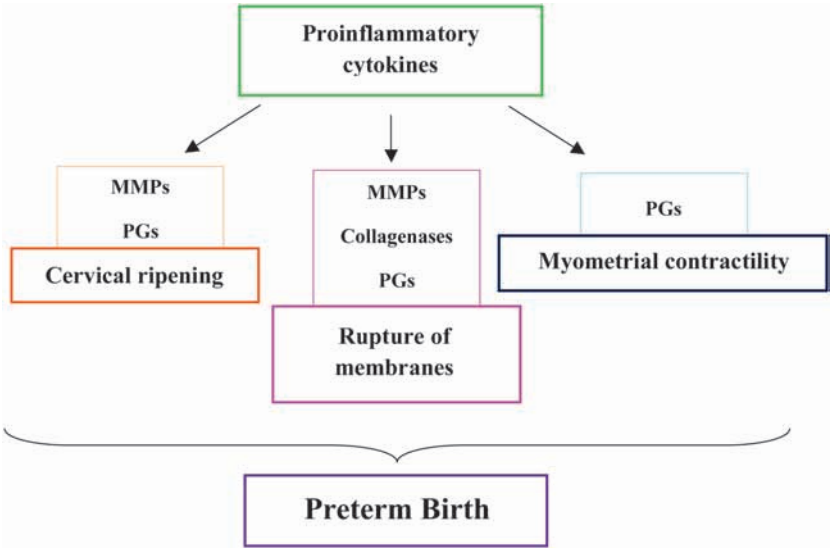

Fig. 2. — Common mechanisms leading to preterm birth

when an inflammatory process occurs, a placental expression of stress-related pathways is activated. Several evidences indicate that during pregnancy the secretion of CRH from intrauterine sources may be influenced by maternal and/or fetal physiological and pathological stress conditions (Pike, 2005). In fact, a relationship exists between stressful life events or poor social circumstances, psychological aspects, environment (eg, poverty, minority or unmarried status, and loss of employment, housing, or partner) and PTB, suggesting that external environmental events are responsible of this syndrome (Petraglia et al, 2001). Psychosocial stress may initiate preterm labor modulating placental products, such as ACTH, cortisol and prostaglandins (Challis et al., 2000; Petraglia et al., 2010). In fact, prematurity seems to be more common among mothers reporting increased stress and anxiety (Lockwood, 1999a) because of maternal stress is associated with PTB, the aberrant regulation of CRH and consequently the production of inflammatory cytokines could represent the pathophysiologic basis of this association. Maternal stress may act via different pathways: (a) a neuroendocrine pathway resulting in the activation of maternal-placental-fetal endocrine systems promoting parturition; (b) an immune/inflammatory pathway wherein maternal stress may modulate placental-decidual immunity thus increasing susceptibility to intrauterine and fetal inflammatory processes. In conclusion, the resultant increase in CRH production may represent a critical factor that could contribute to the early initiation and activation of uterine contractility and PTB (Wadhwa et al., 2001).

2. Identify women at risk

The prediction of spontaneous PTD represents a topic goal with the aim to reduce fetal and neonatal morbidity and mortality. To identify women at risk is a fundamental part of strategies to reduce the PTB in the general population (Goldenberg et al., 2005a). The major objectives to prevent PTB are represented by: (1) better understand the pathways leading to PTB; (2) identify a high risk population that could benefits from particular treatment and identify a low risk population to avoid unnecessary and costly interventions (Behrman et al., 2007) and (3) to initiate risk specific treatment.

Once the pathogenesis is addressed, interventions may be directed to prevent and reduce, before or during pregnancy, both the risk, morbidity and mortality related to PTB (Iams et al., 2008; Petraglia and Visser, 2009).

Many maternal or fetal characteristics have been considered as associated risk factors with PTB (Goldenberg et al, 2005a), and they were classified as periconceptiona, obstetric history or pregnancy associated risk factors (Fig. 3).

a) *Periconceptional risk factors*

- *Socioeconomic characteristics:* Black women are three times more likely to have a PTB than women from other racial (Goldenberg et al., 1996). Low socioeconomic status, low and high maternal ages, and single marital status are associated with PTB (Smith et al., 2007).
- *Nutritional status:* A low BMI, low serum concentrations of iron, folate or zinc are associated with high risk of spontaneous PTB (Bloomfield, 2011).
- *Stress:* Mothers experiencing psychological stress are at increased risk of PTB(Copper et al, 1996), and a role of CRH is proposed (Challis et al., 2000).
- *Systemic diseases:* Thyroid diseases, asthma, diabetes, and hypertension, are associated with increased rates of PTB (Goldenberg et al., 2005b).

b) *Obstetric history*

- *Previous PTB:* The recurrence risk in women with a previous PTB ranges between 15%-50%, depending on number and gestational age of previous deliveries and the risk is inversely related to the gestational age of previous PTB (Goldenberg et al., 2006).
- *Interval between pregnancies:* An interpregnancy interval of less than 6 months confers a increased risk of PTB (Smith et al., 2003a).
- *Previous uterine surgery:* History of myomectomy, cervical cone biopsy or loop electrocautery excision procedures have been associated with an increase in spontaneous PTB (Jakobsson et al., 2007).
- *Smoke:* Tobacco use increases the risk of PTB. Both nicotine and carbon monoxide are powerful vasoconstrictors, and are associated with placental damage and decreased uteroplacental blood flow, leading to fetal growth restriction and PTB (Ebrahim et al., 2000; Aliyu et al., 2010).

c) *Pregnancy associated risk factors*

- *Local or systemic infections:* Infections, such as pyelonephritis and asymptomatic bacteriuria are associated with PTB (Goldenberg et al., 2005). Peridontal disease has received widespread scrutiny suggesting an increased risk independent of other factors (Offenbacher et al., 2006).
- *Intrauterine infection:* Intrauterine infection is an important mechanism leading to PTB and related

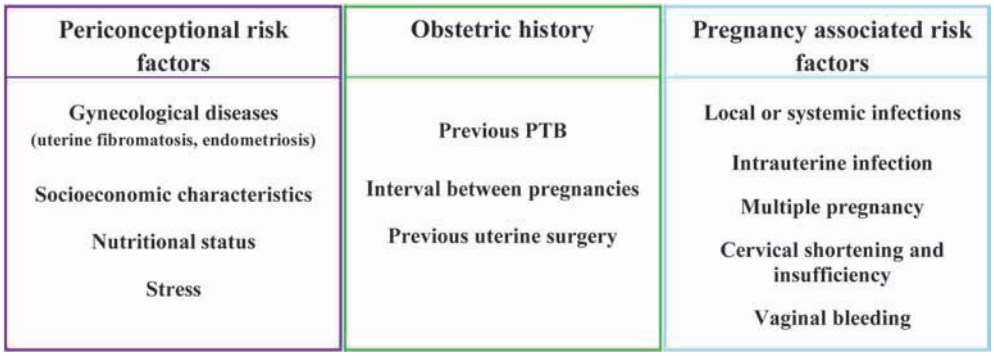

Fig. 3. — Risk factors of preterm delivery

to activation of the innate immune system. The microorganisms most commonly reported are genital Mycoplasma (Larsen and Hwang, 2010). The mechanism by which bacterial vaginosis is associated with PTB is unknown, but microorganisms probably ascend into the uterus before or early during pregnancy (Hillier et al., 1994).

- *Multiple pregnancy:* Multiple gestations carry a risk of PTB, and result in 15-20% of all PTB. Uterine overdistension, resulting in contractions and P-PROM, is the causative mechanism for the rate of increased risk (Stock and Norman, 2010).
- *Cervical shortening and insufficiency:* Cervical shortening is a risk factor for PTB. So, the shorter cervix, the greater the risk of preterm delivery (Di Renzo, 2009).
- *Vaginal bleeding:* Vaginal bleeding caused by placental abruption or placenta praevia is associated with a very high risk of PTB (Krupa et al., 2006).

Clinical assessment of risk

To support clinical practice, several biophysical and biochemical markers have been proposed in addition to the evaluation of the obstetric history and maternal risk factors, to identify patients at risk of spontaneous PTB (Lamont, 2006). Patients with a short cervix could have a higher rate of PTB and they may benefit from targeted interventions (Di Renzo, 2009). Therefore, accurate prediction of the risk of PTB among asymptomatic pregnant women and those symptomatic with threatened preterm labor may offer the opportunity to target care at those most likely to benefit.

Vaginal digital examination to assess the cervix in pregnancy is simple to do but suffers from large variation among examiners (Holcomb and Smeltzer, 1991). Therefore, an useful tool in detecting patients at risk of PTB is the measurement of cervical length (CL) by transvaginal ultrasonography, a reproducible method of examination during pregnancy (Sonek et al., 1990). The assessment of CL has been well standardized (Sonek and Shellhaas, 1998) and it is reproducible among different examinators (Burger et al, 1997). The length of the cervix is directly related to the duration of pregnancy: the shorter the cervix, the greater the likelihood of PTB (Iams et al., 1996; Severi et al., 2003).

In the highest risk women, a CL of 25 mm has a positive predictive value of 70% for PTB < 35 weeks when detected at 14-18 weeks, and of 40% when detected at 18-22 weeks of pregnancy (Mella and Berghella, 2009).

In clinical practice in addition to the use of digital examination and CL evaluation, the identification of other possible biophysical markers should be considered: membrane thickness (Severi et al., 2008) and estimated fetal weight (EFW) (Severi et al., 2012).

It was demonstrated, a positive correlation between membrane thickness and the time of delivery. Indeed, in women at risk for PTB, when this parameter is above the cut-off value of 1.2 mm, the probability to delivery preterm is higher (likelihood ratios of 3.3), with a sensitivity and specificity of 100% (95% CI, 80.3-100) and 69.5% (95% CI, 61.2-77.0) respectively (Severi et al., 2008).

Moreover, a relationship between ultrasound EFW at third trimester and the risk of PTB after premature onset of labor exists. In asymptomatic women between 28 and 36 weeks gestation, EFW lower than 0.90 MoM increases 4.6 times the risk of spontaneous PTB (Severi et al., 2012).

Regarding biomarkers in a biologic fluids a clinical usefulness to predict PTB (Menon et al., 2011) has been shown:

i) Fetal fibronectin (fFN): a glycoprotein found in the extracellular substance of the decidua basalis next to the intervillous space (Kiefer and Vintzileos 2008; Lookwood et al., 1999b). A positive fFN test in vaginal fluids is the most accurate investigation in predicting spontaneous PTB within 710 days after testing among women with symptoms of threatened

PTB (Honest et al., 2002) and improves the predictive value of cervical ultrasonography to identify patients at risk (Honest et al., 2009). A fFN positive test represents a powerful marker of PTB when measured at or after 22-24 weeks of gestation in asymptomatic high risk women (Kurtzman, 2009; Berghella et al., 2008).

ii) CRH and its binding protein (CRH-BP): maternal plasma CRH in the second trimester might be of use in identifying women at high risk of PTB before 34 weeks, detecting about 40% of women who will deliver preterm (McLean and Smith, 2001). Recently, it is found that maternal serum CRH beyond 28 weeks, in presence of intact membranes, is the most accurate biomarkers in predicting preterm birth within 48 hours (Hill et al., 2008). Maternal serum CRH-BP in asymptomatic women at high risk for PTB decreases as parturition approaches, suggesting that CRH bioavailability increases at delivery (Berkowitz et al., 1996).

iii) Ratio of E3/E2: An increase of estriol production is related to the timing of the onset of labor, however, a pivotal event of parturition is represented by a change in the ratio of the E2 and E3 as labor approaches, leading to a more than 10-fold excess of E3. Since E3/E2 increased in the month before delivery, creating an estrogenic environment at the onset of labor, the E3 increase and the altered E3/E2 ratios may be clinically useful in predicting PTB (Smith et al., 2009).

iv) Salivary estriol: hight salivary estriol concentrations from 24 to 34 weeks may be clinically helpful in the identification women with singleton pregnancies at risk of PTB who delivering preterm. An increase in salivary estriol concentrations occurs about 3-4 weeks before the onset of labor in women delivering preterm, with a sensitivity of 71% and specificity of 77% when exceeding a 2.3 ng/ml (McGregor et al., 1995).

Nevertheless, current knowledge of risk factors and biomarkers in predicting the PTB has not been successful in reducing the rate of PTB which continues to rise in the last decades.

3. Management of women at risk of preterm birth

a) *Periconceptional interventions*

- **Periconceptional interventions for women without risk factors**

As many as 50% of PTB occur in women who do not have known risk factors (Mercer et al., 2005). Smoking cessation, optimal nutrition, and periodontal care are commonly recommended. Interventions targeted to reproductive-age women include improved public awareness of the lifetime consequences of PTB (Masset et al., 2003) and the increased risk of PTB that accompanies pregnancies conceived after assisted reproductive technology (ACOG, 2005). Women programming a pregnancy are routinely advised to initiate prenatal vitamins prior to conception and to avoid known risk factors such as cigarette smoking (ACOG, 2005). A reduction in maternal smoking may reduce the rate of PTB (Burguet et al., 2004). The risk attributable to cigarette smoking is greater than 25% for PTB (Shah and Bracken, 2000) and it is about 5% for infant mortality (Salihu et al., 2003). A review of prior pregnancies could be useful to identify opportunities to reduce risks, including periconceptional intervention such as correction of Mullerian anomalies (Patton et al., 2004).

- **Periconceptional interventions for women who have a prior preterm birth**

Women with a prior PTB have an increased risk of recurrence of PTB (Iams and Berghella, 2010). Women with increased medical risks for PTB might benefit from periconceptional interventions such as control of medical disorders (eg, diabetes, seizures, asthma, or hypertension) or interventions that influence prenatal care such as prophylactic progesterone or cerclage before the cervix has dilated (cervical cerclage helps to prevent second trimester loss or PTB in women with at least three previous second trimester losses or PTB (with history indicated cerclage placed at 12 to 14 weeks) (Berghella and Seibel-Seamon, 2007).

b) *Postconceptional interventions*

- **Prenatal care and social support**

The rate of PTB is high in women who do not receive prenatal care. Although perhaps beneficial in adolescents (Quinlivan and Evans, 2004), enhanced prenatal care including social support, home visits, and PTB education have not reduced PTB (Klerman et al., 2001).

- **Modification of maternal activity**

Bed rest is frequently recommended for women whose pregnancies are at risk for PTB, but there is no evidence that it is beneficial (Sosa et al., 2004). Limitation of work and sexual activity is commonly recommended despite a lack of supporting evidence (Yost et al., 2006).

- **Nutritional supplements**

Dietary supplementation of omega-3 polyunsaturated fatty acids is associated with reduced production of inflammatory mediators, and omega-3 supplementation in women at risk for PTB decreases rate of preterm (Barger, 2010).

- **Periodontal care**

The risk of PTB rises with increased severity of periodontal disease and when periodontal disease progresses in pregnancy (Offenbacher et al., 2006). Although postulated to arise from hematogenous transmission of oral microbial pathogens to the genital tract, it is more likely the result of variations in the inflammatory response to resident microflora (Stamilio et al., 2007).

- **Antibiotic treatment**

Treatment of asymptomatic bacteriuria reduces the rate of PTB (Smaill, 2007). The current consensus is that symptomatic vaginal infections with bacterial vaginosis should be treated but screening and prophylaxis are not recommended (ACOG, 2001).

- **Progesterone supplementation**

Progesterone supplementation for women at risk for PTB has been investigated on the basis of several mechanisms of action, including reduced gap junction formation and oxytocin antagonism leading to relaxation of smooth muscle, maintenance of cervical integrity, and anti-inflammatory effects (Dodd et al., 2006; Romero et al., 2011).

- **Cervical cerclage**

The cervix normally stays tightly closed during pregnancy. Since cervical incompetence is considered one of the causes of the PTB, the evaluation of cervical characteristics may provide informations and allows action on a one cause of the syndrome (Mancuso and Owen, 2009). Cervical cerclage (Bergella et al., 2005) is a frequent intervention proposed to prevent PTB once the short CL has been detected or in women with prior spontaneous PTB (Eskandar et al., 2007). Placement of a therapeutic cerclage may reduce the incidence of PTB at <34 weeks' gestation among high-risk patients. A cervical cerclage may be inserted prophylactically before pregnancy or during the first trimester **in women at high risk** or it may be placed therapeutically after detection of cervical changes (Althuisius et al, 2000). It is a surgical treatment to insert a suture to keep the cervix closed. There are a number of proposed treatments (Shirodkar and McDonald techniques) (Shirodkar, 1995; McDonald, 1957) designed to keep the cervix closed until the expected time of birth.

Elective cervical cerclage carries risks for the pregnancy: surgical manipulation of the cervix can cause uterine contractions, bleeding, infection with pyrexia which may lead to miscarriage or preterm labor (Drassinower et al., 2011).

In the last few years the use of cervical pessary (a silicone ring to close the cervix) was introduced in clinical practice. Cervical pessary is a simple and non-invasive alternative that might replace the cervical stitch operation and it could be removed at around 37 weeks. Arabin pessary is a flexible, ring-like silicone pessary available in different sizes (Arabin et al., 2003), relatively non-invasive. It is easy to use, operator independent, it do not require anaesthesia, and it is easily removed when necessary. A possible complication with the use of pessary is represented by changes in vaginal flora during pregnancy (Arabin et al., 2003). However, there is evidence from non-randomised trials concerning some benefit with the use of cervical pessary in preventing PTB (Abdel-Aleem et al., 2010).

4. Management of preterm birth

Prevention and treatment of PTB are crucial interventions to improve fetal/neonatal outcome. In many cases, it may not be appropriate to consider interventions as tocolysis, such as in advanced labor or in case of intrauterine infection or placental abruption (RCOG, 2011). It remains plausible that, for selected women, such as those who require transfer for neonatal care or time to complete a course of corticosteroids, there may be benefit associated with tocolysis (RCOG, 2011).

Thus, management of PTB is oriented to the stabilization both of the mother and the fetus and to the transport of the fetus in uterus to allow delivery in hospitals with resources to properly support preterm infants; it consist in:

- monitoring fetal heart and uterine contractions;
- considering antenatal transfer of the mother and fetus to equipped hospital to care preterm infants;
- administering antibiotics in case of PPROM;
- administering corticosteroid;
- if < 32 weeks, consider tocolysis.

Antibiotics and Preterm Premature Rupture of Fetal Membranes

Preterm premature rupture of membranes (PPROM) complicates 2% of pregnancies but is associated with 40% of PTB and can result in neonatal morbidity and mortality (Merenstein and Weisman, 1996), due to prematurity, sepsis and pulmonary hypoplasia (RCOG, 2006) and in maternal infection and consequently increased risk of chorioamnionitis. Maternal infections are reduced in women exposed to antibiotics (King and Flenady, 2002) and antibiotic treatment is associated with a significant reduction in maternal infections and chorioamnionitis (Kenyon et al., 2003), and with improved neonatal outcome (Mercer et al., 2003) without side effect on childhood outcomes and children health at 7 years of age (Kenyon et al., 2008a). Unlike pPROM, antibiotic therapy is not recommended in cases of intact membranes, as associated with an increase in functional impairment among children at 7 years follow-up

(Kenyon et al., 2008b). Erythromycin or penicillin are the antibiotic of choice, since are associated with a reduction in the numbers of babies born within 48 hours and who had positive blood cultures. Delivery should be considered at 34 weeks of gestation; the expectant management requires an assessment of the risks related to the development of intrauterine infection (RCOG, 2006).

Antenatal Corticosteroids

Antenatal steroids are associated with a significant reduction in rates of neonatal death within the first 24 hours, respiratory distress syndrome (RDS), intraventricular haemorrhage, necrotising enterocolitis, and patent ductus arteriosus (Roberts and Dalziel, 2006), and are safe for the mother. In the lung, corticosteroids promote surfactant synthesis, increase lung compliance, reduce vascular permeability, and generate a greater response to postnatal surfactant treatment. Antenatal corticosteroids have maximal effectiveness in preventing neonatal complications of prematurity when delivery is within 2-7 days after administration (Crowley, 2000).

The most extensively studied and used regimens of corticosteroid treatment for the prevention of RDS is represented by two doses of betamethasone 12 mg given intramuscularly 24 hours apart or four doses of dexamethasone 6 mg given intramuscularly 12 hours apart (RCOG, 2010).

Tocolysis

In some cases tocolytics are administered to the mother to temporarily (48 h) delay threatened spontaneous PTB, facilitating the maternal antenatal corticosteroid administration and the transfer to a specialist unit (Greenfield and Lamont, 2000). The use of tocolytic drugs is associated with a prolongation of pregnancy for up to 7 days and is not associated with a reduction in perinatal or neonatal morbidity (Petraglia and Visser, 2009).

There are a variety of tocolytic agents in use. The most common are oxytocin-receptor antagonists, betamimetics, magnesium sulphate, cyclo-oxygenase inhibitors and calcium channel blockers (Table I). In the absence of any clear evidence that one tocolytic is more efficacious than another, relative safety (Table II) is the main reason for choosing one over the other.

4.1. *Oxytocin-receptor antagonists*

Oxytocin receptor antagonists block oxytocin receptors in the myometrium, preventing a rise in intracellular calcium and thereby relaxing the myometrium. Atosiban is an oxytocin receptor antagonist specifically developed for the treatment of preterm labor (Usta et al., 2011). Potential maternal side-effects are relatively moderate: adverse injection side reaction, nausea, vomiting, headache, chest pain and hypotension (Moutquin et al., 2000).

4.2. *β-adrenergic-receptor agonists*

β-adrenergic-receptor agonists have been the most widely used agents to treat threatened preterm labor (Anotayanonth et al., 2004). The β-adrenergic-receptor agonists cause myometrial relaxation by binding to β2-adrenergic receptors and subsequently increasing the levels of intracellular cyclic AMP. An increase in intracellular cyclic AMP activates protein

Table I. — Tocolytic Agents.

Class of tocolytics	Types	Mechanism(s) of action
Oxytocin-receptor antagonists	Atosiban	Inhibits myometrial contractions and induces uterine quiescence
β-adrenergic-receptor agonists	Ritodrine, Salbutamol, Terbutaline	Bind to β-adrenergic-receptors increasing intracellular levels of cAMP; increase in cAMP initiate reduction in intracellular calcium, in turn inhibiting muscle contraction
Magnesium sulphate		At high levels displaces calcium fromsarcoplasmic reticulum, thereby increasing repolarization time between contractions and decreasing force of contractions
Cyclo-oxygenase inhibitors	Indomethacin	Inhibits production of prostaglandins
Calcium-channel antagonists	Nicardipine, Nifedipine	Prevent entry of calcium in smooth muscle cells by blocking calcium channels and suppressing release of intracellular calcium stores

Table II. — Side-Effect Profiles of Tocolytic Agents.

Class of tocolytics	Maternal side effects	Fetal side effects	Controindications
Oxytocin-receptor antagonists	Hypersensitivity, injection-site reactions	Increased rate of fetal or infant death	None
β-adrenergic-receptor agonists	Tachycardia and hypotension, Tremor, palpitations, shortness of breath, chest discomfort, pulmonary edema, hypokalemia, hyperglycemia	Tachycardia	Tachycardia-sensitive maternal cardiac disease, poorly controlled diabetes mellitus
Magnesium sulphate	Flushing, diaphoresis, nausea, loss of deep-tendon reflexes, respiratory paralysis, cardiac arrest, suppression of heart rate, contractility, and left ventricular systolic pressure, and neuromuscular blockade	Conficting data	Myasthenia gravis
Cyclo-oxygenase inhibitors	Nausea, esophageal reflux, gastritis and emesis, platelet dysfunction	In utero closure of ductus arteriosus, oligohydramnios, patent ductus arteriosus in neonate (conflicting data)	Platelet dysfunction or bleeding disorder, hepatic or renal dysfunction, gastrointestinal or ulcerative disease, asthma
Calcium-channel antagonists	Dizziness, flushing, hypotension, suppression of heart rate, contractility, and left ventricular systolic pressure, neuromuscular blockade, elevation of hepatic aminotransferase levels		Hypotension, preloaddependent cardiac lesions

kinase, which inactivates myosin light-chain kinase, thus diminishing myometrial contractility (Caritis et al, 1991). The most common β-adrenergic-receptor agonists employed in clinical practice is ritodrine (CPIG, 1992). b2-Agonists lack uterine selectivity and can cause metabolic and cardiovascular side-effects. Most serious maternal side-effects are pulmonary edema, myocardial ischemia and heart failure (Smith, 2003b).

4.3. *Magnesium sulphate*

Magnesium sulphate is commonly used to treat pregnancy-induced hypertension and though unlicensed, is widely used for tocolysis in the USA (Greenfield and Lamont, 2000). It decreases uterine activity; one possible mechanism of action is represented by its competition with calcium for entry into the myometrial cells through voltage-gated channels (Mercer and Merlino, 2009).

4.4. *Cyclo-oxygenase (COX) inhibitors*

COX enzymes are fundamental to the production of PGs and inhibitors reduce uterine contractions (Loudon et al, 2003). COX inhibitors are easily administered with few maternal side-effects. COX inhibitors, however, freely cross the placenta and can interfere with prostaglandin homeostasis in the fetus (Moise et al., 1988). The administration of indomenthacin (the most commonly used COX inhibitor) causes any adverse effects in the fetus during tocolysis (Cordero et al., 2007), that include oligohydramnios, renal failure, premature closure of the ductus arteriosus with consequent pulmonary hypertension, persistent patent ductus arteriosus, necrotising enterocolitis, and intraventricular haemorrhage (Vermillion et al., 1997). In fact, they can be administered before 32 weeks and for a maximum of 72 hours.

4.5. *Calcium-channel antagonists*

Calcium-channel antagonists act inhibiting calcium ions influx across the cell membrane, thereby decreasing the tone in the smooth muscle vasculature (Sanborn, 1995). Nifedipine is the most commonly used to inhibit labor; although has been shown to have a more favourable neonatal outcome and better prolongation of gestation, it is not licensed for tocolysis (Blea et al., 1997).

5. Delivery of preterm infants

Routine caesarean delivery of preterm or very-low birthweight infants is controversial, but most evidence does not support the practice (Deulofeut et al., 2005; Riskin et al., 2004). Neonatal intracranial haemorrhage seems to arise commonly before, after and during labor and delivery. For infants in breech presentation, caesarean delivery might avoid trapping of the aftercoming head and other manipulations that could lead to trauma or hypoxia. Optimum delivery of very-low birthweight babies might nevertheless appropriately lead to a caesarean section without labor because of complications associated with growth restriction, ruptured membranes, cord prolapse, bleeding, or expected difficulty with vaginal breech delivery (Grant et al., 1996).

References

Abdel-Aleem H, Shaaban OM, Abdel-Aleem MA. Cervical pessary for preventing preterm birth. Cochrane Database Syst Rev. 2010 Sep 8;(9):CD007873. Review.

ACOG Practice Bulletin number 31. Assessment of risk factors for preterm birth. Obstet Gynecol. 2001;98:709-16.

ACOG Committee Opinion 313. The importance of preconception care in the continuum of women's health care. Obstet Gynecol. 2005;106:665-6.

ACOG Committee Opinion 324. Perinatal risks associated with assisted reproductive technology. Obstet Gynecol. 2005;106:1143-6.

Aliyu MH, Lynch O, Saidu R et al. Intrauterine exposure to tobacco and risk of medically indicated and spontaneous preterm birth. Am J Perinatol.. 2010;27:405-10.

Althuisius SM, Dekker GA, van Geijn HP et al. Cervical Incompetence Prevention Randomized Cerclage Trial (CIPRACT): Study design and preliminary results. Am J Obstet Gynecol. 2000;183:823-9.

Anotayanonth S, Subhedar NV, Garner P et al. Betamimetics for inhibiting preterm labour. Cochrane Database Syst Rev. 2004 Oct 18;(4):CD004352. Review.

Arabin B, Halbesma JR, Vork F et al. Is treatment with vaginal pessaries an option in patients with a sonographically detected short cervix? J Perinat Med. 2003;31:122-33.

Barger MK. Maternal nutrition and perinatal outcomes. J Midwifery Womens Health. 2010;55:502-11.

Behrman RE, Butler AS (Editors). Preterm Birth: Causes, Consequences, and Prevention. Institute of Medicine (US) Committee on Understanding Premature Birth and Assuring Healthy Outcomes Washington (DC): National Academies Press (US); 2007.

Berghella V, Odibo AO, To MS et al. Cerclage for short cervix on ultrasonography: meta-analysis of trials using individual patient-level data. Obstet Gynecol. 2005;106:181-9.

Berghella V, Hayes E, Visintine J et al. Fetal fibronectin testing for reducing the risk of preterm birth. Cochrane Database Syst Rev. 2008;8:CD006843. Review.

Berghella V, Seibel-Seamon J. Contemporary use of cervical cerclage. Clin Obstet Gynecol. 2007;50:468-77.

Berkowitz GS, Lapinski RH, Lockwood CJ et al. Corticotropin-releasing factor and its binding protein: maternal serum levels in term and preterm deliveries. Am J Obstet Gynecol. 1996;174:1477-83.

Blea CW, Barnard JM, Magness RR et al. Effect of nifedipine on fetal and maternal hemodynamics and blood gases in the pregnant ewe. Am J Obstet Gynecol. 1997;176:922-30.

Bloomfield FH. How is maternal nutrition related to preterm birth? Annu Rev Nutr. 2011;31:235-61.

Burger M, Weber-Rossler T, Willmann M: Measurement of the pregnant cervix by transvaginal sonography: an interobserver study and new standards to improve the interobserver variability. Ultrasound Obstet Gynecol. 1997;9:188-93.

Burguet A, Kaminski M, Abraham-Lerat L et al. The complex relationship between smoking in pregnancy and very preterm delivery: results of the Epipage study. BJOG. 2004; 111: 258-65.

Canadian Preterm Labor Investigators Group (CPIG). Treatment of preterm labor with the beta-adrenergic agonist ritodrine. N Engl J Med. 1992;327:308-12.

Caritis SN, Chiao JP, Kridgen P. Comparison of pulsatile and continuous ritodrine administration: effects on uterine contractility and beta-adrenergic receptor cascade. Am J Obstet Gynecol. 1991;164:1005-11.

Challis JRG, Matthews SG, Gibb W et al. Endocrine and paracrine regulation of birth at term and preterm. Endocr Rev. 2000;21:514-50.

Challis JRG, Lockwood CJ, Myatt L et al. Inflammation and pregnancy. Reprod Sci. 2009;16:206-15. Review.

Copper RL, Goldenberg RL, Das A et al. The preterm prediction study: maternal stress is associated with spontaneous preterm birth at less than 35 weeks' gestation. Am J Obstet Gynecol. 1996;175:1286-92.

Cordero L, Nankervis CA, Gardner D et al. The effects of indomethacin tocolysis on the postnatal response of the ductus arteriosus to indomethacin in extremely low birth weight infants. J Perinatol. 2007;27:22-7.

Crowley P. Prophylactic corticosteroids for preterm birth. Cochrane Database Syst Rev. 2000;(2):CD000065. Review.

Deulofeut R, Sola A, Lee B et al. The impact of vaginal delivery in premature infants weighing less than 1251 grams. Obstet Gynecol. 2005;105:525-31.

Di Renzo GC. The great obstetrical syndromes. J Matern Fetal Neonatal Med. 2009;22:633-5.

Dodd JM, Flenady V, Cincotta R et al. Prenatal administration of progesterone for preventing preterm birth. Cochrane Database Syst Rev. 2006 Jan 25;(1):CD004947. Review.

Drassinower D, Poggi SH, Landy HJ et al. Perioperative complications of history-indicated and ultrasound-indicated cervical cerclage. Am J Obstet Gynecol. 2011;205:53.

Ebrahim SH, Floyd RL, Merritt RK et al. Trends in pregnancy related smoking rates in the United States, 1987-1996. JAMA. 2000; 283:361-6.

Eskandar M, Shafiq H, Almushait MA et al. Cervical cerclage for prevention of preterm birth in women with twin pregnancy. Int J Gynecol Obstet. 2007;99:110-2.

Goldenberg RL, Cliver SP, Mulvihill FX et al. Medical, psychosocial, and behavioural risk factors do not explain the increased risk for low birth weight among blackwomen. Am J Obstet Gynecol. 1996;175:1317-24.

Goldenberg RL, Culhane JF. Pregnancy health status and the risk of preterm delivery. Arch Pediatr Adolesc Med. 2005; 159:89-90.

Goldenberg RL, Goepfert AR, Ramsey PS. Biochemical markers for the prediction of preterm birth. Am J Obstet Gynecol. 2005a;192:36-46.

Goldenberg RL, Culhane JF, Johnson DC. Maternal infection and adverse fetal and neonatal outcomes. Clin Perinatol. 2005b; 32:523-59.

Goldenberg RL, Andrews WW, Faye- O et al. The Alabama preterm birth project: placental histology in recurrent spontaneous and indicated preterm birth. Am J Obstet Gynecol. 2006;195:792-6.

Grant A, Penn ZJ, Steer PJ. Elective or selective caesarean delivery of the small baby? A systematic review of the controlled trials. Br J Obstet Gynaecol. 1996;103:1197-200.

Greenfield PJ, Lamont RF. The contemporary use of tocolytics. Current Obstet Gynaecol. 2000;10:218-24.

Hill JL, Campbell MK, Zou GY et al. Prediction of preterm birth in symptomatic women using decision tree modeling for biomarkers. Am J Obstet Gynecol. 2008;198:468.e1-7.
Hillier SL, Krohn MA, Cassen E et al. The role of bacterial vaginosis and vaginal bacteria in amniotic fluid infection in women in preterm labor with intact fetal membranes. Clin Infect Dis. 1994;20:276-8.
Holcom WL Jr, Smeltzer JS. Cervical effacement: variation in belief among clinicians. Obstet Gynecol. 1991;78:43-5.
Honest H, Bachmann LM, Gupta JK et al. Accuracy of cervicovaginal fetal fibronectin test in predicting risk of spontaneous preterm birth: systematic review. BMJ. 2002;325:301. Review.
Honest H, Forbes CA, Durée KH et al. Screening to prevent spontaneous preterm birth: systematic reviews of accuracy and effectiveness literature with economic modeling. Health Technol Assess. 2009;13:1-627. Review.
Iams JD, Goldenberg RL, Meis PJ et al. The length of the cervix and the risk of spontaneous premature delivery. National Institute of Child Health and Human Development Maternal Fetal Medicine Unit Network. N Engl J Med. 1996;334:567-72.
Iams JD, Romero R, Culhane JF et al. Primary, secondary, and tertiary interventions to reduce the morbidity and mortality of preterm birth. Lancet. 2008;371:164-75.
Iams JD, Berghella V. Care for women with prior preterm birth. Am J Obstet Gynecol. 2010;203:89-100.
Jakobsson M, Gissler M, Sainio S et al. Preterm delivery after surgical treatment for cervical intraepithelial neoplasia. Obstet Gynecol. 2007;109:309-13.
Kenyon S, Boulvain M, Neilson J. Antibiotics for preterm rupture of membranes. The Cochrane Database Syst Rev. 2003;(2):CD001058. Review.
Kenyon S, Pike K, Jones DR et al. Childhood outcomes after prescription of antibiotics to pregnant women with preterm rupture of the membranes: 7-year follow-up of the ORACLE I trial. Lancet. 2008a;372:1310-8.
Kenyon S, Pike K, Jones DR et al. Childhood outcomes after prescription of antibiotics to pregnant women with spontaneous preterm labour: 7-year follow-up of the ORACLE II trial. Lancet. 2008b;372:1319-27.
Kiefer DG, Vintzileos AM. The utility of fetal fibronectin in the prediction and prevention of spontaneous preterm birth. Rev Obstet Gynecol. 2008;1:106-12.
King J, Flenady V. Antibiotics for inhibiting preterm labour with intact membranes. The Cochrane Database Syst Rev. 2002; (3):CD001807. Review.
Klerman LV, Ramey SL, Goldenberg RL et al. A randomized trial of augmented prenatal care for multiple-risk, Medicaid-eligible African American women. Am J Public Health. 2001; 91:105-11.
Krupa FG, Faltin D, Cecatti JG, et al. Predictors of preterm birth. Int J Gynaecol Obstet. 2006;94:5-11.
Kurtzman J, Chandiramani M, Briley A et al. Quantitative fetal fibronectin screening in asymptomatic high-risk patients and the spectrum of risk for recurrent preterm delivery. Am J Obstet Gynecol. 2009;200:263.e1-6.
Lamont RF. Setting up a preterm prevention clinic: a practical guide. BJOG. 2006;113:86-92. Review.
Larsen B, Hwang J. Mycoplasma, Ureaplasma, and adverse pregnancy outcomes: a fresh look. Infect Dis Obstet Gynecol. 2010; pii: 521921.
Lockwood CJ. Stress-associated preterm delivery: The role of corticotropinreleasing hormone. Am J Obstet Gynecol. 1999a;180:264-6.
Lockwood CJ, Senyei AE, Dische MR et al. Fetal fibronectin in cervical and vaginal secretions as a predictor of preterm delivery. N Engl J Med. 1999b;325:669-74.
Lockwood CJ. Predicting premature delivery-no easy task. N Engl J Med. 2002;346: 282-4.
Loudon JA, Groom KM, Bennett PR. Prostaglandin inhibitors in preterm labour. Best Pract Res Clin Obstet Gynaecol. 2003;17:731-44.
Mancuso MS and Owen J. Prevention of Preterm Birth Based on a Short Cervix: Cerclage. Semin Perinatol. 2009;33:325-33.
Massett HA, Greenup M, Ryan CE et al. Public perceptions about prematurity: a national survey. Am J Prev Med. 2003;24:120-7.
McDonald IA. Suture of the cervix for inevitable miscarriage. J Obstet Gynaecol Br Emp. 1957;146:346-50.
McGregor JA, Jackson GM, Lachelin GC et al. Salivary estriol as risk assessment for preterm labor: a prospective trial. Am J Obstet Gynecol. 1995;173:1337-42.
McLean M and Smith R. Corticotrophin-releasing hormone and human parturition. Reproduction. 2001;121:493-501. Review.
Mercer BM, Goldenberg RL, Das AF et al. What we have learned regarding antibiotic therapy for the reduction of infant morbidity after preterm premature rupture of the membranes. Semin Perinatol. 2003;27:217-30.
Mercer B, Milluzzi C, Collin M. Periviable birth at 20 to 26 weeks of gestation: proximate causes, previous obstetric history and recurrence risk. Am J Obstet Gynecol. 2005; 193:1175-80.
Mercer BM, Merlino AA. Magnesium sulfate for preterm labor and preterm birth. Obstet Gynecol. 2009;114:650-68.
Merenstein GB,Weisman LE. Premature rupture of the membranes: neonatal consequences. Semin Perinatol. 1996; 20:375-80.
Mella MT, Berghella V. Prediction of preterm birth: cervical sonography. Semin Perinatol 2009;33:317-24. Review.
Menon R, Torloni MR, Voltolini C et al. Biomarkers of spontaneous preterm birth: an overview of the literature in the last four decades. Reprod Sci. 2011;18:1046-70.
Moise KJ, Huhta JC, Sharif DS et al. Indomethacin in the treatment of premature labor. Effects on the fetal ductus arteriosus. N Engl J Med. 1988;319:327-31.
Moutquin JM, Sherman D, Cohen H et al. Double-blind, randomized, controlled trial of atosiban and ritodrine in the treatment of preterm labor: a multicenter effectiveness and safety study. Am J Obstet Gynecol. 2000;182:1191-9.
Offenbacher S, Boggess KA, Murtha AP et al. Progressive periodontal disease and risk of very preterm delivery. Obstet Gynecol. 2006;107:29-36.
Patton PE, Novy MJ, Lee OM et al. The diagnosis and reproductive outcome after surgical treatment of the complete septate uterus, duplicated cervix and vaginal septum. Am J Obstet Gynecol. 2004;190:1669-75.
Petraglia F, Sawchenko PE, Rivier J et al. Evidence for local stimulation of ACTH secretion by corticotropin-releasing factor in human placenta. Nature. 1987;328:717-9.
Petraglia F, Garuti GC, De Ramundo B et al. Mechanism of action of interleukin-1 beta in increasing corticotropin-releasing factor and adrenocorticotropin hormone release from cultured human placental cells. Am J Obstet Gynecol. 1990;163:1307-12.
Petraglia F, Aguzzoli L, Florio P et al. Maternal plasma and placental immunoreactive corticotrophin-releasing factor concentrations in infection associated term and pre-term delivery. Placenta. 1995;16:157-64.
Petraglia F, Hatch MC, Lapinski R et al. Lack of effect of psychosocial stress on maternal corticotropin-releasing factor and catecholamine levels at 28 weeks' gestation. J Soc Gynecol Investig. 2001;8:83-8.
Petraglia F, Visser GH. Prevention and management of preterm labour. J Matern Fetal Neonatal Med. 2009;22:24-30.
Petraglia F, Imperatore A, Challis JR. Neuroendocrine mechanisms in pregnancy and parturition. Endocr Rev. 2010; 31:783-816. Review.
Pike IL. Maternal stress and fetal responses: evolutionary perspectives on preterm delivery. Am J Hum Biol. 2005;17: 55-65.
Quinlivan JA, Evans SF. Teenage antenatal clinics may reduce the rate of preterm birth: a prospective study. BJOG. 2004;111:571-8.

Rauk PN, Friebe-Hoffmann U, Winebrenner LD et al. Interleukin-6 up-regulates the oxytocin receptor in cultured uterine smooth muscle cells. Am J Reprod Immunol. 2001; 45:148-53.
RCOG. Preterm Prelabour Rupture of Membranes Green-top Guideline No. 44; 2006.
RCOG.Antenatal Corticosteroids to Reduce Neonatal Morbidity and Mortality. Green-top Guideline No. 7; 2010.
RCOG. Tocolysis for Women in Preterm Labour. Green-top Guideline No.1b; 2011.
Riskin A, Riskin-Mashiah S, Lusky A et al. The relationship between delivery mode and mortality in very low birthweight singleton vertex-presenting infants. BJOG. 2004;111:1365-71.
Roberts D, Dalziel S. Antenatal corticosteroids for accelerating fetal lung maturation for women at risk of preterm birth. Cochrane Database Syst Rev. 2006 Jul 19;(3):CD004454. Review.
Romero R, Espinoza J, Chaiworapongsa T et al. Infection and prematurity and the role of preventive strategies. Semin Neonatol. 2002; 7:259-274.
Romero R, Espinoza J, Kusanovic JP et al. The preterm parturition syndrome. BJOG. 2006;113:17-35.
Romero R, Espinoza J, Gonçalves LF et al. The role of inflammation and infection in preterm birth. Semin Reprod Med. 2007;25:21-39.
Romero R. Vaginal progesterone to reduce the rate of preterm birth and neonatal morbidity: a solution at last. Womens Health (Lond Engl). 2011;7:501-4.
Salihu HM, Pierre-Louis BJ, Alexander GR. Levels of excess infant deaths attributable to maternal smoking during pregnancy in the United States. Matern Child Health J. 2003; 7:219-27.
Sanborn BM. Ion channels and the control of myometrial electrical activity. Semin Perinatol. 1995;19:31-40.
Sennström MB, Ekman G, Westergren-Thorsson G et al. Human cervical ripening, an inflammatory process mediated by cytokines. Mol Hum Reprod. 2000;6:375-81.
Severi FM, Bocchi C, Florio P et al. Comparison of two-dimensional and three-dimensional ultrasound in the assessment of the cervix to predict preterm delivery. Ultrasound Med Biol. 2003;29:1261-5.
Severi FM, Bocchi C, Voltolini C et al. Thickness of fetal membranes: a possible ultrasound marker for preterm delivery. Ultrasound Obstet Gynecol. 2008;32:205-9.
Severi FM, Bocchi C, Imperatore A et al. Ultrasound estimated fetal weight slightly below the median is associated with increased risk of spontaneous preterm birth. Prenatal Diagnosis. 2012; 32:588-91.
Shah NR, Bracken MB. A systematic review and meta-analysis of prospective studies in the association between maternal cigarette smoking and preterm delivery. Am J Obstet Gynecol 2000; 182:465-72.
Shirodkar VN. A new method of operative treatment for habitual abortions in the second trimester of pregnancy. Antiseptic. 1955;52:299-300.
Slater DM, Dennes WJ, Campa JS et al. Expression of cyclooxygenase types-1 and -2 in human myometrium throughout pregnancy. Mol Hum Reprod. 1999;5:880-4.
Smaill F, Vazquez JC. Antibiotics for asymptomatic bacteriuria in pregnancy. Cochrane Database Syst Rev. 2007 Apr 18;(2): CD000490. Review.
Smith GC, Pell JP, Dobbie R. Interpregnancy interval and risk of preterm birth and neonatal death: retrospective cohort study. BMJ. 2003a;327:313.
Smith GN. What are the realistic expectations of tocolytics? BJOG. 2003b;110:103-6.
Smith LK, Draper ES, Manktelow BN et al. Socioeconomic inequalities in very preterm birth rates. Arch Dis Child Fetal Neonatal Ed. 2007;92:11-4.
Smith R, Smith JI, Shen X et al. Patterns of plasma corticotropin-releasing hormone, progesterone, estradiol, and estriol change and the onset of human labor. J Clin Endocrinol Metab. 2009;94:2066-74.
Sonek JD, Iams JD, Blumenfeld M et al. Measurement of cervical length in pregnancy: comparison between vaginal ultrasonography and digital examination. Obstet Gynecol. 1990;76:172-5.
Sonek J, Shellhaas C: Cervical sonography: a review. Ultras Obstet Gynecol. 1998;11:71-78.
Sosa C, Althabe F, Belizan J et al. Bed rest in singleton pregnancies for preventing preterm birth. Cochrane Database Syst Rev. 2004;(1):CD003581. Review.
Stamilio OM, Chang JJ, Macones GA. Periodontal disease and preterm birth: do the data have enough teeth to recommend screening and preventative treatment? Am J Obstet Gynecol. 2007;196:93-4.
Stock S, Norman J. Preterm and term labour in multiple pregnancies. Semin Fetal Neonatal Med. 2010;15:336-41.
Torricelli M, Giovannelli A, Leucci E et al. Labor (term and preterm) is associated with changes in the placental mRNA expression of corticotrophin-releasing factor. Reprod Sci. 2007;14:241-5.
Torricelli M, Novembri R, Bloise E et al. Changes in placental CRH, urocortins, and CRH-receptor mRNA expression associated with preterm delivery and chorioamnionitis J Clin Endocrinol Metab. 2011;96:534-40.
Usta IM, Khalil A, Nassar AH. Oxytocin Antagonists for the Management of Preterm Birth: A Review. Am J Perinatol. 2011;28:449-60.
Vermillion ST, Scardo JA, Lashus AG et al. The effect of indomethacin tocolysis on fetal ductus arteriosus constriction with advancing gestational age. Am J Obstet Gynecol. 1997;177:256-9.
Wadhwa PD, Culhane JF, Rauh V et al. Stress, infection and preterm birth: a biobehavioural perspective. Paediatr Perinat Epidemiol. 2001;15:17-29.
Wolfe CD, Patel SP, Linton EA et al. Plasma corticotrophin-releasing factor (CRF) in abnormal pregnancy. Br J Obstet Gynaecol. 1988;95:1003-6.
Yost NP, Owen J, Berghella V et al. Effect of coitus on recurrent preterm birth. Obstet Gynecol. 2006;107:793-7.
Xu P, Alfaidy N, Challis JR. Expression of matrix metalloproteinase (MMP)-2 and MMP-9 in human placenta and fetal membranes in relation to preterm and term labor. J Clin Endocrinol Metab. 2002; 87:1353-61.

The uterine myocyte as a target for prevention of preterm birth

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Abstract

Preterm birth (PTB) remains the most common cause of neonatal morbidity and mortality as well as long-term disability. Current strategies to prevent or arrest spontaneous preterm labor (SPTL) have limited success. For almost three decades, there have been no novel pharmacological agents used clinically to address this important obstetrical complication. In this review, we focus on the uterine myocyte as a target for prevention of spontaneous PTB. After presenting an overview of intracellular signaling pathways that are important in regulation of smooth muscle contractility, we discuss previous and current pharmacological approaches to manage SPTL. We also present recent evidence from our own laboratories suggesting a potentially novel and uterine-specific approach to maintain or impose uterine relaxation. Finally, we briefly discuss extrinsic systems that might affect uterine activity and reinforce the concept that SPTL represents a syndrome that is the end result of a variety of pathophysiologic etiologies leading to PTB. We conclude by emphasizing the need for much more research to provide sufficient understanding of the mechanisms of SPTL and to make inroads towards reducing the incidence and adverse consequences of this common and serious syndrome.

Key words: **Prematurity, preterm labour, myosin regulatory light chain, rhoA-associated kinase, tocolysis, uterine contractility.**

Introduction

Preterm birth (PTB) is widely recognized as the major cause of neonatal mortality and long-term disability. There are three broad categories of PTB. Approximately one-third of cases are associated with and result from intrauterine infection. Current obstetrical practice is to terminate these pregnancies immediately, regardless of gestational age, because of potential serious complication to either the baby or the mother. The second and increasingly large category is the iatrogenic group where concerns about maternal or fetal health dictate that the outcome of the fetus would be better with immediate delivery, again regardless of gestational age. The third category, and the one most relevant to this discussion is the group that enters spontaneous preterm labor (SPTL) without evidence of maternal or fetal factors that would preclude attempts to prolong gestation. These are the pregnancies where the prevention of SPTL or treatment to arrest the premature contractions (tocolysis) could logically diminish the occurrence of complications due to PTB and generally enhance neonatal outcomes. This group is the focus of this paper.

Despite increasing awareness within the scientific community of the importance of SPTL (Behrman et al., 2007; Howson et al., 2012), there has been little advancement towards understanding the mechanisms that determine the timing of birth. The result of this relative lack of progress is a dearth of novel strategies to prevent PTB. Indeed, over the past three decades, there have been no new, clinically proven pharmacological approaches to prevent PTB arising from SPTL.

The objective of this paper is to discuss potential new strategies for the prevention of PTB. We will begin by describing the general mechanisms that regulate smooth muscle contractility. We have recently reviewed this topic in more detail than will be presented here (Aguilar and Mitchell, 2010). Then we will consider potential mechanisms that are

intrinsic to the smooth muscle myocyte and could act either by inhibition of pro-contractile mechanisms or stimulation of pro-relaxant pathways. We will then discuss the concept of specific regulatory mechanisms intrinsic to uterine myocytes that might support development of targets specifically to affect the uterus but without risk of adverse effects on other smooth muscle beds, particularly the cardiovascular system. Finally, we will discuss the potential of targeting pathways extrinsic to the uterine myocyte that could affect uterine contractility.

Regulation of smooth muscle contractility

The pivotal event for triggering the contractile machinery in smooth muscle is the phosphorylation of myosin regulatory light chains (RLC). As illustrated in Figure 1, this reaction is catalyzed by myosin regulatory light chain kinase (MLCK). The reverse reaction, leading to muscle relaxation, is mediated by myosin regulatory light chain phosphatase (MLCP). Oxytocin (OT) is the best-characterized and most potent uterine agonist. This hormone is produced in the hypothalamus and stored in the neurohypophysis of both mother and fetus. Perhaps more relevant to parturition, OT also is produced in the maternal decidua immediately adjacent to the uterine smooth muscle (myometrium) (Chibbar et al., 1993). Other potent agonists include endothelin-1 and prostaglandin (PG) $F_{2\alpha}$, which also are produced by maternal decidua (Arthur et al., 2008). Each of these agonists interact with specific membrane receptors linked to signaling pathways through small heterotrimeric GTPase proteins (G-proteins). The Gα subunits are key to determining which pathway will be activated. The $G\alpha_{q/11}$ subunit mediates activation of adjacent phospholipase C_β, which hydrolyses membrane phosphatidylinositol bisphosphate into two small 'second messengers,' inositol trisphosphate (IP_3) and diacyl glycerol (DAG) (Berridge, 1993). IP_3 stimulates the release of Ca^{2+} from the intracellular sarcoplasmic reticulum stores resulting in increased free intracellular Ca^{2+}. The resultant depolarization activates and opens the voltage-dependent L-type Ca^{2+} channels on the myocyte membrane. Since the extracellular concentration of Ca^{2+} is approximately 5 orders of magnitude greater than the intracellular Ca^{2+} concentration, this results in rapid and massive influx of Ca^{2+} from the extracellular space to the intracellular space. The free intracellular Ca^{2+} binds to calmodulin (CaM) and the Ca^{2+}-CaM complex subsequently binds to the activation site on MLCK, resulting in phosphorylation of RLC (here referred to as 'pRLC') and activation of the contractile apparatus. Because of this central role for Ca^{2+} in the initiation of myocyte shortening, this is known as the 'Ca^{2+}-dependent' pathway of smooth muscle contraction.

More recently, there has been growing awareness of a 'Ca^{2+}-independent' pathway. In the classical description of this pathway (Kozasa et al., 1998), the $G\alpha_{12\text{-}14}$ subunit family initiates the signaling by activation, through a yet unknown mechanism, of a guanidine nucleotide exchange factor (GEF). This results in activation of the small monomeric G-protein rhoA-GDP by exchanging GTP for GDP. The major target of activated rhoA-GTP is rhoA-associated kinase (ROK), which is the principal effector of this pathway. A major target of ROK is the substrate binding subunit of MLCP. Phosphorylation of the targeting subunit prevents targeting to and dephosphorylation of pRLC by MLCP, thus promoting muscle contraction. This mechanism has been recognized as the explanation of the phenomenon of 'Ca^{2+}-sensitization,' which describes a situation where the rise in intracellular Ca^{2+} concentration produces a larger-than-expected contraction. This effect is thought to be the result of simultaneous MLCP inhibition alongside MLCK activation (Somlyo and Somlyo, 2003). As we will discuss later, we believe this pathway may represent an important target for future tocolytics.

A third potential pathway for uterine myocyte stimulation is mediated by $G\alpha_i$. This pathway does not appear to be a major regulator of uterine contractility but may be involved in transduction of signals arising from the PGE_2 receptor isoform EP_3 (Kotani et al., 2000) and OT (Phaneuf et al., 1996).

Heterotrimeric G-proteins also mediate a major mechanism for uterine relaxation. In this instance, the predominant isoform is $G\alpha_s$. Activation of this subunit causes increased activity of membrane adenylyl cyclase (AC), which converts adenosine trisphosphate (ATP) to cyclic-5'-adenosine monophosphate (cAMP) and this in turn activates protein kinase A (PKA). This pathway is stimulated by endogenous and exogenous β-adrenergic agonists to cause uterine relaxation. Similarly, nitric oxide (NO) can directly activate cytosolic guanylyl cyclase (GC) in uterine myocytes to stimulate production of cyclic-5'-guanosine monophosphate (cGMP) with subsequent activation of PKG. PKA and PKG exhibit their relaxant effects on smooth muscle by acting on at least three targets. PKA mediates phosphorylation of the Ca^{2+}-CaM binding site on MLCK, thereby hindering enzyme activation. PKA and PKG also increase the activity of the Ca^{2+}-ATPases in the plasma membrane (PMCA) and sarcoplasmic and endoplasmic reticulum (SERCA) resulting in active extrusion of Ca^{2+} from the intracellular compartment and repolarization of the plasma membrane. Lastly, PKA and PKG can phosphorylate the MLCP

targeting subunit at a site adjacent to the ROK target site, thus rendering MLCP insensitive to the inhibitory effects of ROK.

Non-specific mechanisms of tocolysis

Essentially all the above pathways regulating uterine contractility have been exploited in attempts to develop a useful tocolytic drug to arrest SPTL. Here, we will provide only a brief overview of these trials. More authoritative and in-depth reviews are available in the Cochrane Collaboration databases. Unfortunately, most of these pathways are active in all types of smooth muscle. Therefore, use of doses that will arrest uterine activity is often associated with serious off-target side effects, most notably hypotension and related cardiovascular complications.

The β-adrenergic agonists are the best-studied tocolytic class of drugs. Multiple good-quality randomized control trials (RCTs) demonstrated efficacy to delay delivery for at least 48-72 hours (King et al., 1988) but there is no compelling evidence of improved infant outcome. The maternal cardiovascular complications, such as hypotension and others noted above, were common and often led to serious adverse outcomes, including mortality. Use of lower doses to avoid these complications has not been shown to produce effective tocolysis. In addition, these drugs mimic the metabolic effects of β-adrenergic agents that can lead to hyperinsulinemia and hypokalemia, further potentiating the cardiovascular complications. These drugs are uncommonly used in current practice, though they have been introduced and are being promoted in developing countries.

Drugs that block Ca^{2+} channels, for example nifedipine, are among the most commonly used tocolytic agents today (King et al., 2003). Unfortunately, their use is not supported by clinical trial comparison to a placebo. This is an important consideration since, depending on the criteria for diagnosing SPTL and the rigor of the diagnosis, the success rate of placebo treatment might be well over 50%. Since there is no evidence that uterine myoctes in SPTL have any greater sensitivity to Ca^{2+} channel blockers than do vascular myocytes, it seems likely that concentrations required to provide effective tocolysis would be associated with similar cardiovascular complications as the β-adrenergic drugs. Indeed, safety concerns regarding the use of these drugs in pregnancy is the focus of a recent systematic review (Khan et al., 2010).

Another pharmacological approach has targeted the synthesis of $PGF_{2\alpha}$, which may activate the uterus, ripen the cervix or directly stimulate contractions. Most studies have used non-specific inhibitors of cyclooxygenase (COX-1 and COX-2), most commonly the non-steroidal anti-inflammatory drug (NSAID) indomethacin. Although the original studies showed dramatic beneficial effects (Zuckerman et al., 1974; Zuckerman et al., 1984), more recent studies have not demonstrated such strong effects. Other studies pointed to major fetal-neonatal side effects including oligohydramnios, patent ductus arteriosus, cerebral hemorrhage and necrotizing enterocolitis (Norton et al., 1993). Again, there has been no evidence suggesting improved outcome for the infant. Other NSAIDs, including COX-2-specific inhibitors have been used but have not been shown to be safer or more effective (Groom et al., 2005). The COX inhibitors block production of all prostanoids, including PGE_2 and PGI_2. The physiological roles of these hormones on human uterine function are extremely complex and may differ in the upper and lower segments of the uterus. It is likely that they affect uterine myocytes as well as connective tissue cells. When the roles of the various PGs are more clearly understood, it is possible that development of more selective drugs targeting enzymes or receptors in the PG pathway will be useful for clinical control of uterine contractility. At present, the NSAIDs appear not to have fulfilled their initial promise (Mitchell and Olson, 2004).

Another approach to tocolysis involves the use of NO donors to induce activation of PKG, as described above (Smith et al., 2007). Once again, due to the lack of uterine specificity of this approach, it is likely that significant uterine relaxation will be accompanied by vascular relaxation and increased maternal side effects, including the potential for decreased uterine blood flow.

Potential uterine-specific mechanisms of tocolysis

Oxytocin antagonists

The most potent contractile agonist for uterine myocytes appears to be OT. Relative to other tissues, OT receptor (OTR) is most highly expressed in the myometrium, decidua and myoepithelial cells of the breast. Therefore, regarding smooth muscle targets, OTR is relatively tissue specific for the uterus. The development of OTR antagonists began approximately 25 years ago and clinical trials demonstrated their efficacy just over a decade ago. In general, these drugs are short polypeptides that mimic the native molecule and interfere with OT binding to OTR. Not surprisingly, there is overlap with the receptor for vasopressin, which is a hormone with similar structure to OT. These drugs are expensive to produce and, as peptides, need to be administered parenterally. Though there is strong evidence of beneficial effects and few side effects in comparison

to other tocolytic drugs, atosiban never received approval for use in the United States, perhaps because of a flaw in the placebo-controlled RCT to demonstrate the efficacy of the drug. In this trial (Romero et al., 2000), there was a very significant imbalance in the number of extremely preterm pregnancies (22-26 weeks) randomized to receive atosiban. The death rate in these babies at the brink of viability was very high. Because of this, when all babies were considered, there was an overall increase in the perinatal mortality rate of the babies receiving the drug compared to the placebo. The drug has been approved and is in common use in Europe. There are non-peptide OTR agonists that can be administered orally but trials of these in humans have not yet been published (Pettibone et al., 1995).

More recently, a different approach has been taken in the development of receptor antagonists. Rather than interfering with ligand binding, these 'allosteric' inhibitors interfere with other extracellular components of the seven transmembrane receptors and interfere with the signal transduction mechanisms. Indeed, such an inhibitor has been developed for the $PGF_2\alpha$ receptor. Interestingly, this experimental drug (THG113.31) had little effect on $PGF_2\alpha$-induced contractions in human uterine strips *ex vivo* but was a good inhibitor of OT-induced contractions (Friel et al., 2005). Further studies demonstrated that this inhibitor has an inhibitory effect on the $G\alpha_{12\text{-}14}$ signaling cascade but appears to enhance the $G\alpha_{q/11}$ pathway (Goupil et al., 2010). This emphasizes that uterine contractility is regulated by two distinct and counterbalanced systems – the Ca^{2+}-dependent, MLCK-mediated phosphorylation of RLC and the Ca^{2+}-independent, MLCP-mediated dephosphorylation of pRLC. Development of effective tocolytic drugs requires consideration of both pathways. To date, most drug development has focused on the former. Our recent research has examined regulation of MLCP and, we believe, has uncovered a potentially important and unique feature of uterine myocytes that could provide an effective and specific pharmacological target for tocolysis.

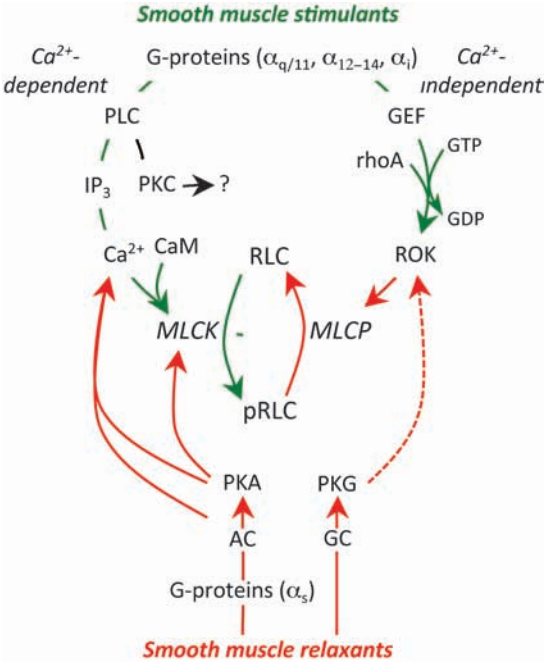

Fig. 1. — Regulation of smooth muscle contractility. Uterine stimulants generally stimulate specific G-protein coupled receptors (GPCR) on the myocyte membrane. This triggers two stimulatory pathways. The $G\alpha_q$ subunit activates phospholipase C (PLC) in the adjacent membrane and this results in hydrolysis of membrane phosphoinositides to from inositol trisphosphate (IP_3) and diacyl glycerol, which can activate protein kinase C (PKC; see text). IP_3 stimulates release of Ca^{2+} from the sarcoplasmic reticulum and the resulting decrease in cell membrane resting potential leads to massive influx of Ca^{2+} from the extracellular space through voltage–gated L-type Ca^{2+}-channels. Ca^{2+} binds to calmodulin (CaM). The Ca^{2+}-CaM complex interacts with and activates myosin regulatory light chain kinase (MLCK). MLCK mediates the phosphorylation of myosin regulatory lights chains (RLC) and initiates the contractile apparatus. The second stimulatory pathway is mediated through $G\alpha_{12\text{-}14}$. This causes activation of a guanidine nucleotide exchange factor (GEF), which activates membrane rhoA-GDP by substituting GTP for the GDP. The rhoA-GTP activates rhoA-associated kinase (ROK). ROK phosphorylates myosin regulatory light chain phosphatase (MLCP), which inhibits its activity. Since MLCP is normally responsible for removing the phosphate from pRLC to cause uterine relaxation, enhanced ROK activity prolongs the effectiveness of pRLC and promotes enhanced contractile activity. The major inhibitory pathways might also be mediated by GPCR, linked through $G\alpha_s$ resulting in activation of adenylate cyclase (AC) or direct activation of guanylate cyclase (GC), which in turn activates protein kinase A (PKA) or PKG. These kinases inhibit contractility through a variety of mechanisms described in the text. The green lines indicate stimulatory activity and the red lines inhibitory. The broken line indicates an indirect effect.

Inhibition of the MLCP pathway

As would be expected from the schematic in Figure 1, inhibitors of ROK reduce the inhibitory restraints on MLCP, promote dephosphorylation of pRLC, and thereby lead to diminished contractility of smooth muscle. Several studies have shown inhibition of basal and OT-stimulated uterine contractions both *in vivo* and *ex vivo* in human and rat myometrium (Niiro et al., 1997; Kupittayanant et al., 2001; Moran et al., 2002; Woodcock et al., 2004; Woodcock et al., 2006). Unfortunately, interruption of the ROK pathway also affects vascular smooth muscle so this may represent a non-specific pathway and the potential use of ROK inhibitors for prevention or treatment of SPTL would be complicated by intolerable off-target effects in vascular or other smooth muscle.

We considered that the logical focus of our approach to modification of uterine myocyte activity would be the reversible phosphorylation of RLC. We

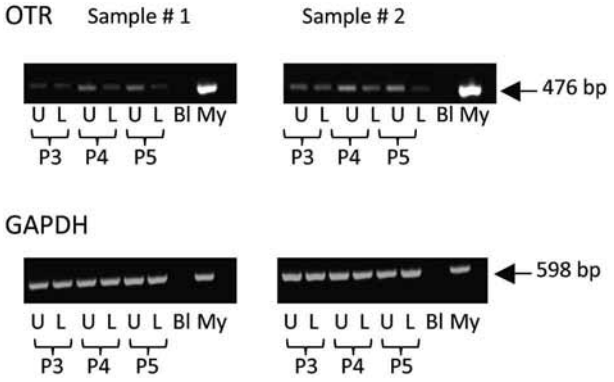

Fig. 2. — Expression of mRNA for oxytocin receptor in myocytes derived from upper and lower uterine segments. PCR of extracts from primary cultures of uterine myocytes derived from paired samples of uterine muscle (n = 2 patients) obtained from the upper fundal portion of the uterus (U) or the lower uterine segment (L) along with molecular weight markers (M), blanks (Bl) and extracts from myometrial tissues (My). The cells were used at passage 3, 4 and 5 (P3-P5). The upper panels demonstrate expression of OTR (476 bp) and the bottom panels show expression of GAPDH (598 bp) as a control. OTR is expressed in upper and lower segment myocytes and does not change with passage number.

developed relatively high throughput assays to assess this reaction using human uterine myocytes (Aguilar et al., 2010; Aguilar., 2011). Since we were primarily interested in uterine contractility during late pregnancy, we obtained uterine biopsies from the upper flap of the lower segment transverse uterine incision at the time of elective cesarean section at term but prior to clinical labor onset. Since it has been suggested that there might be regional differences in physiological function for myocytes located in the upper more contractile part of the uterus compared myocytes located in the lower segment, we isolated myocytes from both uterine regions to validate that the cells from the lower segment were representative of those in the fundus. For these experiments, we obtained paired (upper and lower) uterine biopsies. The lower segment biopsies were taken as described above. The fundal biopsy was obtained by first excising the overlying decidua, then excising a small myometrial biopsy using iris scissors.

For the first step in validation, we used RT-PCR to examine expression of mRNA for OTR in primary cultures of uterine myocytes from the upper (fundal) and lower parts of the uterus at several passages. In Figure 2 it is seen that OTR expression is similar in both regions and is maintained through several passages of cells. A Ca^{2+} assay was then used to investigate the response of these cells to OT (Fig. 3). Treatment of fundal and lower segment myocytes with OT resulted in a concentration-dependent increase in intracellular Ca^{2+}. There was no difference in the responses between the fundal and lower segment cells.

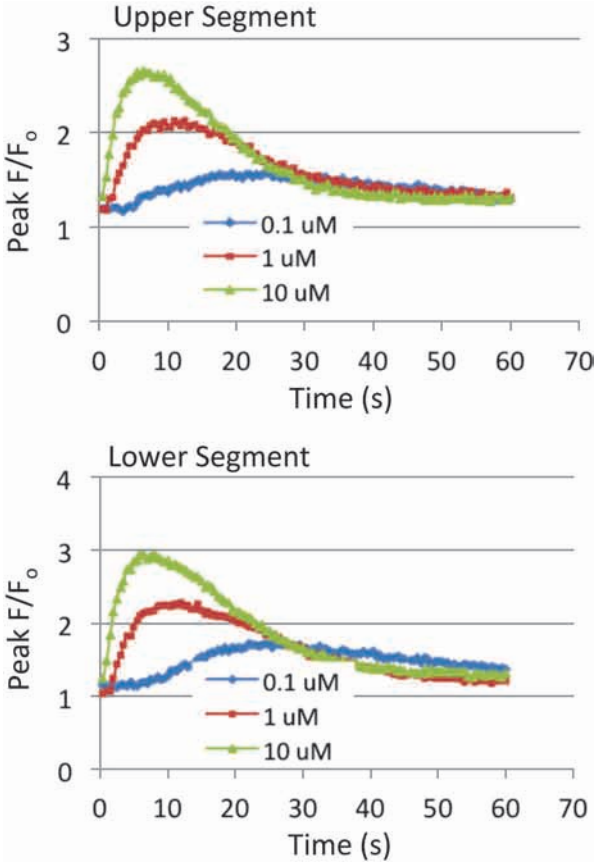

Fig. 3. — Intracellular concentrations of Ca^{2+} following stimulation of uterine myocytes with oxytocin. Primary cultures of fundal (upper panel) and lower (lower panel) segment uterine myocytes were plated in Greiner Bio-One black plates (5.0×10^4 cells/ml) and grown for 48 hours. Cells were then loaded with Fluo-4 dye and incubated at 37°C for 30 minutes. OT (0.1 μM, 1 μM, 10 μM) was added to the cells and fluorescence was measured. Data are presented as peak fluorescence over baseline fluorescence (F/F_0; mean ± SEM; n = 4 patients).

We then demonstrated (Fig. 4) concentration-dependent, and statistically significant increases in concentrations of pRLC following stimulation with OT. These responses were similar in cells from fundal and lower segment biopsies. The peak responses in phosphorylation of RLC occurred within the first 20 s, in accord with the Ca^{2+} data in Figure 3. We noted that the potent and specific ROK inhibitor glycyl-H1152 (g-H) caused a 40-50% reduction of resting pRLC concentrations but the response to OT was maintained. A similar response profile was demonstrated using the MLCK inhibitor ML7. Again, these responses were similar in cells from the fundal and lower uterine segments. We also measured responses for diphosphorylated RLC (ppRLC; Fig. 5) and again noted significant responses to OT, which were similar in the cells from fundal and lower segments. Treatment with the inhibitors of ROK or MLCK again caused suppression of basal ppRLC concentrations. However, unlike pRLC, the

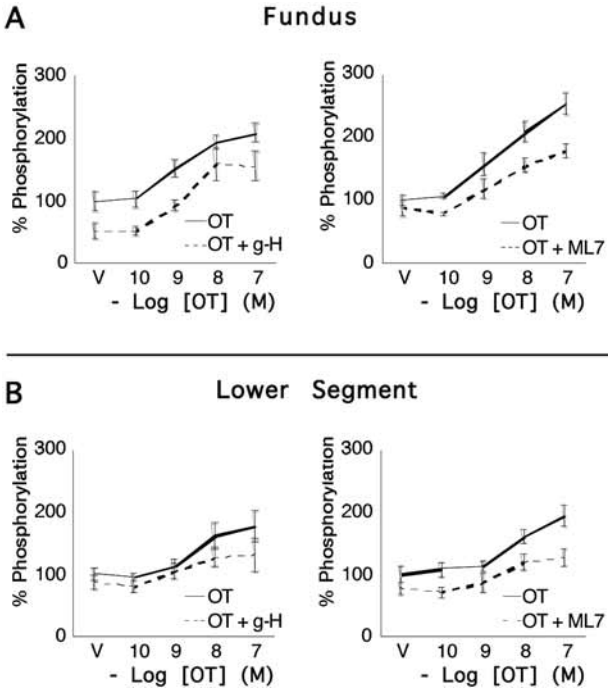

Fig. 4. — Concentrations of pRLC in uterine myocytes from upper and lower uterine segments following stimulation by oxytocin. Primary cultures of myocytes from the uterine fundus and lower uterine segments (n = 5 patients) were treated with increasing concentrations of OT for 20 s and the response measured using a specific antibody for pRLC phosphorylated at S19 in an *in-cell western* assay. There were significant, concentration-dependent increases that were similar in cells from upper and lower segments. 15-minute pretreatment with the rho-kinase inhibitor (g-H; 1 μM) or the myosin regulatory light chain kinase inhibitor (ML7; 25 μM) caused approximately 20-30% suppression of basal pRLC but the response to OT was maintained.

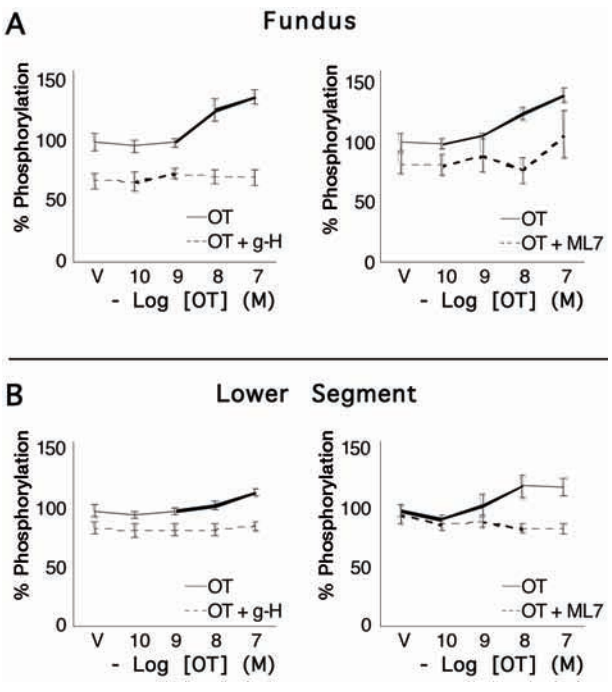

Fig. 5. — Concentrations of ppRLC in uterine myocytes from upper and lower uterine segments following stimulation by oxytocin. Primary cultures of myocytes from the uterine fundus and lower uterine segments (n = 5 patients) were treated with increasing concentrations of OT for 20 s and the response measured using a specific antibody for ppRLC phosphorylated at T18 and S19 in an *in-cell western* assay. There were significant, concentration-dependent increases in ppRLC in cells from upper and lower segments. As for pRLC, 15-mimute pretreatment with the rho-kinase inhibitor (g-H; 1 μM) or the myosin regulatory light chain kinase inhibitor (ML7; 25 μM) caused approximately 20-30% suppression of basal ppRLC. However, in contrast to the results for pRLC, the ppRLC responses to OT were completely abolished in the presence of the kinase inhibitors.

responses of ppRLC to OT were blocked by either of the inhibitors, again similarly in cells from the fundal and lower segments.

We have reported previously on the results of a series of experiments specifically exploring the role of ROK and MLCK in mono- and diphosphorylation of RLC in uterine myocytes following stimulation with physiological uterine agonists (Aguilar et al., 2010; 2011; 2012). We made specific comparisons to the responses of vascular myocytes to physiological agonists. There were two major conclusions from these experiments. The first conclusion was that uterine myocytes respond to stimulation with significant increases in both pRLC and ppRLC whereas the vascular myocytes respond only with pRLC. The responses in pRLC are due to the well-described effects of physiological agonists: increased Ca^{2+}-dependent MLCK-mediated phosphorylation of RLC and increased ROK-mediated inhibition of MLCP.

The second major conclusion of these studies was a novel and potentially important finding. The increased ppRLC was produced by ROK-mediated phosphorylation of pRLC to ppRLC. Since this biochemical conversion of pRLC to ppRLC was not noted in the vascular myocytes, we speculated that it might be a unique feature of smooth muscle with a phasic contractile pattern, such as the uterus. Since ppRLC causes 3-fold greater activation of myosin ATPase, which provides the energy for the contractile apparatus (Ikebe et al., 1986; 1988), we reasoned that diphosphorylation of RLC may represent an evolutionary adaptation of phasic smooth muscle driven by the need to produce periodic but powerful contractions, in contrast to tonic smooth muscle such as vascular, which requires long-term stable tension with relatively little change in amplitude. Importantly, the ROK inhibitor prevented the ppRLC response to OT in uterine myocytes *in vitro* at concentrations that were 100-fold lower that those required to decrease the concentration of pRLC in vascular myocytes. Thus, inhibition of ROK has potential to be a clinically useful and utero-specific target to mediate uterine relaxation while avoiding

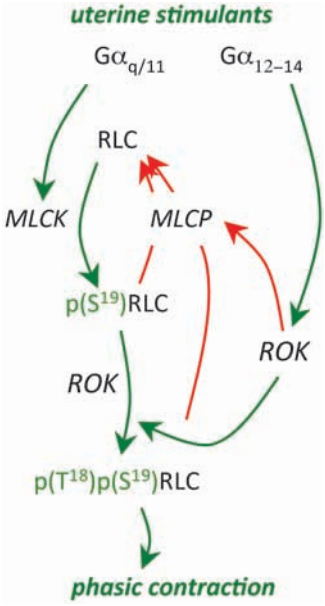

Fig. 6. — Schematic diagram illustrating the unique pattern of phosphorylation of RLC in uterine myocytes. As in all smooth muscle, myosin regulatory light chain (RLC) is phosphorylated by myosin regulatory light chain kinase (MLCK) at S19. However, in uterine myocytes, pRLC is additionally phosphorylated at T18 to form ppRLC, a more potent activator of the myosin ATPase that supplies energy for the contraction. This diphosphorylation pathway might be a unique adaptive pathway for smooth muscle that contract in a phasic pattern. The green lines indicate stimulatory activity and the red lines inhibitory.

vascular compromise. Our current concept of the mechanisms regulating phosphorylation of RLC specifically in uterine myocytes is presented in the schematic in Figure 6.

Extrinsic influences on uterine myocytes

The previous discussions have considered mechanisms that are intrinsic to the uterine myocyte. Clearly, there are mechanisms extrinsic to the myocyte that will influence myocyte responses to agonists or antagonists. Indeed, SPTL is likely the end result of many differing pathophysiologic pathways that involve intrinsic and/or extrinsic pathways. As such, SPTL represents a syndrome rather than a specific disease entity. Here, we discuss some of the mechanisms extrinsic to the uterine myocyte that could provide pharmacological targets to influence uterine contractility.

Concept of uterine activation and stimulation

Several years ago, a useful concept was proposed suggesting that the status of uterine contractility during pregnancy could be considered in three categories (Challis et al., 2000). Uterine *quiescence* was the period that began at implantation and lasted most of pregnancy, during which the uterus remained relatively quiet. Next was the period of uterine *activation*, which occurred over a few days prior to clinical labor onset. During this time, the density of 'contraction associated proteins' (CAPs) increased to transform the uterus into a sensitive contractile organ capable of responding to agonists with powerful, coordinated contractions. Included among the CAPs are the receptors for uterine agonists (OT, $PGF_2\alpha$, etc.), a variety of ion channels, and the major gap junction protein connexin 43. Uterine *stimulation* occurs due to the actions of endogenous agonists such as OT, $PGF_2\alpha$, etc. There remains only poor understanding of the identity of the key endogenous agonist(s). Regulation of these processes can be considered 'upstream' of the uterine myocyte, though the mechanisms are similarly unclear. These mechanisms might represent earlier targets for interruption of the cascade of events leading to parturition. The following sections deal with two examples of such potential extrinsic regulatory systems.

Estrogen/progesterone ratio

Over the past half century, it has been demonstrated that, in most mammalian species, withdrawal of progesterone from the maternal circulation is necessary and sufficient to cause parturition. However, in higher order primates, including humans, there is no such decline in maternal serum progesterone at the time of parturition. It has been proposed that, in these species, progesterone still may be an essential progestational hormone but its withdrawal at the time of parturition occurs on a more local, intrauterine basis that is not reflected in maternal serum. Various mechanisms have been suggested for a 'functional progesterone withdrawal.' These include decreased synthesis or increased metabolism within the amnion, chorion and decidua (Mitchell and Powell, 1984). These tissues lay immediately adjacent to the myometrium and thus might have significant influence over progesterone effects on uterine myocytes. Similarly, changes in expression and activity of progesterone receptors within the uterine myocyte could regulate progesterone-dependent transcriptional activity (Mesiano et al., 2002; Condon et al., 2003).

Over the past decade, there has been renewed interest in the use of supplemental progesterone to prevent PTB in women at high-risk. Based on the results of a 30-year old trial (Johnson et al., 1975), an RCT demonstrated some efficacy in preventing PTB using prophylactic progestin supplementation over the last half of gestation (Meis et al., 2003). The drug used was 17-hydroxyprogesterone caproate, which clearly is not progesterone and its mechanism of action is not fully understood. However, the results were supported by an additional RCT using

natural progesterone administered by vaginal suppository, which also showed a statistically significant improvement on the rates of PTB (da Fonseca et al., 2003). Though there is increasing use of this prophylactic regimen for woman at high risk of PTB, it remains unclear what the outcomes will be regarding the health of the newborn. Prophylactic use of progestin also has been shown to be efficacious in women with a short cervix demonstrated on routine mid-pregnancy ultrasound (Romero et al., 2012). The cost-effectiveness of this strategy of ultrasound screening and progestin administration and the potential longer-term effects of this approach remain to be determined.

Research from several animal species has also suggested that estrogen plays an important role in the timing of parturition. In general, its effects tend to oppose those of progesterone. This has given rise to the concept of the ratio of estrogen/progesterone as a key factor. In particular, estrogen has been demonstrated to enhance transcription of many of the genes that code for CAP proteins while progesterone can block the increase in CAP mRNA expression (Ou et al., 1997; 1998; 2000). As with progesterone, local estrogen synthesis and metabolism might be of primary importance (Mitchell & Wong, 1993; Romero et al., 1988). Estrogen antagonists will cause delay in rat parturition but this is complicated by decreased growth rate of the pups (Fang et al., 1996). In theory, the potentially important negative effects of administration of estrogen antagonists outweigh their potential to delay birth. In addition, there is no evidence that they are effective in SPTL.

Immune system

It has been known for decades that parturition, whether at term or preterm, is accompanied by an inflammatory response resulting in an invasion of leukocytes into intrauterine tissues (Liggins, 1981). Abundant recent research has extensively characterized the pro-inflammatory responses that accompany parturition, either with or without infection at term or preterm. However, the purpose of this inflammatory response remains unclear. Some have reasoned that this enhanced activity of the immune system is focused on healing the placental site following parturition and mediating the process of uterine involution (Mitchell and Taggart, 2009). Others have suggested that the inflammatory response initiates the process of parturition. It is possible that both lines of thought could be correct. From the evolutionary perspective, activation of the healing response concurrently with the process that leads to increased uterine contractility would be logical and beneficial. Linking the two processes such that they are interdependent would ensure the two distinct and vital activities act together. This might be analogous to the situation in sheep where increased activity of the uterus is linked to the same mechanisms that mature the fetal lungs in preparation for extrauterine existence.

To date, the role of the immune system in the initiation of parturition, though extensively studied, remains unresolved. It is clear that, in the presence of intrauterine infection, this inflammatory reaction is markedly enhanced. There is convincing evidence that a severe intrauterine inflammatory event, such as infection or chemical irritation can provoke parturition at any stage of gestation. However, the role of the pro-inflammatory reactions in regulation of term labor and SPTL require further clarification. Similarly, there has been elegant demonstration of the effects of uterine stretch on pathways thought to be important in regulating parturition, such as the up-regulation of CAPs (Ou et al., 1997; Terzidou et al., 2005). However, again, the role of such mechanisms in determining the occurrence of labor at term or preterm is not well understood.

At present, the research has yet to produce clinically effective therapeutic tools for tocolysis. Historically, the extensive RCTs using large doses of glucocorticoids administered to women with SPTL to accelerate fetal lung maturation have demonstrated clearly that these potent anti-inflammatory agents had no clinically significant effect on the timing of birth. In the cases of infection-associated PTL, where the inflammatory response may be the driving force to PTB, current practice is to hasten delivery and avoid the use of tocolytics. In summary, despite massive research activity, the role of immunomodulators in the management of SPTL has yet to be defined.

Conclusions

It is clear that PTB can arise from many distinct etiologic pathways involving a myriad of physiological and pathophysiological mechanisms. Some of these arise from within the target cells – the uterine myocytes – and some involve systemic extrinsic systems that modulate uterine myocyte activity. The mechanistic pathways concern many membrane proteins of the uterine myocyte including GPCR and ion channels, among many others. Many intracellular signal transduction pathways, including the Ca^{2+}-CaM-MLCK and the rhoA-ROK-MLCP pathways, are likely to be important. Extrinsic influences such as the estrogen/progesterone ratio, uterine stretch and the immune system are likely to play important modulatory or even causative roles. We have

considered each of these pathways, and undoubtedly many more, to be *modules* that affect uterine contractions. Though not included in this discussion, there are many more modules that potentially regulate the process of cervical ripening. When a critical number of modules are activated, labor will occur: this is the concept of modular accumulation of physiological systems (MAPS) that we have proposed earlier (Mitchell and Taggart, 2009).

In view of this probable heterogeneity of pathways contributing to the occurrence of SPTL, future attempts at tocolytic therapy might need to be broad-based and include combinations of targets. Future research using genomic, proteomic and metabolomic technologies holds the promise of predicting women at risk of SPTL and hopefully indicate the modules that are prematurely activated. This will facilitate a preventative rather than a therapeutic (tocolytic) approach, which is likely to have many advantages. Combination pharmacological regimens will necessitate even more attention to uterine-specific therapies to avoid off-target effects that will preclude the ability to use effective doses. Drug interactions will then become even more important considerations and pharmacogenomic approaches may help resolve these issues. Whereas the past has been disappointing with respect to prevention of PTB, the future hold new promise that every baby can be born at its optimal time.

References

Aguilar HN, Mitchell BF. Physiological Pathways and Molecular Mechanisms Regulating Uterine Contractility. Hum Reprod Update. 2010;16:725-44.

Aguilar HN, Tracey CN, Tsang SC et al. Phos-Tag-Based Analysis of Myosin Regulatory Light Chain Phosphorylation in Human Uterine Myocytes. PLoS One. 2011;6:e20903.

Aguilar HN, Tracey CN, Zielnik B, Mitchell BF. Quantification of Rapid Myosin Regulatory Light Chain Phosphorylation Using High-Throughput in-Cell Western Assays: Comparison to Western Immunoblots. PLoS One. 2010;5:e9965.

Aguilar HN. J Cell Mol Med. 2012;in press.

Arthur P, Taggart MJ, Zielnik B, Mitchell BF. Relationship between Gene Expression and Function of Uterotonic Systems in the Rat During Gestation, Uterine Activation and Both Term and Preterm Labour. J Physiol. 2008;586:6063-76.

Behrman RE, Butler AS. Preterm Birth: Causes, Consequences, and Prevention. *Preterm Birth: Causes, Consequences, and Prevention*, 2007, National Academies Press, Washington.

Berridge M J. Inositol Trisphosphate and Calcium Signalling. Nature. 1993;361:315-25.

Challis JRG, Matthews SG, Gibb W *et al*. Endocrine and Paracrine Regulation of Birth at Term and Preterm. Endocr Rev. 2000;21:514-50.

Chibbar R, Miller FD, Mitchell BF. Synthesis of Oxytocin in Amnion, Chorion, and Decidua May Influence the Timing of Human Parturition. J Clin Invest 1993;91:185-92.

Condon JC, Jeyasuria P, Faust JM et al. A Decline in the Levels of Progesterone Receptor Coactivators in the Pregnant Uterus at Term May Antagonize Progesterone Receptor Function and Contribute to the Initiation of Parturition. Proc Natl Acad Sci USA. 2003;100:9518-23.

da Fonseca EB, Bittar RE, Carvalho MH et al. Prophylactic Administration of Progesterone by Vaginal Suppository to Reduce the Incidence of Spontaneous Preterm Birth in Women at Increased Risk: A Randomized Placebo-Controlled Double-Blind Study. Am J Obstet Gynecol. 2003; 188:419-24.

Fang X, Wong S, Mitchell BF. Relationships among Sex Steroids, Oxytocin, and Their Receptors in the Rat Uterus During Late Gestation and at Parturition. Endocrinology. 1996;137:3213-9.

Friel AM, O'Reilly MW, Sexton DJ et al. Specific Pgf(2alpha) Receptor (Fp) Antagonism and Human Uterine Contractility in Vitro. BJOG 2005;112:1034-42.

Goupil E, Tassy D, Bourguet C et al. A Novel Biased Allosteric Compound Inhibitor of Parturition Selectively Impedes the Prostaglandin F2alpha-Mediated Rho/Rock Signaling Pathway. J Biol Chem. 2010;285:25624-36.

Groom KM, Shennan AH, Jones BA et al. Tocox – a Randomised, Double-Blind, Placebo-Controlled Trial of Rofecoxib (a Cox-2-Specific Prostaglandin Inhibitor) for the Prevention of Preterm Delivery in Women at High Risk. BJOG. 2005;112:725-30.

Howson CP, Kinney MV, Lawn JE. *Born Too Soon: The Global Action Report on Preterm Birth*. March of Dimes, PMNCH, Save the Children, Geneva. 2012.

Ikebe M, Hartshorne DJ, Elzinga M. Identification, Phosphorylation, and Dephosphorylation of a Second Site for Myosin Light Chain Kinase on the 20,000-Dalton Light Chain of Smooth Muscle Myosin. J Biol Chem. 1986;261:36-9.

Ikebe M, Koretz J, Hartshorne DJ. Effects of Phosphorylation of Light Chain Residues Threonine 18 and Serine 19 on the Properties and Conformation of Smooth Muscle Myosin. J Biol Chem 1988;263:6432-7.

Johnson JW, Austin KL, Jones GS et al. Efficacy of 17alpha-Hydroxyprogesterone Caproate in the Prevention of Premature Labor. N Engl J Med. 1975;293:675-80.

Khan K, Zamora J, Lamont RF et al. Safety concernsfor the use of calcium channel blockersin pregnancy for the treatment of spontaneous preterm labour and hyprtension: a systematic review and regression analysis. J Mat-Fetal Neonatal Med. 2010;23(9);1030-8.

King JF, Flenady VJ, Papatsonis DN et al. Calcium Channel Blockers for Inhibiting Preterm Labour. Cochrane Database Syst Rev. 2003:CD002255.

King JF, Grant A, Keirse MJ et al. Beta-Mimetics in Preterm Labour: An Overview of the Randomized Controlled Trials. Br J Obstet Gynaecol. 1988;95:211-22.

Kotani M, Tanaka I, Ogawa Y et al. Multiple Signal Transduction Pathways through Two Prostaglandin E Receptor Ep3 Subtype Isoforms Expressed in Human Uterus. J Clin Endocrinol Metab. 2000;85:4315-22.

Kozasa T, Jiang X, Hart MJ et al. P115 Rhogef, a Gtpase Activating Protein for Galpha12 and Galpha13. Science. 1998;280:2109-11.

Kupittayanant S, Burdyga T, Wray S The Effects of Inhibiting Rho-Associated Kinase with Y-27632 on Force and Intracellular Calcium in Human Myometrium. Pflugers Arch. 2001;443:112-4.

Liggins GC. Cervical Ripening as an Inflammatory Reaction. In Elwood DA, Anderson ABM (ed), *The Cervix in Pregnancy and Labour, Clinical and Biochemical Investigations*. Churchill Livigstone, Edinburgh. 1981.

Meis PJ, Klebanoff M, Thom E et al. Prevention of Recurrent Preterm Delivery by 17 Alpha-Hydroxyprogesterone Caproate. N Engl J Med. 2003;348:2379-85.

Mesiano S, Chan EC, Fitter JT et al. Progesterone Withdrawal and Estrogen Activation in Human Parturition Are Coordinated by Progesterone Receptor a Expression in the Myometrium. J Clin Endocrinol Metab. 2002;87:2924-30.

Mitchell BF, Olson DM. Prostaglandin Endoperoxide H Synthase Inhibitors and Other Tocolytics in Preterm Labour. Prostaglandins Leukot Essent Fatty Acids. 2004;70:167-87.

Mitchell BF, Powell WA. Progesterone Production by Human Fetal Membranes: An in Vitro Incubation System for Studying Hormone Production and Metabolism. Am J Obstet Gynecol. 1984;148:303-9.

Mitchell BF, Taggart MJ. Are Animal Models Relevant to Key Aspects of Human Parturition? Am J Physiol Regul Integr Comp Physiol. 2009;297:R525-45.

Mitchell BF, Wong S. Changes in 17 Beta,20 Alpha-Hydroxysteroid Dehydrogenase Activity Supporting an Increase in the Estrogen/Progesterone Ratio of Human Fetal Membranes at Parturition. Am J Obstet Gynecol.1993;168:1377-85.

Moran CJ, Friel AM, Smith,TJ et al. Expression and Modulation of Rho Kinase in Human Pregnant Myometrium. Mol Hum Reprod. 2002;8:196-200.

Niiro N, Nishimura J, Sakihara C et al. Up-Regulation of Rho a and Rho-Kinase mRnas in the Rat Myometrium During Pregnancy. Biochem Biophys Res Commun. 1997;230:356-9.

Norton ME, Merrill J, Cooper BA et al. Neonatal Complications after the Administration of Indomethacin for Preterm Labor. N Engl J Med. 1993;329:1602-7.

Ou CW, Chen ZQ, Qi S et al. Expression and Regulation of the Messenger Ribonucleic Acid Encoding the Prostaglandin F(2alpha) Receptor in the Rat Myometrium During Pregnancy and Labor. Am J Obstet Gynecol. 2000;182: 919-25.

Ou CW, Chen ZQ, Qi S et al. Increased Expression of the Rat Myometrial Oxytocin Receptor Messenger Ribonucleic Acid During Labor Requires Both Mechanical and Hormonal Signals. Biol Reprod. 1998;59:1055-61.

Ou CW, Orsino A, Lye SJ. Expression of Connexin-43 and Connexin-26 in the Rat Myometrium During Pregnancy and Labor Is Differentially Regulated by Mechanical and Hormonal Signals. Endocrinology. 1997;138:5398-407.

Pettibone DJ, Guidotti M, Harrell CM et al. Progress in the Development of Oxytocin Antagonists for Use in Preterm Labor. Adv Exp Med Biol. 1995;395:601-12.

Phaneuf S, Carrasco MP, Europe-Finner GN et al. Multiple G Proteins and Phospholipase C Isoforms in Human Myometrial Cells: Implication for Oxytocin Action. J Clin Endocrinol Metab. 1996;81:2098-103.

Romero R, Nicolaides K, Conde-Agudelo A et al. Vaginal Progesterone in Women with an Asymptomatic Sonographic Short Cervix in the Midtrimester Decreases Preterm Delivery and Neonatal Morbidity: A Systematic Review and Meta-analysis of Individual Patient Data. Am J Obstet Gynecol. 2012;206:124. e1-19.

Romero R, Sibai BM, Sanchez-Ramos L et al. An oxytocin receptor antagonist (atosiban) in the treatment of preterm labor: a randomized, double-blind, placebo-controlled trial with tocolytic rescue. Am J Obstet Gynecol. 2000;182:1173-83.

Romero R, Scoccia B, Mazor M et al. Evidence for a Local Change in the Progesterone/Estrogen Ratio in Human Parturition at Term. Am J Obstet Gynecol. 1988;159:657-60.

Smith GN, Walker, MC, Ohlsson A et al. Randomized Double-Blind Placebo-Controlled Trial of Transdermal Nitroglycerin for Preterm Labor. Am J Obstet Gynecol 2007;196:37 e31-38.

Somlyo AP, Somlyo AV. Ca2+ Sensitivity of Smooth Muscle and Nonmuscle Myosin Ii: Modulated by G Proteins, Kinases, and Myosin Phosphatase. Physiol Rev. 2003;83:1325-58.

Terzidou V, Sooranna SR, Kim LU et al. Mechanical Stretch up-Regulates the Human Oxytocin Receptor in Primary Human Uterine Myocytes. J Clin Endocrinol Metab. 2005;90:237-46.

Woodcock NA, Taylor W, Thornton S. Effect of an Oxytocin Receptor Antagonist and Rho Kinase Inhibitor on the [Ca++]I Sensitivity of Human Myometrium. Am J Obstet Gynecol. 2004;190:222-8.

Woodcock NA, Taylor CW, Thornton S. Prostaglandin F2alpha Increases the Sensitivity of the Contractile Proteins to Ca2+ in Human Myometrium. Am J Obstet Gynecol. 2006;195: 1404-6.

Zuckerman H, Reiss U, Rubinstein I. Inhibition of Human Premature Labor by Indomethacin. Obstet Gynecol. 1974; 44:787-92.

Zuckerman H, Shalev E, Gilad G et al. Further Study of the Inhibition of Premature Labor by Indomethacin. Part I. J Perinat Med. 1984;12:19-23.

Metabolic Modulation of the Myometrium and Labour, Term and Preterm

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Abstract

An understanding of the physiological and pharmacological factors regulating myometrial contractility is essential to development of therapeutic interventions aimed at addressing the clinical disorders associated with labor. Preterm labor and dystocia are the clinical conditions associated with dysfunctional myometrial contractility. Both conditions are seen in increased frequency in the presence of maternal obesity. This review serves to address the role of adipocytokines, secretory products of adipose tissue, on uterine smooth muscle contractility in pregnancy. These adipocytokines include leptin, ghrelin and apelin. The impact of hypercholesterolemia, hyperlipidemia and other conditions associated with obesity such as diabetes mellitus on myometrial contractile performance is also examined.

Key words: **Adipocytokines, cholesterol, dysfunctional labor, hyperlipidemia, myometrium, obesity, preterm labor.**

Introduction

An understanding of the physiological and pharmacological factors regulating myometrial contractility is essential to development of therapeutic interventions aimed at addressing the clinical disorders associated with labor. Myometrial dysfunction leads to adverse clinical outcomes namely preterm labor or dystocia. These conditions impact on fetal outcome and mode of delivery. Both preterm labor and dystocia have been seen with increased frequency in obese parturients. The purpose of this review is to investigate potential mechanisms leading to preterm labor or dystocia in women in high body mass index categories.

Preterm Labor

Preterm labor, and its clinical sequelae, represents the largest challenge in perinatal medicine, from the perspectives of morbidity and economic cost. Preterm labour is the leading cause of adverse perinatal outcome accounting for 75% of perinatal mortality and more than half of long-term morbidity. (McCormick, 1985). Preterm infants have higher rates of cerebral palsy, sensory deficits, learning disabilities and respiratory illnesses compared with children born at term. The morbidity associated with preterm birth often extends to later life, resulting in enormous physical, psychological and economic costs (Petrou, 2003; 2005). Estimates indicate that in 2005 the costs to the United States of America alone in terms of medical and educational expenditure associated with preterm birth were more than US$ 26.2 billion (Peristats, 2006). In the USA approximately 1 in 8 infants is delivered before 37 completed weeks while in Europe the reported rate of preterm babies is of the order of 5-9% (Martin et al., 2010; Beck, 2010). Current knowledge of the pathophysiology of preterm labor is poor and, as a result, measures to prevent preterm labour have been largely unsuccessful. Previously described causes include spontaneous preterm labor (31-50%), multiple pregnancy and associated complications (12-28%), preterm prelabor rupture of membranes (6-40%), hypertensive disorders of pregnancy (12%), intrauterine growth restriction (2-4%), ante partum haemorrhage (6-9%) and a small

contribution from anatomical abnormalities (8-9%) (Slattery, 2002).

Epidemiological studies have identified a number of risk factors including low socioeconomic status, black race, extremes of maternal age, multiple pregnancy, low maternal BMI, maternal nutritional status, substance misuse and infection (Goldenberg, 2008). It is, however, often difficult to establish causality in many of these associations due to confounding variables. The molecular and cellular processes involved in the onset and maintenance of human parturition are complex and incompletely understood. Many of the important physiological pathways, associated with onset of the labour process, have been elucidated in studies in recent years (Aguilar and Mitchell, 2010; Arthur et al., 2007; Lopez, 2003) and although some of these mechanisms are reasonably well understood the exact pathophysiology that underlies preterm labour remains unknown (Menon, 2008).

In the last two decades significant research resources have been dedicated to elucidation of the molecular and physiological aspects of uterine function. As a result, our understanding of myometrial contractility, and its regulatory factors, has greatly improved. These extensive efforts have however not yielded results that have made a meaningful impact on either the prevention or treatment of preterm birth, and, sadly, the adverse perinatal outcome has remained unchanged (Costeloe, 2000; Kenyon and Peebles, 2011).

For tocolytic drugs, formerly the mainstay of treatment for preterm labour, recent research findings have established both their clear limitations in terms of benefit, and also highlighted their major adverse maternal and fetal effects (Tsatsaris et al., 2001; Berkman et al., 2003; Anotayanonth et al., 2003; Papatsonis et al., 2005).

Historically, β2 adrenergic agonists had been the most widely used agents in clinical practice (de Heus et al., 2008). In more recent times many newer agents with effects on uterine contractility and efficacy in the maintenance of uterine quiescence have been investigated (Terrone et al., 2000; Bisits et al., 2004; Gill et al., 2006; Conde-Agudelo et al., 2011; Usta et al., 2011). Of these agents, β-mimetics, the oxytocin antagonist atosiban, and the Ca2+ channel blocker, nifedipine, have all been shown to significantly delay delivery after the onset of preterm labour for longer than 24 h and 48 h compared with placebo or no treatment (Romero et al., 2000; Tsatsaris et al., 2001; Wex et al., 2011; Derbent et al., 2011; Nassar et al., 2011). However, neonatal outcome, as measured by perinatal death, respiratory distress syndrome, birthweight, patent ductus arteriosus, necrotising enterocolitis, intraventricular haemorrhages, seizures, hypoglycaemia, or neonatal sepsis did not differ greatly in treatment and control groups.

No definitive tocolytic compound, with the correct balance of efficacy, side-effect profile and positive effect on neonatal outcome, has emerged despite intensive research effort (Kam and Lamont, 2008). For this reason, significant scientific and clinical resources have continued to be invested into the development of novel tocolytic agents (Yuan and Lopez Bernal, 2007; Bourguet et al., 2011). In order to develop these new tocolytics, an improved understanding of the factors governing myometrial physiology is necessary and for this reason the focus of attention in recent years has also moved to endogenous factors regulating myometrial contractility (Crankshaw and Morrison, 2011) alongside the appreciation that metabolic factors play a significant role (Elmes et al., 2011). For these reasons, this chapter will explore the potential role of metabolic factors in regulation of myometrial contractility, and analyse the relevance of extremes of body mass index, particularly obesity, and other metabolic states, to the timing and control of parturition.

Obesity and Dystocia

Obesity is an increasing challenge in obstetric practice. The physical and metabolic sequelae of obesity are associated with a large number of pregnancy and delivery related complications (Weiss et al., 2004; Ovesen et al., 2011). It is also evident that the prevalence and extent of obesity in women of childbearing age has increased in recent years (Kanaglingam et al., 2005; Lynch et al., 2008). Increased BMI makes practical clinical procedures such as clinical examination, phlebotomy and intravenous cannulation challenging. There is also an increasing body of evidence to suggest that obesity plays a role in adverse fetal conditions such as neural tube defects, congenital heart defects, stillbirth, intrauterine growth restriction and macrosomia (Abrams and Laros et al., 1986; Seidman et al., 1989; Jensen et al., 2003; Arendas et al., 2008; Rasmussen et al., 2008; Flenady et al., 2011). Recent studies have shown that intra-partum complications such as dysfunctional labour, increased caesarean delivery, macrosomia, shoulder dystocia and fetal brachial plexus injury are increased in women who are overweight or obese (Weis et al., 2004; Bhattarcarya et al., 2007; Owens et al, 2010). Previous hypotheses have suggested that excessive adipose tissue distribution in the maternal pelvis, or around the fetal shoulders and abdomen, or inadequate maternal effort in the second stage of labor, may be responsible for the dystocic and dysfunctional labour seen in

obese parturients. However the idea that metabolic modulation of uterine contractility may play a role in the dysfunctional labour observed in this group of women has also been raised (Lowe and Corwin, 2011). This theory hypothesizes that women with a higher body mass index are more likely to have metabolic factors contributing to inefficient uterine action, and higher caesarean section rates, all of which have been observed in these women (Weiss et al., 2004).

In recent years much research interest has focused on a new range of secretory products from adipose tissue, collectively known as adipocytokines (Briana and Malamitsi-Puchner, 2010), many of which are now known to be closely linked to metabolic regulation of important systemic factors such as glucose tolerance, insulin resistance, cardiac and vascular function, and many other neuro-endocrine mediated processes (Xu et al., 2010; Torres-Leal et al., 2010). Adipocytokines such as leptin, adiponectin, resistin, ghrelin and apelin have been shown to be implicated in fetal growth and in utero development though their exact role in this complex process must still be completely elucidated (Malamitsi-Puchner et al., 2007; Chanoine et al., 2009; Higgins and Mc Auliffe, 2010). What is conclusive is that serum concentrations of most adipocytokines are altered in the pregnant state and that these compounds are expressed in significant concentrations by the feto - placental unit (Zhao et al., 2004; O'Brien et al., 2010; Van Mieghem et al., 2010). This indicates that adipocytokines play a central role in the development and maintenance of normal pregnancy and may also have an impact on myometrial function. The outline below will examine the effects of adipocytokines, and other metabolic factors, in relation to their effects on myometrial contractility.

Adipocytokines

Leptin

Leptin is a peptide with 167 amino acid residues which is secreted from adipose tissue under the control of the obesity gene (Haynes et al., 1998) and is known to have regulatory effects on neuronal tissue, vascular smooth muscle and non-vascular smooth muscle systems (Yuan et al., 2004). Serum leptin concentrations have been shown to rise significantly with increasing percentage body fat (Considine et al., 1996) and obese individuals have markedly increased leptin production (Hamilton et al., 1995). The role of leptin in human reproduction is not clear. While leptin is most likely involved in the regulation of ovarian function, oocyte maturation, embryo development and implantation (Haynes et al., 1998), the role of leptin during pregnancy has not been clearly established. The placenta is a major source of leptin production during pregnancy (Domali and Messinis, 2002; Zhao et al., 2004), and leptin may play a role in the pathophysiology of preeclampsia (Anderson et al., 2005) and hypertensive disorders of pregnancy (Ozkan et al., 2005). Leptin and leptin receptor genes are also expressed in human umbilical cord, fetal membranes and uterine tissue (Akerman et al., 2002).

In the human (Krizova et al., 2004), as well as in rat (Chien et al., 1997) and higher mammalian models (Wang et al., 2005), serum leptin levels are markedly elevated during pregnancy and demonstrate a significant decrease just before or at the time of parturition.

Leptin has been demonstrated to decrease myometrial contractility in an in vitro model (Moynihan et al., 2006). These findings are demonstrable and similar for both spontaneous and oxytocin induced contractions. This relaxant effect was observed at relatively low concentrations for in vitro experiments and was apparently cumulative in nature. Leptin was found to exert a mean maximal inhibition (MMI) of contractility of 46.794 ± 5.133% (n = 6) on spontaneous myometrial contractions. Figure 1 shows the effect of leptin on spontaneous myometrial contractility in vitro (figure reproduced with permission from Elsevier Science). When this effect was investigated in oxytocin-induced contractions, similar results were seen. Leptin was found to be responsible for a mean maximal inhibition (MMI) of 42.323 ± 3.642 % (n = 6). Both of these inhibitory responses were found to be statistically significant when compared with simultaneously run vehicle controls. This physiological inhibitory effect of leptin on uterine contractility may play a role in the dysfunctional labor process associated with maternal obesity. Furthermore these findings raise the possibility that metabolic factors may play a role in the dysfunctional labours seen in women in high BMI categories.

Ghrelin

Ghrelin is a 28-amino acid peptide and is the endogenous ligand for the growth hormone secretagogue receptor. It is centrally involved in the control of food intake, energy balance and utilization of fat during pregnancy (Budak et al., 2006). When first described,it was thought that, ghrelin was produced predominantly in the stomach (Kojima et al., 1999) However it is now reported that ghrelin production takes place in many other tissues including the placenta, ovary, and testis, of both rats and humans (Gualillo et al., 2001; Gaytan et al., 2005). In human

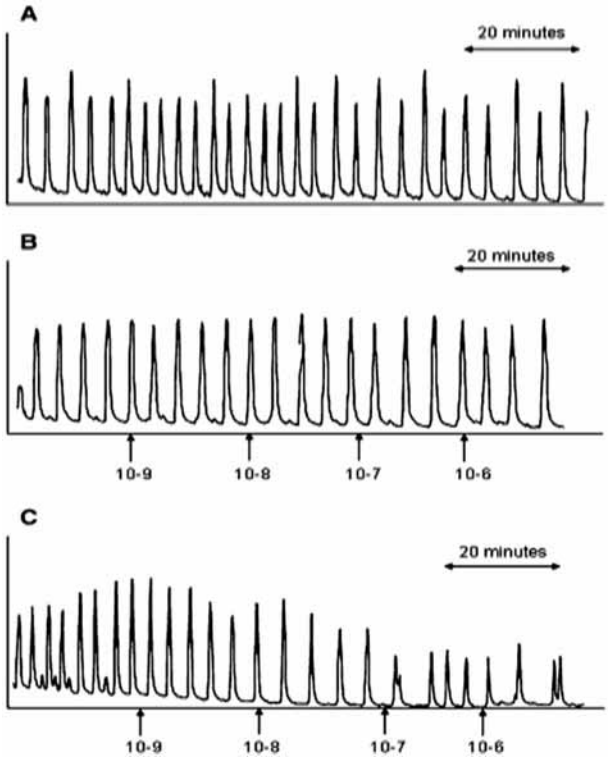

Fig. 1. — Effects of leptin on spontaneous myometrial contractility in pregnant nonlaboring tissue. Representative recordings demonstrating **A**, spontaneous contractions in a control strip, **B**, spontaneous contractions treated with vehicle only, and **C**, the effects of cumulative additions of leptin (1 nmol/l-1 μmol/L) in 20-minute intervals are shown.

pregnancy it is established that serum ghrelin levels peak around mid-gestation and fall to their lowest levels in the third trimester (Fuglsang et al., 2005). In general, ghrelin levels are reduced in obese non-pregnant adults, but are increased in subjects (adults and children), of low body mass index (Soriano-Guillen et al., 2004).

In an in vitro setting ghrelin has been shown to exert an influence on uterine contractility (Hehir et al., 2008). Ghrelin was found to exert an inhibitory effect on contractility, compared to simultaneously run control strips. The mean maximal inhibition values were found to be 33.66 ± 2.63% for spontaneous contractions ($n = 6$; $P < 0.05$), and 31.55 ± 4.64% for oxytocin-induced contractions ($n = 6$; $P < 0.05$).

This inhibitory effect of ghrelin on uterine contractions suggests it plays a physiologic role in regulation of myometrial activity. This is consistent with the low levels of ghrelin, which are observed in the third trimester. These results highlight the potential role of metabolic modulation of myometrium, and particularly at extremes of BMI measurements.

Apelin

Apelin is an adipocytokine, which is a novel bioactive peptide identified as the endogenous ligand of the orphan G protein-coupled receptor, APJ (Tatemoto et al., 1998). The physiologically active apelin molecule is a 36 amino-acid peptide. In humans it is secreted by many systems such as vascular smooth muscle, pituitary and pancreatic tissues (Brailoiu et al., 2002; Ringström et al., 2010; Maenhaut and Van de Voorde, 2011). Apelin has also been shown to be expressed in the placenta (Van Mieghem, 2010), however it is primarily expressed by adipose tissue and serum levels are found to be increase in the obese state (Boucher et al., 2055). Serum apelin levels are also found to decrease in a fasting state and increase upon re-feeding, suggesting that insulin may regulate apelin gene expression and secretion.[81] Physiologically apelin has also been shown to be involved in the regulation of cardiovascular function and fluid homeostasis (Reaux et al., 2001; Quazi et al., 2009;). Serum apelin levels have been shown to be decreased in the second trimester of pregnancy compared with non-pregnant controls however fetal levels have been shown to be markedly increased on Day 1 and Day 4 of life (Malamitsi-Puchner et al., 2007; Kourtis et al., 2011).

We have demonstrated that apelin exerts a relaxant effect on human myometrial contractility in vitro (Hehir and Morrison, 2012). A mean maximal inhibition in contractility (MMI) of 36.8% ± 6.4 was recorded in spontaneous contractions ($n = 6$) ($P < 0.05$). In the case of agonist - induced contractions a mean maximal inhibition (MMI) of 30.4% ± 4.6 ($n = 6$) ($P < 0.05$) was recorded. Similar to other adipocytokines apelin has been found to exert an inhibitory effect on human myometrial contractility in vitro, in tissue obtained during pregnancy. The cellular mechanisms of this, however, and the potential clinical implications, are not fully clear. However, these findings in addition to those mentioned previously further contribute to the concept of metabolic regulation of human myometrial function during pregnancy.

Cholesterol and Lipids

During pregnancy, complex changes occur in lipid profiles. From the 12th week of gestation, phospholipids, cholesterol (total, LDL, HDL), and triglycerides increase in response to estrogen stimulation and insulin resistance (Piechota and Staszewski, 1992; Toescu et al., 2004; Ghio et al., 2011). These changes in lipid balance have been suggested to provide anabolic support for the fetus. In a fasting maternal state adipose tissue lipolytic activity is highly enhanced, and its products, free fatty acids (FFA) and glycerol, are mainly driven to maternal liver, where FFA are converted to ketone bodies and

glycerol to glucose, which easily cross the placenta and sustain fetal metabolism (Herrera, 2002). Recent work has suggested that these alterations affect lipid rafts and caveolae (the omega-shaped invaginations of cell membranes found in uterine smooth muscle, which are stabilized by the cholesterol- binding protein caveolin) and thereby uterine signalling cascades and contractility (Smith et al., 2005). Cholesterol, an essential component of caveolae, has also been shown to have an important role in controlling smooth muscle contractility (Dreja et al., 2002; Noble et al., 2006). Body mass index and hypercholesterolemia have been shown to be positively associated (Gostynski et al., 2004). There is also evidence that cholesterol levels are particularly raised in obese pregnant women's serum (Podedinsky, 1987) and in the membranes of obese pregnant women's myometrium (Pulkkinen et al., 1998).

In an *in vitro* model both cholesterol was found to decrease both the amplitude and the amount of force produced in unit time of spontaneous myometrial contractility. This relaxant effect was also seen in contractions elicited with the addition of oxytocin. These contractile changes are related to changes in intracellular calcium (Jie Zhang et al., 2007). The elevated cholesterol seen in pregnancy may therefore contribute to uterine quiescence prior to parturition but could cause difficulties in labor in obese and/or dyslipidemic women, consistent with their increased cesarean delivery rates.

Obesity

As alluded to above women with a raised BMI have been shown to have an increased rate of caesarean section (Weiss et al., 2004). The increased rate of caesarean section seen in this group of parturient has been shown to be mainly due to a failure in the progression of the labour in the first stage (Crane et al., 1997; Young and Woodmansee, 2002). Recent studies have also demonstrated that obese women delivering vaginally are at increased risk of prolonged first stage of labour and that overweight and obese women had a significantly slower labour progression before 7-cm dilation and an increased rate of first-stage caesarean section (Vahratian et al., 2004). Myometrium from obese women has been shown, in an *in vitro* model, to contract with less force and frequency than that from normal-weight women. Measurements of intracellular calcium indicate that alterations in calcium are responsible for these changes in contractility (Zhang et al., 2007) increased rates of post - partum haemorrhage was also seen in this group of parturients and this is consistent with the hypothesis posed, namely that inadequate uterine activity is associated with obesity. In addition to these findings obesity has been shown to alter the properties of the feto-placental vasculature, namely by affecting the tone of the umbilical artery in vitro (Hehir et al., 2009). These results have implications for fetal growth and development in utero.

Diabetes Mellitus

Diabetes in pregnancy is associated with significant fetal and maternal morbidity and mortality. The rate of caesarean section is higher in women with diabetes (Ehrenberg et al., 2004; Jensen et al., 2004), with the Confidential Enquiry into Maternal and Child Health reporting a rate of 67.4% (Department of Health, 2010). Caesarean section in women with diabetes in pregnancy is associated with a 2.5-fold increased risk of wound infection (Takoudes et al., 2004), and an increased risk of thrombosis and postpartum haemorrhage (Dunne et al., 2003). Despite the significant morbidity associated with CS in diabetic pregnancies, the high CS rate in diabetic pregnancies is not understood. Some authors suggest that the increased CS rate is due to the confounding factor of obesity (Langer et al., 2005), however, other analyses have found diabetes mellitus to be an independent risk for CS (Bo et al., 2003; Kjos et al., 2004). Although macrosomia has been reported as a risk factor for cesarean delivery studies have found that a reduction in macrosomia does not result in a concomitant reduction in CS rate (Crowther et al., 2005). Recent studies analysing the contractile performance of myometrium from diabetic mothers versus that of non-diabetic women have shown that there is significantly decreased contraction amplitude and duration in myometrium from diabetic compared with control patients, even when possible confounders such as BMI were analysed (Al-Qahtani et al., 2012). Reduced intracellular calcium signals and expression of calcium channels were found in uteruses from diabetic patients, which, along with a reduction in muscle content found on histological examination, could explain these findings. In the presence of oxytocin myometrium from diabetic patients did not reach the levels found in non-diabetic patients (Al Qahtani et al., 2012). These findings may well contribute to the increased emergency caesarean section rate seen in diabetic mothers.

Conclusion

The complications associated with obesity in an obstetric population have been widely reported. The hypothesis that a woman's metabolism may have an effect on her ability to labor successfully is novel and

by no means fully elucidated. Previous studies have suggested that dysfunctional labors seen in women from increased BMI categories were due to cephalopelvic disproportion secondary to increased pelvic soft tissue (Young and Woodmansee, 2002), however the increasing evidence outlined in this review would suggest that physiology and endocrinology also play a role in this process.

The adipocytokines are a particularly interesting group of compounds which may well hold potential in unlocking the reasoning for the poor labor performance of obese women. The adipocytokines were all found to have an effect on myometrial contractility in vitro and this increases the evidence in favour of metabolic modulation of uterine smooth muscle contractility. The exact mechanism by which these compounds cause their effect on contractile performance are hitherto, however, uninvestigated. This is an area with potential for future investigation, as is the discovery, and examination of the effects, of more novel products of adipose tissue on myometrial contractility. Novel adipocytokines which are altered in accordance with BMI include adiponectin (Arita et al., 1999), and visfatin (Fukuhara et al., 2005) and these compounds may provide grounds for future research.

Cholesterol and other lipids are altered during the pregnant state, however in the presence of obesity this increase can be exaggerated and may lead to increased serum concentrations which may in turn influence uterine contractility. The mechanism of this action is clearer and can be attributed to alterations in intracellular calcium.

The presence of metabolic states such as obesity and diabetes mellitus have been shown to influence myometrial contractility in an in vitro model and this suggests that education about these conditions and decreasing their incidence may benefit people in labour, the exact mechanism whereby these conditions exert their effects remain unclear but the in vitro findings are in broad agreement with the suggestion that obese and diabetic women labour poorly leading to a much higher caesarean section rate in this patient group. In conclusion, this review provides valuable insight into the potential for metabolic modulation to influence myometrial activity and hence labour outcome, but this is a novel area of research, and one which still poses many questions to be addressed in the future.

References

Abrams B, Laros R Jr: Prepregnancy weight, weight gain, and birth weight. Am J Obstet Gynaecol. 1986, 154:503-9.

Aguilar H, Mitchell B. Physiological pathways and molecular mechanisms regulating uterine contractility. Hum Reprod Update. 2010;16(6):725-44.

Akerman F, Lei Z, Rao C. Human umbilical cord and fetal membranes co-express leptin and its receptor genes. Gynecol Endocrinol. 2002;16(4):299-306

Al-Qahtani S, Heath A, Quenby S et al. Diabetes is associated with impairment of uterine contractility and high Caesarean section rate. Diabetologia. 2012 ;55(2):489-98.

Anderson C, Lopez F, Zhang H et al. Characterization of changes in leptin and leptin receptors in a rat model of preeclampsia. Am J Obstet Gynecol. 2005;193(1):267-72.

Anotayanonth S, Subhedar N, Garner P et al. Betamimetics for inhibiting preterm labour. Cochrane Database Syst Rev. 2004 Oct 18;(4):CD004352.

Arendas K, Qiu Q, Gruslin A. Obesity in pregnancy: preconceptional to postpartum consequences. J Obstet Gynaecol Can. 2008;30(6):477-88.

Arita Y, Kihara S, Ouchi N et al. Paradoxical decrease of an adipose-specific protein, adiponectin, in obesity. Biochem Biophys Res Commun. 1999;257:79-83.

Arthur P, Taggart M, Mitchell B. Oxytocin and parturition: a role for increased myometrial calcium and calcium sensitization? Front Biosci. 2007;12:619-33.

Beck S, Wojdyla D, Say L et al. The worldwide incidence of preterm birth: a systematic review of maternal mortality and morbidity. Bull World Health Organ. 2010;88(1):31-8.

Berkman N, Thorp J Jr, Lohr K et al. Tocolytic treatment for the management of preterm labor: a review of the evidence. Am J Obstet Gynecol. 2003;188(6):1648-59.

Bhattacharya S, Campbell D, Liston W et al. Effect of Body Mass Index on pregnancy outcomes in nulliparous women delivering singleton babies. BMC Public Health. 2007;7:168.

Bisits A, Madsen G, Knox M et al. The Randomized Nitric Oxide Tocolysis Trial (RNOTT) for the treatment of preterm labor. Am J Obstet Gynecol. 2004;191(3):683-90.

Bo S, Menato G, Signorile A et al. Obesity or diabetes: what is worse for the mother and for the baby? Diabetes Metab. 2003;29:175-8.

Boucher J, Masri B, Daviaud D et al. Apelin, a newly identified adipokine up-regulated by insulin and obesity. Endocrinology. 2005;146(4):1764-71.

Bourguet C, Goupil E, Tassy D et al. Targeting the prostaglandin F2α receptor for preventing preterm labor with azapeptide tocolytics. J Med Chem. 2011;54(17):6085-97.

Brailoiu G, Dun S, Yang J et al. Apelin-immunoreactivity in the rat hypothalamus and pituitary. Neurosci Lett. 2002;327(3): 193-7.

Briana D, Malamitsi-Puchner A. The role of adipocytokines in fetal growth. Ann N Y Acad Sci. 2010;1205:82-7.

Budak E, Fernandez Sanchez M, Bellver J et al. Interactions of the hormones leptin, ghrelin, adiponectin, resistin, and PYY3-36 with the reproductive system. Fertil Steril. 2006; 85(6):1563-81.

Chanoine J, De Waele K, Walia P. Ghrelin and the growth hormone secretagogue receptor in growth and development. Int J Obes (Lond). 2009;33 Suppl 1:S48-52.

Chien E, Hara M, Rouard M et al. Increase in serum leptin and uterine leptin receptor messenger RNA levels during pregnancy in rats. Biochem Biophys Res Commun. 1997;237: 476-80.

Conde-Agudelo A, Romero R, Kusanovic JP. Nifedipine in the management of preterm labor: a systematic review and meta-analysis. Am J Obstet Gynecol. 2011;204(2):134.e1-20.

Considine R, Sinha M, Heiman M et al. Serum immunoreactive leptin concentrations in normal-weight and obese humans. N Engl J Med. 1996;334:292-5.

Costeloe K, Hennessy E, Gibson A et al. The EPICure study: outcomes to discharge from hospital for infants born at the threshold of viability. Pediatrics. 2000;106:659-71.

Crane S, Wojtowycz M, Dye T et al. Association between prepregnancy obesity and the risk of cesarean delivery. Obstet Gynecol. 1997;89:213-6.

Crankshaw D, Morrison J. Methodology and pharmacological analysis of effects of uterotonic compounds in human

myometrium in vitro. Am J Obstet Gynecol. 2011;205(2): 155.e1-6.
Crowther C, Hiller J, Moss Jet al. . Effect of treatment of gestational diabetes mellitus on pregnancy outcomes. N Engl J Med. 2005;352:2477-86.
de Heus R, Mulder EJ, Derks JB et al. Acute tocolysis for uterine activity reduction in term labor: a review. Obstet Gynecol Surv. 2008;63(6):383-8.
Department of Health (2010) NHS Maternity Statistics, England. 2002-2003 Statistical Bulletin.
Derbent A, Simavli S, Gümüş II et al. Nifedipine for the treatment of preterm labor in twin and singleton pregnancies. Arch Gynecol Obstet. 2011;284(4):821-6.
Domali E, Messinis I. Leptin in pregnancy. J Matern Fetal Neonatal Med. 2002;12(4):222-30.
Dreja K, Voldstedlund M, Vinten J et al. Cholesterol depletion disrupts caveolae and differentially impairs agonist-induced arterial contraction. Arterioscler Thromb Vasc Biol. 2002; 22(8):1267-72.
Dunne F, Brydon P, Smith K et al. Pregnancy in women with Type 2 diabetes: 12 years outcome data 1990-2002. Diabet Med. 2003;20:734-8.
Ehrenberg H, Durnwald C, Catalano P et al. The influence of obesity and diabetes on the risk of cesarean delivery. Am J Obstet Gynecol. 2004;191:969-74.
Elmes M, Tan D, Cheng Z et al. The effects of a high-fat, high-cholesterol diet on markers of uterine contractility during parturition in the rat. Reproduction. 2011;141(2):283-90.
Flenady V, Koopmans L, Middleton P et al. Major risk factors for stillbirth in high-income countries: a systematic review and meta-analysis. Lancet. 2011;377(9774):1331-40.
Fuglsang J, Skjaerbaek C, Espelund U et al. Ghrelin and its relationship to growth hormones during normal pregnancy. Clin Endocrinol. 2005;62:554-9.
Fukuhara A, Matsuda M, Nishizawa M et al. Visfatin: a protein secreted by visceral fat that mimics the effects of insulin. Science. 2005;307:426-30.
Gaytan F, Barreiro M, Chopin L et al. Immunolocalization of ghrelin and its functional receptor, the type Ia growth hormone secretagogue receptor, in the cyclic human ovary. J Clin Endocrinol Metab. 2005;90(3):1798-1804.
Ghio A, Bertolotto A, Resi V et al. Triglyceride metabolism in pregnancy. Adv Clin Chem. 2011;55:133-53.
Gill A, Madsen G, Knox M et al. Neonatal neurodevelopmental outcomes following tocolysis with glycerol trinitrate patches. Am J Obstet Gynecol. 2006;195(2):484-7.
Goldenberg R, Culhane J, Iams J et al. Epidemiology and causes of preterm birth. Lancet. 2008;371(9606):75-84.
Gostynski M, Gutzwiller F, Kuulasmaa K, et al. Analysis of the relationship between total cholesterol, age, body mass index among males and females in the WHO MONICA Project. Int J Obes Relat Metab Disord. 2004;28(8):1082-90.
Gualillo O, Caminos J, Blanco M et al. Ghrelin a novel placental-derived hormone. Endocrinology. 2001;142(2) :788-94.
Hamilton B, Paglia D, Kwan AYM et al. Increased obese mRNA expression in omental fat cells from massively obese humans. Nat Med.1995;1:953-6
Haynes W, Morgan D, Walsh S et al. Cardiovascular consequences of obesity: role of leptin. Clin Exp Pharmacol Physio. 1998;25(1):65-9.
Hehir M, Glavey S, Morrison J. Uterorelaxant effect of ghrelin on human myometrial contractility. Am J Obstet Gynecol. 2008;198(3):323.e1-5.
Hehir M, Morrison J. The adipokine apelin and human uterine contractility. Am J Obstet Gynecol. 2012;206(4):359.e1-5.
Hehir M, Moynihan A, Glavey S et al. Umbilical artery tone in maternal obesity. Reprod Biol Endocrinol. 2009;7:6.
Herrera E. Implications of dietary fatty acids during pregnancy on placental, fetal and postnatal development – a review. Placenta. 2002;23 Suppl A:S9-19.
Higgins M, Mc Auliffe F. A review of maternal and fetal growth factors in diabetic pregnancy. Curr Diabetes Rev. 2010;6(2): 116-25.
Jensen D, Damm P, Moelsted-Pedersen L et al. Outcomes in type 1 diabetic pregnancies: a nationwide, population-based study. Diabetes Care. 2004; 27:2819-23.
Jensen D, Damm P, Sorensen B et al. Pregnancy outcome and prepregnancy body mass index in 2459 glucose-tolerant Danish women. Am J Obstet Gynecol. 2003;189(1):239-44.
Jie Zhang, Kendrick A, Quenby S et al. Contractility and calcium signaling of human myometrium are profoundly affected by cholesterol manipulation: implications for labor? Reprod Sci. 2007;14(5):456-66.
Kam K, Lamont R. Developments in the pharmacotherapeutic management of spontaneous preterm labor. Expert Opin Pharmacother. 2008;9(7):1153-68.
Kanaglingam M, Forouhi N, Greer I et al. Changes in booking BMI over a decade: retrospective analysis from a Glasgow Maternity Hospital. BJOG. 2005;112(10):1431-3.
Kenyon A, Peebles D. Myth: tocolysis for prevention of preterm birth has a major role in modern obstetrics. Semin Fetal Neonatal Med. 2011;16(5):242-6.
Kjos S, Berkowitz K, Xiang A. Independent predictors of cesarean delivery in women with diabetes. J Matern Fetal Neonatal Med. 2004;15:61-7.
Kojima M, Hosoda H, Date Y et al. Ghrelin is a growth-hormone releasing acetylated peptide from the stomach. Nature. 1999; 402:656-60.
Kourtis A, Giomisi A, Mouzaki M et al. Apelin levels in normal pregnancy. Clin Endocrinol (Oxf). 2011;164(4):579-83.
Krizova J, Eretova V, Haluzikova D et al. Soluble leptin receptor and leptin levels in pregnant women before and after delivery. Endocr Res. 2004;30(3):379-85.
Langer O, Yogev Y, Xenakis EM et al. Overweight and obese in gestational diabetes: the impact on pregnancy outcome. Am J Obstet Gynecol. 2005;192:1768-76.
López Bernal A. Mechanisms of labour – biochemical aspects. BJOG. 2003;110 Suppl 20:39-45.
Lowe N, Corwin E. Proposed biological linkages between obesity, stress, and inefficient uterine contractility during labor in humans. Med Hypotheses. 2011;76(5):755-60.
Lynch C, Sexton D, Hession M et al. Obesity and mode of delivery in primigravid and multigravid women. Am J Perinatol. 2008;25(3);163-7.
Maenhaut N, Van de Voorde J. Regulation of vascular tone by adipocytes. BMC Med. 2011;9:25. Review.
Malamitsi-Puchner A, Gourgiotis D, Boutsikou M et al. Circulating apelin concentrations in mother/infant pairs at term. Acta Paediatr. 2007;96(12):1751-4.
Martin J, Osterman M, Sutton P. Are preterm births on the decline in the United States? Recent data from the National Vital Statistics System. NCHS data brief, no 39. Hyattsville, MD: National Center for Health Statistics. 2010.
McCormick, M. The contribution of low birth weight to infant mortality and childhood morbidity. N Engl J Med. 1985; 312:82-90.
Menon R. Spontaneous preterm birth, a clinical dilemma: etiologic, pathophysiologic and genetic heterogeneities and racial disparity. Acta Obstet Gynecol Scand. 2008;87(6):590-600.
Moynihan A, Hehir M, Glavey S et al. Inhibitory effect of leptin on human uterine contractility in vitro. Am J Obstet Gynecol. 2006;195(2):504-9.
Nassar A, Aoun J, Usta I. Calcium channel blockers for the management of preterm birth: a review. Am J Perinatol. 2011;28(1):57-66.
Noble K, Zhang J,Wray S. Lipid rafts, the sarcoplasmic reticulum and uterine calcium signalling: an integrated approach. J Physiol. 2006;570(pt 1):29-35.
O'Brien M, Earley P, Morrison J et al. Ghrelin in the human myometrium. Reprod Biol Endocrinol. 2010;28;8:55.
Ovesen P, Rasmussen S, Kesmodel U. Effect of prepregnancy maternal overweight and obesity on pregnancy outcome. Obstet Gynecol. 2011;118:305-12.
Owens L, O'Sullivan E, Kirwan B et al. ATLANTIC DIP Collaborators. ATLANTIC DIP: the impact of obesity on

pregnancy outcome in glucose-tolerant women. Diabetes Care. 2010;33(3):577-9.
Ozkan S, Erel C, Madazli et al. Serum leptin levels in hypertensive disorder of pregnancy. Eur J Obstet Gynecol Reprod Biol. 2005;120(2):158-63.
Papatsonis D, Flenady V, Cole S et al. Oxytocin receptor antagonists for inhibiting preterm labour. Cochrane Database Syst Rev. 2005 Jul 20;(3):CD004452.
PeriStats [online database]. White Plains, NY: March of Dimes; 2006.
Petrou S, Mehta Z, Hockley C et al. The impact of preterm birth on hospital inpatient admissions and costs during the first 5 years of life. Pediatrics. 2003; 112:1290-7.
Petrou S. The economic consequences of preterm birth during the first 10 years of life. BJOG. 2005;112 Suppl 1:10-5.
Piechota W, Staszewski A. Reference ranges of lipids and apolipoproteins in pregnancy. Eur J Obstet Gynecol Reprod Biol. 1992;45:27-35.
Podedinsky N. Serum lipid composition in obese pregnant patients. Akusherstvo I Ginekologiia Moskva. 1987;6:22-6.
Pulkkinen M, Nyman S, Hamalainen MM et al. Proton NMR spectroscopy of the phospholipids in human uterine smooth muscle and placenta. Gynecol Obstet Invest. 1998;46(4):220-4.
Quazi R, Palaniswamy C, Frishman WH. The emerging role of apelin in cardiovascular disease and health. Cardiol Rev. 2009;17(6):283-6.
Rasmussen S, Chu S, Kim S, et al. Maternal obesity and risk of neural tube defects: a metaanalysis. Am J Obstet Gynecol. 2008;198:611-9.
Reaux A, De Mota N, Skultetyova I, et al. Physiological role of a novel neuropeptide, apelin, and its receptor in the rat brain. J Neurochem. 2001;77(4):1085-96.
Ringström C, Nitert M, Bennet H et al. Apelin is a novel islet peptide. Regul Pept. 2010 ;162(1-3):44-51.
Romero R, Sibai B, Sanchez-Ramos L et al. An oxytocin receptor antagonist (atosiban) in the treatment of preterm labor: a randomized, double-blind, placebo-controlled trial with tocolytic rescue. Am J Obstet Gynecol. 2000;182: 1173-83.
Seidman D, Ever-Hadani P, Gale R. The effect of maternal weight gain in pregnancy on birth weight. Obstet Gynecol. 1989;74(2):240-6.
Slattery M, Morrison J, Preterm delivery. Lancet. 2002; 360(9344):1489-97.
Smith R, Babiychuk E, Noble K et al. Increased cholesterol decreases uterine activity: functional effects of cholesterol alteration in pregnant rat myometrium. Am J Physiol Cell Physiol. 2005;288(5):C982-8.
Soriano-Guillen L, Barrios V, Campos-Baros A et al. Ghrelin levels in obesity and anorexia nervosa: effect of weight reduction or recuperation. J Pediatr. 2004;144:36-42.
Takoudes T, Weitzen S, Slocum J et al. Risk of cesarean wound complications in diabetic gestations. Am J Obstet Gynecol. 2004;191:958-63.
Tatemoto K, Hosoya M, Habata Y et al. Isolation and characterization of a novel endogenous peptide ligand for the human APJ receptor. Biochem Biophys Res Commun. 1998;251(2): 471-6.
Terrone D, Rinehart B, Kimmel E et al. A prospective randomized, controlled trial of high and low maintenance doses of magnesium sulfate for acute tocolysis. Am J Obstet Gynecol. 2000;182:1477-82.
Toescu V, Nuttall S, Martin U et al. Changes in plasma lipids and markers of oxidative stress in normal pregnancy and pregnancies complicated by diabetes. Clin Sci (Colch). 2004; 106:93-8.
Torres-Leal F, Fonseca-Alaniz M, Rogero M et al. The role of inflamed adipose tissue in the insulin resistance. Cell Biochem Funct. 2010;28(8):623-31.
Tsatsaris V, Papatsonis D, Goffinet F et al. Tocolysis with nifedipine or beta-adrenergic agonists: a meta-analysis. Obstet Gynecol. 2001;97:840-7.
Usta I, Khalil A, Nassar A. Oxytocin antagonists for the management of preterm birth: a review. Am J Perinatol. 2011; 28(6):449-60.
Vahratian A, Zhang J, Troendle J et al. Maternal prepregnancy overweight and obesity and the pattern of labor progression in term nulliparous women. Obstet Gynecol. 2004;104:943-51.
Van Mieghem T, van Bree R, Van Herck E et al. Maternal apelin physiology during rat pregnancy: the role of the placenta. Placenta. 2010;31(8):725-30.
Wang C, Medan M, Shimizu K et al. Secretion of leptin throughout pregnancy and early postpartum period in Japanese monkeys: placenta as another source of leptin. Endocrine. 2005;27(1):75-82.
Weiss J, Malone F, Emig D et al. Obesity, obstetric complications and cesarean delivery rate – A population-based screening study. Am J Obstet Gynecol. 2004;190:1091-7.
Wex J, Abou-Setta A, Clerici G et al. Atosiban versus beta-mimetics in the treatment of preterm labour in Italy: clinical and economic importance of side-effects. Eur J Obstet Gynecol Reprod Biol. 2011;157(2):128-35. Review.
Xu A, Wang Y, Lam K et al. Vascular actions of adipokines molecular mechanisms and therapeutic implications. Adv Pharmacol. 2010;60:229-55. Review.
Xu S, Tsao P, Yue P. Apelin and insulin resistance: another arrow for the quiver? J Diabetes. 2011;3(3):225-31.
Young T, Woodmansee B. Factors that are associated with cesarean delivery in a large private practice: the importance of prepregnancy body mass index and weight gain. Am J Obstet Gynecol. 2002;187:312-8
Yuan S, Chung Y, Chen H et al. Aberrant expression and possible involvement of the leptin receptor in bladder cancer. Urology. 2004;63:408-13.
Yuan W, López Bernal A. Cyclic AMP signalling pathways in the regulation of uterine relaxation. BMC Pregnancy Childbirth. 2007;7 Suppl 1:S10. Review
Zhang J, Bricker L, Wray S et al. Poor uterine contractility in obese women. BJOG. 2007;114(3):343-8.
Zhao J, Townsend K, Schulz L et al. Leptin receptor expression increases in placenta, but not hypothalamus, during gestation in Mus musculus and Myotis lucifungus. Placenta. 2004; 25:712-22.

Ion channels in uterine smooth muscle cells to regulate contractions during term and preterm delivery: - Role of non-selective cation channels and gap junctions.

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Abstract

Uterine contractions are generated by calcium (Ca^{2+}) influx into myometrial cells which is regulated by the membrane potential of myometrial cells. Non-selective cation channels (NSCCs) associated with membrane potential were studied in rat myometrial cells using the patch-clamp method and molecular biological techniques. Two types of NSCC current in myometrial cells were detected. One is lanthanum (La) sensitive NSCC current and the other is adenosine triphosphate (ATP) induced current. Purinergic P2X7 receptor was determined to be the functional channel for ATP induced currents. These NSCCs are suggested to be mechanisms to generate spontaneous depolarizations, leading to rhythmical uterine contractions. Blocking of these NSCCs by magnesium ions (Mg^{2+}) may be one of the mechanisms for inhibiting uterine contractions in tocolysis. Cell-to-cell couplings through gap junction (GJ) channels increase at the end of pregnancy. We used the double patch-clamp technique on myometrial paired cells and characterized GJ currents. Most properties were comparable to those of connexin 43, indicating that connexin 43 is the main component of myometrial GJ. Junctional conductance between myometrial cells was enhanced sixfold in preterm delivering compared with preterm non-delivering rats. This enhancement is caused by an increase in the number of GJ channels. Finally, the expression of P2X receptors was examined in the myometrium, and P2X4 and P2X7 were expressed most strongly. The expression levels of these receptors increases in the late stages of pregnancy. Lipopolysaccharide, an endotoxin, significantly increased these levels, showing that inflammation is associated with preterm delivery.

Key words: **Uterine smooth muscle cells, ion channel, non-selective cation channel, ATP receptor, gap junction, magnesium.**

Introduction

Electrical excitability of uterine smooth muscle during delivery is relatively high comparing with various other smooth muscles. Uterine contractions are generated by an increase in the concentration of intracellular Ca^{2+} of myometrial cells. The main source of contractile Ca^{2+} in uterine smooth muscle is thought to be Ca^{2+} influx through L-type Ca^{2+} channels rather than release of sequestered calcium from sarcoplasmic reticulum (Kasai et al., 1994; Kwarabayashi, 1997). On the other hand, the regulation of uterine contractile waves is not fully understood and there is a need to search for other sources of contractile Ca^{2+}. Some types of receptor-operated non-selective cation channel (NSCC) have been identified in various smooth muscle cells (Inoue and Isenberg, 1990; Loirand et al., 1991;

Sims, 1992; Vogalis and Sanders 1990; Wang and Large 1991) and have been suggested to be another pathway for Ca^{2+}influx.

The opening of L-type Ca^{2+} channels is regulated by the membrane potential of uterine smooth muscle cells. The membrane potential of smooth muscle is determined by various types of potassium (K) channels. We reported three types of K channels, delayed rectifier K^+ channel, Ca-activated K^+ channel and transient K^+ channel in rat myometrial cells using the whole cell patch-clamp method (Miyoshi et al., 1991). These K^+ channel are found to repolarize the membrane potential after action potentials and terminate muscle contractions. The opening of K^+ channels induces hyperpolarization of the cell to keep the uterine muscle during pregnancy quiescent.

Myometrial NSCCs also play an important role to change the membrane potential of the cell and the depolarization induces spontaneous uterine contractions. We have reported the presence of two types of NSCC, lanthanum (La) sensitive NSCC and purinergic P2X receptors in pregnant rat uterus which are alternative pathways of Ca^{2+} influx (Miyoshi et al., 2004; Miyoshi et al., 2010). NSCCs also induce depolarization of the cell membrane and are possible mechanism for automaticity in the myometrium.

Extracellular magnesium ions (Mg^{2+}) are well known to inhibit uterine contractions and have been reported to suppress the spontaneous depolarization at physiological concentrations. In fact, Mg^{2+} is clinically used to treat uterine contraction of preterm labor. However, the mechanism of Mg^{2+} blockade of uterine contractions is not known. We reported that myometrial NSCCs were blocked at a physiological concentration of external Mg^{2+} and are thought to be targets of Mg^{2+} (Miyoshi et al., 2004; Miyoshi et al., 2010).

Uterine contraction during labor is characterized not only by its automaticity but also by highly synchronized cell contractions in the whole uterus. Cell couplings through gap junction (GJ) channels between myometrial cells are increased dramatically at the end of pregnancy, leading to the onset of labor. This increase has been well established morphologically and functionally (Garfield et al, 1977). We previously reported the change and enhancement of electrical couplings between rat myometrial cells during pregnancy using the double patch-clamp technique (Miyoshi et al., 1996). The enhancement was also observed in a preterm birth model rat treated with antiprogesterone (RU486).

Preterm birth is a major determinant of perinatal morbidity and mortality. (Romero and Mazor, 1988). Inflammatory infiltration of the uterus is known to be one of the causes inducing preterm labor. However, the mechanism of enhancement of uterine contractility in these conditions remains to be elucidated. The Gram-negative bacterial endotoxin, lipopolysaccharide (LPS) is believed to cause adverse pregnancy outcomes (Fidel et al., 1994; Okawa et al., 2001). We have shown that inflammation by LPS treatment on pregnant rat enhanced markedly the level of gene expression of myometrial NSCCs, indicating that increased NSCCs could enhance the excitability of myometrium (Urabe et al., 2009).

Identification of myometrial NSCC currents

The conventional whole-cell patch-clamp method was used in freshly isolated myometrial cells from pregnant rats on day 18-20 of gestation (Miyoshi et al., 2004). NSCC current was isolated by suppressing background K^+ channel currents by tetraethyl-ammonium chloride and L-type Ca^{2+} channel by 5 μM nifedipine. Reduction of external Mg^{2+} to 0.1 mM induced measurable leakage currents. These currents had a linear shape and no time-dependent component as shown in Figure 1. Reduction of sodium ion (Na^+) in the external solution substantially decreased the amplitude of this current, indicating that Na^+ should be the main carrier ion through these channels. This current is blocked by lanthanum (La; blocker of NSCC) ion and Kd was calculated to be 2.4 μM. Also, these channels were observed to have permeability of various monovalent ions and the order of permeability was $K^+ > Cs^+ > Na^+ > Li^+$ (Cs: cesium, Li: lithium). These findings indicate this channel in rat myometrial cells to have the properties of NSCC. The La-sensitive NSCC also had a small but significant conductance for Ca^{2+}, showing that the La-sensitive NSCC may be another pathway for Ca^{2+} leading to uterine contraction.

Possible constituents of La-sensitive NSCC are the transient receptor potential protein (TrpC). The TrpC protein family is suggested to mediate Ca^{2+} entry in myometrial cells. The La-sensitive NSCC have some characteristics similar to TrpC channels such as their ion selectivity and sensitivity to La^{3+}, suggesting the possibility that TrpC serves as a calcium entry pathway in myometrial cells. TrpC-channels, TrpC3 or TrpC4 DNAs were detected in myometrium (unpublished data).

Identification of myometrial P2X7 receptor currents

We found another type of myometrial NSCC current which are induced by external adenosine triphosphate (ATP) in the presence of La^{3+} (Miyoshi et al., 2010). ATP has been reported to enhance the membrane conductance of myometrial cells and uterine contractility. Purinergic P2 receptor

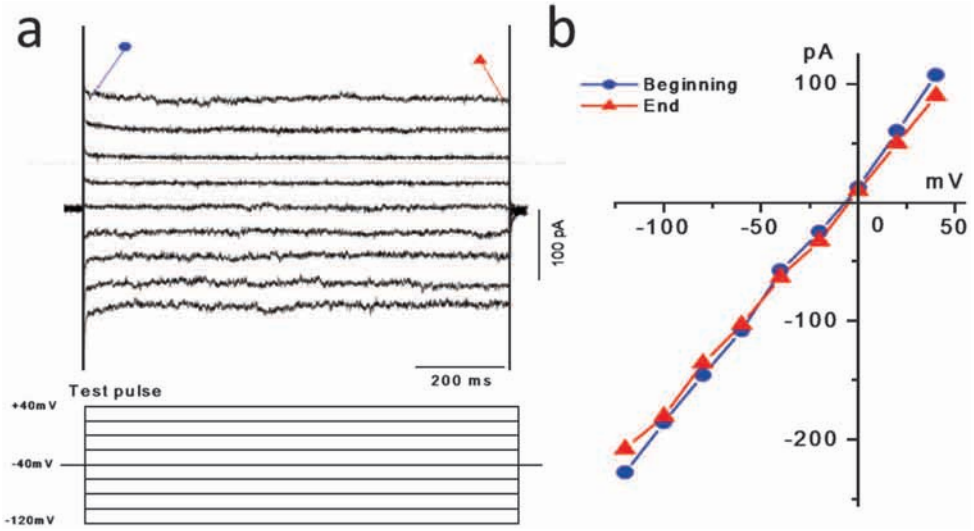

Fig. 1. — Family of NSCC currents obtained from rat myometrial cells and their current-voltage relationship.
a. Superimposed traces of whole-cell currents through NSCC. Note that the currents shown have no time-dependency. Test pulse protocol is shown in the left lower panel. The holding potential was -40 mV. At a membrane potential of -40 mV an inward current was observed. The thin dotted line indicates the zero current level. **b.** The amplitude of the current at the beginning (●) or end (▲) of the test pulse was plotted against the membrane potential. The current-voltage relationships were linear. (H. Miyoshi et al. 2004).

expression has been reported in the myometrium, using molecular biology, but the functional identity of the receptor subtype has not been determined. In our previous study ATP-induced currents were recorded and characterized in single myometrial cells from pregnant rats. Extracellular ATP applied in the range of 10 μM - 1 mM induced currents with an EC_{50} of 74 μM, with no time-dependency, or voltage-dependency as presented in Figure 2. The induced currents carried multiple monovalent cations, with conductances ranked as $K^+ > Cs^+ > Li^+ > Na^+$. They are activated by P2X receptor agonists, with their effectiveness ranked as 2`,3`-O-(4-benzoylbenzoyl)-ATP (Bz-ATP) >> ATP > αβ-methylene ATP (αβ-MeATP) > 2-methylthio ATP (2-MeSATP) ≥ UTP ≥ GTP > ADP. P2X receptor is classified into seven subtypes from P2X1 to P2X7. ATP-induced currents in myometrial cells are not decreased and no desensitization to ATP was observed. These currents are blocked by the selective P2X7 receptor antagonist, 3-(5-(2,3-dichlorophenyl)-1 H-tetrazol-1-yl) methyl pyridine (A438079). We

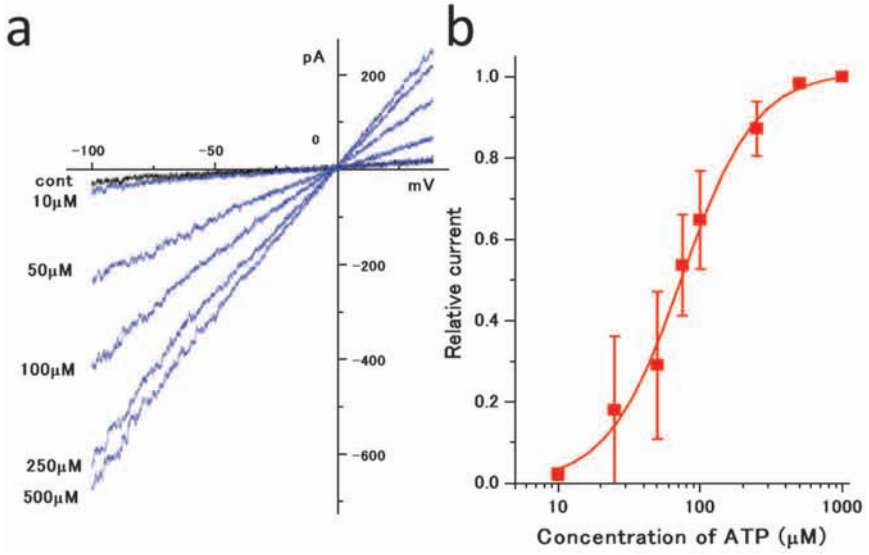

Fig. 2. — Dose-dependency of the currents in response to extracellular ATP.
a. Currents were recorded at ATP concentrations between 10 and 500 μM using the ramp pulse protocol from -100 to +40 mV. The curve labelled "cont" shows a recording in the absence of ATP. Current activation was detected at ATP concentrations as low as 10 μM. **b.** The mean current amplitudes at -80 mV relative to those measured in the presence of 1mM ATP are shown; bars show SDs. EC50 for ATP was estimated to be 74 μM (n = 7) from this curve, which was obtained by fitting the data to the Boltzmann equation. (H. Miyoshi et al. 2010).

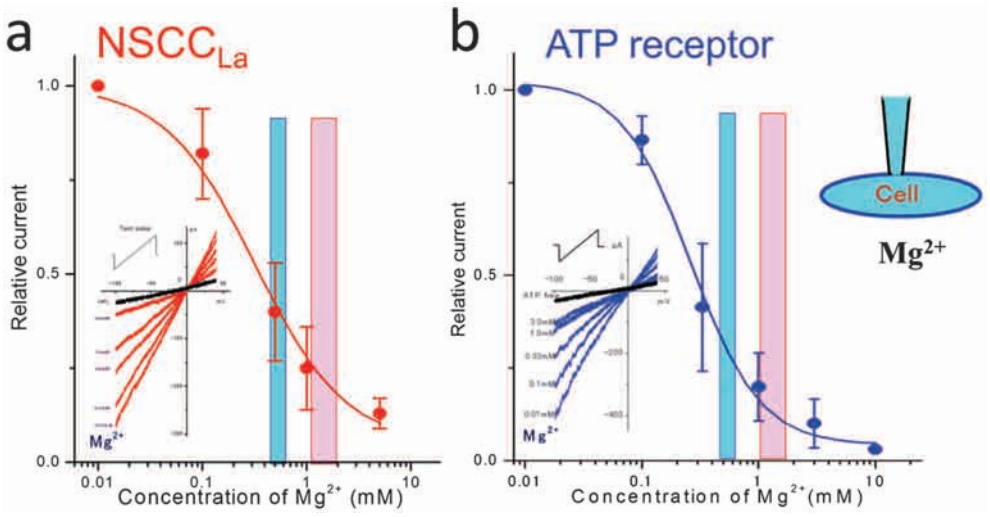

Fig. 3. — Effect of extracellular Mg^{2+} on La-sensitive NSCC and ATP-induced currents.
Effects of Mg^{2+} on the La-sensitive NSCC currents (a: $NSCC_{La}$) and the ATP induced currents (b: ATP receptor) were examined at various Mg^{2+} concentrations. Currents were inhibited by Mg^{2+} in a concentration-dependent manner shown in each small column. Mg^{2+} concentration-effect curves are presented. IC_{50} was calculated to be 0.28 mM for $NSCC_{La}$ in a and 0.26 mM for ATP receptor in b from the curves by fitting the data to the Boltzmann equation. Note that Mg^{2+} did not completely inhibit the current, since 10% of the current persisted at Mg^{2+} concentrations higher than 5 mM. (H. Miyoshi et al. 2004, 2010).

therefore conclude that ATP-induced currents in rat myometrial cells crossed cell membranes via P2X7 receptors.

Blockade of myometrial NSCCs by Mg ion

Consistent with NSCCs of other organs, Mg^{2+} inhibits NSCC currents of rat myometrium in a dose-dependent manner. Extracellular Mg^{2+} inhibits the La-sensitive NSCC currents in a concentration-dependent manner with an IC_{50} value of 0.28 mM as shown in Figure 3a (Miyoshi et al., 2004). We also showed that the P2X7 receptor currents were blocked by Mg^{2+} with an IC_{50} of 0.26 mM as shown in Figure 3b (Miyoshi et al., 2010). These values of IC_{50} are comparable with the physiological concentration of free Mg (Mg^{2+}) in rat serum. In increasing Mg^{2+} from the physiological concentration to the therapeutic range from 1 to 2 mM, the amplitude of myometrial NSCCs current is reduced to around one third. In fact, external application of ATP could induce depolarization of myometrial cells leading to uterine contractions and this depolarization is inhibited significantly by higher concentration of Mg^{2+}. These findings indicate that blocking of myometrial NSCCs is one of mechanism of tocolytic effects by $MgSO_4$.

Clinically, administering extracellular Mg^{2+} is known to inhibit uterine contractions. The observed Mg^{2+} blockade may reasonably explain the inhibitory effect of Mg^{2+} on uterine contractions in the treatment of preterm labor. Further research is needed into the myometrial NSCCs as a therapeutic target in abnormal uterine contraction, as a possible treatment for premature labor.

Enhancement of cell coupling in labor

Gap junction channels play an important role as a pathway of electrical excitation in contraction of the uterus and the regulation of their activities leads to maintenance of uterine quiescence and the onset of labor. In our previous study, the double whole-cell patch-clamp technique was applied to paired cells freshly isolated from pregnant rat myometrium to investigate the functional control during pregnancy (Miyoshi et al., 1996). The macroscopic gap junction currents decayed slowly from an instantaneous, constant-conductance level to a steady-state level described by quasi-symmetrical Boltzmann functions of transjunctional voltage. Unitary junctional conductance was detected to be 85 pS (85-90% of events) and 25 pS under halothane exposure. The properties of gap junction currents in these preparations are almost identical to those of connexin 43. There was no significant difference on the current properties during pregnancy. Junctional conductances as measured in various stages of pregnancy were estimated to be 5 ± 8 nS in preterm, 32 ± 16 nS in term and 7 ± 10 nS in postpartum. The conductance in delivering was six fold greater than that in preterm nondelivering rats and decreased quickly in 24 hours as shown in Figure 4a. An antiprogesterone (RU486) was injected to the rat at the day 18 (D18) to induce preterm labor and gap junctional conductance was measured at D19. The conductance was

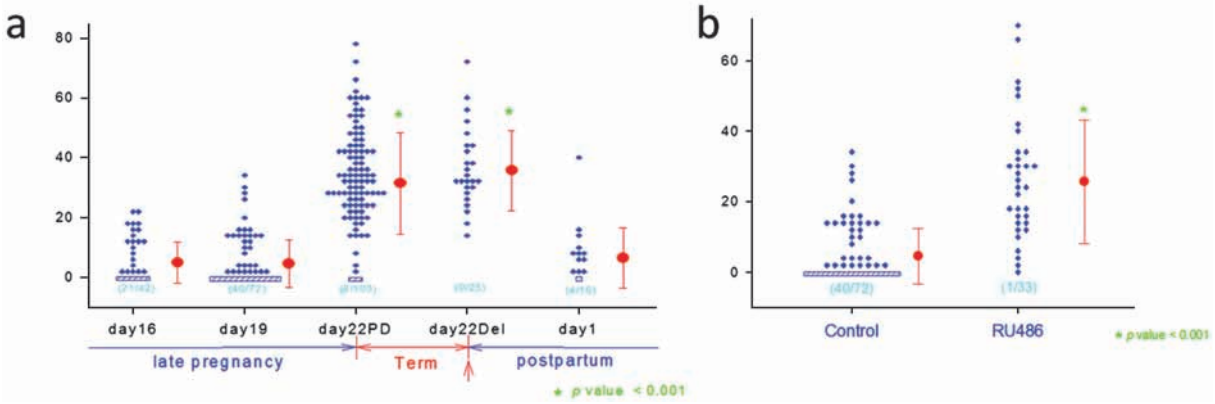

Fig. 4. — Change of junctional conductance during pregnancy.
Individual measurements of junctional conductance from myometrial pairs are plotted for day 16, 19, day 22 predelivery (day22PD), day 22 actively delivering (day22Del), one-day postpartum (PP1) in **a** and day-19 preterm-delivering (RU486) in **b.** Nonzero values are plotted individually as open symbols; for clarity, "zero" values are represented as single open boxes for each group (H. Miyoshi et al. 1996). The mean values of junctional conductance are shown as filled circles (●) with error bars (mean ± SD). Differences among the six groups were significant at the $p < .0001$ level (two-tailed t test).

5 ± 8 nS in control and 26 ± 17 nS in RU486 injected rat, showing that electrical couplings are increased and lead to preterm labor (Fig. 4b). The effect of cyclic AMP dependent agonist on junctional conductance was studied in cell pairs at D22 non delivering. Junctional conductance was decreased to be 78% with isoproterenol of 10 M and 81% with dibutylic c-AMP of 1 mM. We conclude that the enhancement of cell-to-cell couplings at the end of pregnancy and in preterm labor is the increase in numbers of gap junction channels. Junctional conductance is blocked by cyclic AMP dependent agonists.

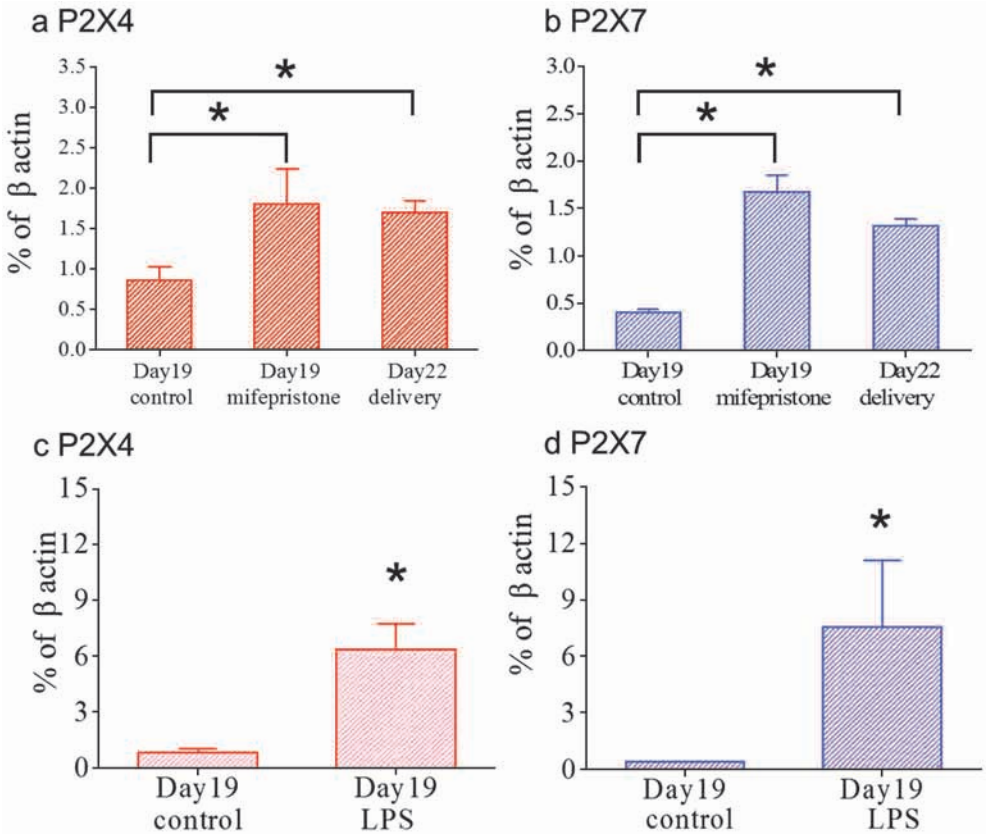

Fig. 5. — Quantitative analysis of P2X4 and P2X7 mRNAs in the myometrium.
a,b) An antiprogesterone mifepristone (RU486) or saline (control; 1 mg for each) was injected subcutaneously at day 18 of gestation. On the next day the uterine tissue was collected. The expression levels of the P2X4 and P2X7 mRNAs were compared with those at delivery on day 22. **c,d)** LPS or saline (C: control; 0.2 mg/kg for each) was injected into the intraperitoneal cavity on day 18 of gestation. The expression levels of the P2X4 and P2X7 mRNAs were shown in **c** and **d** (S. Urabe et al. 2009). The measured data were normalized against those for β-actin. Values are presented as the mean ± S.D (* $P < .01$).

Expression of myometrial NSCCs

We investigated the changes in the expression of myometrial NSCCs during pregnancy and the effect of inflammation in preterm delivery (Urabe et al., 2009). The expression of each subtype of P2X receptor and TrpC channel mRNA was measured by real-time RT-PCR with TaqMan probes (ABI). P2X4 and P2X7 were determined to be dominant subtypes of the P2X channel in the rat myometrium during pregnancy. The expression of P2X4 is increased by 70% in rats delivering on day 22, compared to those delivering on day 15 and that of P2X7 is enhanced by 90% as shown in Figure 5a and 5b. On the other hands, TrpC3 and TrpC4 were detected dominantly. The expression of TrpC3 was increased three times in the late stages of gestation. However, TrpC4 was suppressed by 57% (unpublished data). The mRNA expression of P2X4, P2X7 and TrpC3 channels are increased in the late stages of pregnancy. When the result of the patch-clamp study is considered, the P2X7 channel is suggested to be highly concerned with onset of labor.

The expressions of P2X4 and P2X7 mRNA are enhanced in the preterm model that was treated with antiprogesterone (RU486) at the same level as in labor in comparison to those of the control group as shown in Figure 5a and 5b. This result indicates that these channels are responsible for the hormone-dependent preterm delivery.

As an inflammatory model lipopolysaccharide (LPS; 0.2 mg/kg) was injected into intraperitoneal cavity on day 18 and the myometrium was sampled after six hours. In this model the expressions of P2X4, P2X7 and TrpC3 were enhanced by 7, 18 and 25 times, but TrpC4 was not changed (Fig. 5c and 5d, data for TrpC: unpublished data). The enhancement was much greater than that in normal delivery. This finding therefore suggests that the inflammation in the whole body of the animal may enhance the expression of myometrial NSCCs to accelerate uterine contractility and thereby may induce preterm delivery. The mechanism of preterm delivery with infection may be different from that at the onset of normal delivery at term.

Conclusion

We have revealed various ion channels in myometrial cells concerned with uterine contractility. Regulation of membrane potential of the cell is essential to explain muscle contractility. We present some findings of myometrial NSCCs. However, the role of myometrial NSCCs is still largely unknown and should be the object of further study to clarify the cause of preterm delivery. The expression of some myometrial NSCCs was enhanced remarkably. These NSCCs are likely to be associated with inflammation and may take a part of the cascade of inflammation. New findings on myometrial NSCCs may become the clue to the development of a new treatment for premature birth.

In our studies antiprogesterone was used as a model for preterm delivery. Blocking of progesterone enhanced the expression of myometrial NSCCs and function of gap junctions to increase uterine activities. Although no direct evidence, these findings may reasonably explain the inhibition of preterm birth by progesterone treatment.

References

Fidel Jr PL, Romero R, Wolf N et al. Systemic and local cytokine profiles in endotoxin-induced preterm parturition in mice. Am J Obstet Gynecol. 1994;170:1467-75.

Garfield RE, Sims S, Daniel EE. Gap junctions: their presence and necessity in myometrium during parturition. Science. 1977;198:958-60.

Inoue R, Isenberg G Intracellular calcium ions modulate acetylcholine-induced inward current in guinea-pig ileum. J Physiol (Lond) 1990;424:73-92.

Kasai Y, Iino M, Tsutsumi O et al. Effects of cyclopiazonic acid on rhythmic contractions in uterine smooth muscle bundles of the rat. Br J Pharmacol. 1994;112:1132-6.

Kawarabayashi T, Tsukamoto T, Shojo H et al. Changes in responsiveness of freshly isolated longitudinal muscle cells from rat uterus towards oxytocin during gestation: contractility and calcium signaling. Mol Cell Endocrinol. 1997;128: 77-84.

Loirand G, Pacaud P, Baron A et al. Large conductance calcium-activated non-selective cation channel in smooth muscle cells isolated from rat portal vein. J Physiol (Lond). 1991;437:461-75.

Miyoshi H, Urabe T, Fujiwara el al. A Electrophysiological properties of membrane currents in single myometrial cells isolated from pregnant rats. Pflügers Arch. 1991;419:386-93.

Miyoshi H, Boyle MB, MacKay LB et al. Voltage-clamp studies of gap junctions between uterine muscle cells during term and preterm labor. Biophys J. 1996;71: 1324-34.

Miyoshi H, Yamaoka K, Garfield RE et al. Identification of a non-selective cation channel current in myometrial cells isolated from pregnant rats. Pflügers Arch. 2004;447:457-64.

Miyoshi H, Yamaoka K, Urabe S et al. Functional expression of purinergic P2X7 receptors in pregnant rat myometrium. Am J Physiol Regul Integr Comp Physiol. 2010;298:R1117-24.

Okawa T, Suzuki H, Yaanagida K et al. Effect of lipopolysaccharide on uterine contractions and prostaglandin production in pregnant rats. Am J Obstet Gynecol. 2001;184:84-9.

Romero R, Mazor M. Infection and preterm labor. Clin Obstet Gynecol. 1988;31:553-84.

Sims SM Cholinergic activation of a non-selective cation current in canine gastric smooth muscle is associated with contraction. J Physiol (Lond). 1992;449:377-98.

Urabe S, Miyoshi H, Fujiwara H et al. Enhanced expression of P2X4 and P2X7 purinergic receptors in the myometrium of pregnant rats in preterm delivery models. Reprod Sci. 2009; 16:1186-92.

Vogalis F, Sanders KM. Cholinergic stimulation activates a non-selective cation current in canine pyloric circular muscle cells. J Physiol (Lond). 1990;429:223-36.

Wang Q, Large WA Noradrenaline-evoked cation conductance recorded with the nystatin whole-cell method in rabbit portal vein cells. J Physiol (Lond). 1991;435:21-39.

Use of Non-invasive Uterine Electromyography in the Diagnosis of Preterm Labour

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Abstract

Predictive values of methods currently used in the clinics to diagnose preterm labour are low. This leads to missed opportunities to improve neonatal outcomes and, on the other hand, to unnecessary hospitalizations and treatments. In addition, research of new and potentially more effective preterm labour treatments is hindered by the inability to include only patients in true preterm labour into studies. Uterine electromyography (EMG) detects changes in cell excitability and coupling required for labour and has higher predictive values for preterm delivery than currently available methods. This methodology could also provide a better means to evaluate various therapeutic interventions for preterm labour. Our manuscript presents a review of uterine EMG studies examining the potential clinical value that this technology possesses over what is available to physicians currently. We also evaluated the impact that uterine EMG could have on investigation of preterm labour treatments by calculating sample sizes for studies using EMG vs. current methods to enrol women. Besides helping clinicians to make safer and more cost-effective decisions when managing patients with preterm contractions, implementation of uterine EMG for diagnosis of preterm labour would also greatly reduce sample sizes required for studies of treatments.

Key words: **Cervical length, preterm delivery, preterm labour, preterm birth, tocodynamometry, uterine electromyography.**

Introduction

None of the currently used methods reliably distinguish between true and false preterm labour (Iams, 2003). Up to 50% of women admitted with the diagnosis of preterm labour are subsequently found not to be in true labor (McPheeters et al., 2005). On the other hand, 20% of symptomatic patients that are diagnosed as not being in preterm labour will deliver prematurely (McPheeters et al., 2005). This leads to missed opportunities to improve outcome of premature neonates, and also to unnecessary costs and side effects of treatments. In addition, women in false preterm labour who would not deliver preterm regardless of treatment are constantly included into analyses of treatment's efficacy. Very large studies are therefore needed for these analyses to be adequately powered, which significantly hinders the research of potentially better preterm labour treatments.

Several changes occur in the myometrium prior to preterm labour. Excitability of cells increases, systems that inhibit myometrial activity decrease and, at the same time, systems that stimulate myometrial activity increase (Tezuka et al., 1995; Yuan & Lopez Bernal, 2007; Fuchs et al., 1984). Electrical coupling between myometrial cells also increases and an electrical syncytium required for effective contractions is formed (Balducci et al., 1993; Garfield et al., 1988). Non-invasive measurement of uterine electromyography (EMG) yields information about these changes by measuring the electrical properties of the myometrium (Leman et al., 1999; Maner et al., 2003; Buhimschi et al., 1997; Marque et al., 2007; Rabotti et al., 2010; Lucovnik et al., 2011).

The aim of this manuscript is to review current knowledge on potential clinical value of uterine EMG in the diagnosis of preterm labour. We also evaluated the impact this technology could have on investigation of new preterm labour treatments.

Currently used methods to diagnose preterm

The diagnosis of preterm labour still often relies on presence of contractions. However, contractions occur commonly in normal pregnancy. In fact, they are one of the most common reasons for visits to obstetrical triage (Bennett et al., 1998). The currently available methodology to evaluate contractions – **tocodynamometry (TOCO)** – does not allow clinicians to determine which patient is in true preterm labour and needs to be admitted, treated and possibly transferred to a hospital with a neonatal intensive care unit. Unfortunately, TOCO became a standard of care without ever undergoing vigorous clinical trials, in an age 40 years ago when such trials were in their infancy. TOCO measures the change in shape of the abdominal wall as a function of uterine contractions and, as a result, is a qualitative rather than quantitative method (Freeman, 2002). It has been shown in several studies that monitoring uterine activity with TOCO has a low sensitivity and positive predictive value for preterm delivery (Iams, 2003; Peaceman et al., 1997; Iams et al., 2002).

Cervical dilation, effacement, consistency, position, and station of the presenting part, determined by **manual examination**, are components of the Bishop scoring system. The score was not primarily developed for this purpose, but is often used clinically as a predictor of preterm delivery. However, the assessment of the cervix by digital exam is subjective, and its prognostic values have also been shown to be low (Gomez et al., 1994; Jackson et al., 1992).

There is now substantial evidence that measuring **cervical length** by transvaginal ultrasound and testing for **fetal fibronectin** in cervicovaginal fluid can help to avoid unnecessary treatment due to high negative predictive values of these tests (Iams et al., 1996; Leitich et al., 1999; Fuchs et al., 2004; Tsoi et al., 2005). Although a short cervical length indicates a higher risk for preterm delivery, it does not identify patients in true preterm labour reliably. Many women with short cervices, even those presenting with symptoms of preterm labour, do not deliver prematurely (Iams, 2003; Fuchs et al., 2004; Tsoi et al., 2005). Fetal fibronectin is an extracellular matrix glycoprotein produced by amniocytes and cytotrophoblast that normally resides at the decidual-chorionic interface (Honest et al., 2002). Its presence in the cervicovaginal fluid indicates decidual activation. Similar to cervical length, however, the positive predictive value of fetal fibronectin is low and many patients with a positive test do not deliver preterm (Iams, 2003).

There is consequently a great need for a method with a high positive predictive value for preterm delivery that would accurately identify patients in true preterm labour.

Accuracy of uterine electromyography in prediction of preterm delivery

Myometrial activation, required for effective contractions and true labour, is characterized by molecular changes leading to changes in the EMG activity of the myometrium (Buhimschi et al., 1997; Leman et al., 1999; Maner et al., 2003; Marque et al., 2007; Rabotti et al., 2010; Lucovnik et al., 2011). Extensive studies have been done in the last 60 years to monitor uterine EMG from electrodes placed on the uterus (Figueroa et al., 1990; Devedeux et al., 1993; Wolfs & van Leeuwen, 1997). More recent studies indicate that uterine EMG can be monitored non-invasively from the abdominal surface (Fig. 1) (Buhimschi & Garfield 1996; Buhimschi et al., 1998; Garfield et al., 1998).

Studies demonstrated similar effectiveness of transabdominal uterine EMG as compared to TOCO and intrauterine pressure catheter measurements in detecting contractions (Maul et al., 2004). 'Bursts' of electrical (EMG) signals are responsible for uterine contractions (Fig. 2).

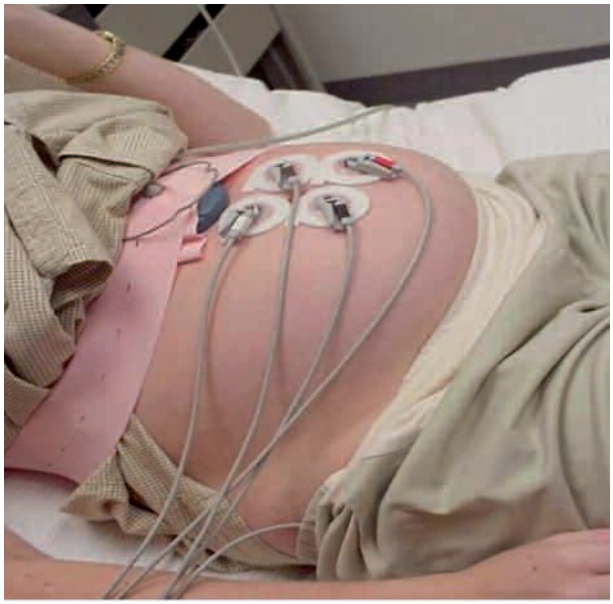

Fig. 1. — Electrode placement on the abdominal surface for non-invasive uterine electromyography (EMG) recording.

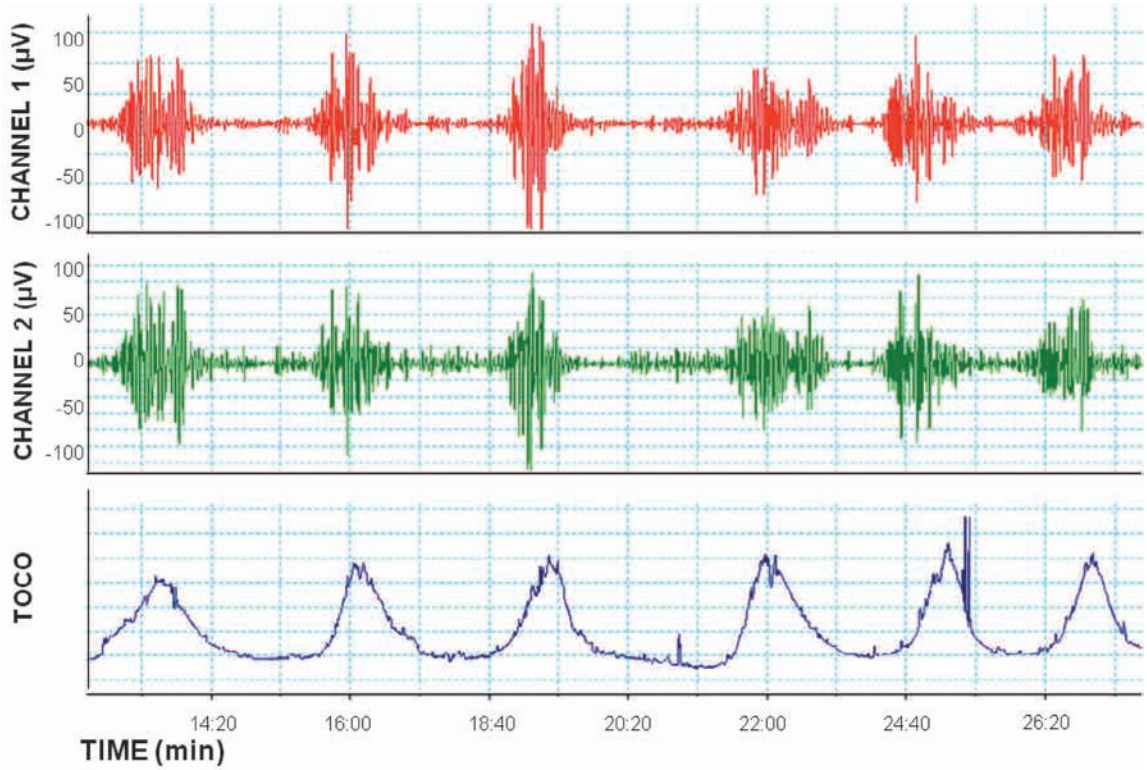

Fig. 2. — Electromyographic (EMG) activity is responsible for uterine contractions. Top traces show a sample EMG recording from two electrode pairs (channels 1&2). Note the excellent temporal correspondence between EMG and mechanical contractile events (measured by TOCO).

In addition, it has been shown that EMG yields valuable information about the changes in the electrical properties of the myometrium. These changes are the direct consequence of increased electrical excitation and coupling between myometrial cells that are required for preterm labour. Several EMG parameters can indicate the onset of labour. EMG bursts have been reported to be more frequent and their duration more constant in true labour (Buhimschi et al., 1997; Maner & Garfield, 2007). An increase in peak amplitude and frequency of EMG signals, assessed by power-spectrum (PS) analysis, has also been observed prior to preterm labour (Buhimschi et al., 1997; Maner et al., 2003). A more recent study showed that propagation velocity (PV) of uterine EMG signals, estimated from the time interval between signal arrivals at adjacent electrode pairs, increases as preterm delivery approaches (Fig. 3) (Lucovnik et al., 2011).

The combination (rescaled sum) of EMG PV and PS peak frequency yielded higher predictive values for preterm delivery than any EMG parameter alone. Receiver-operating-characteristics curve analysis for PV + PS peak frequency had an area under the curve of 0.96 for prediction of preterm delivery within 7 days (Lucovnik et al., 2011). Therefore, uterine EMG has been shown to be much more accurate in diagnosing preterm labour than all the methods currently used clinically (Fig. 4).

Currently used methods versus electromyography to calculate the number of women needed in studies of preterm labour treatments

Although perinatal mortality rate due to prematurity has decreased dramatically over the past four decades in high-income countries, this reduction resulted from improvements in neonatal care for premature babies, and has occurred in spite of our inability to prevent preterm delivery once preterm labour is established (Gyetvai et al., 1999; Hack & Fanaroff 1999; Giles & Bisits 2007). Development of effective preterm labour treatments that would prolong pregnancy sufficiently to allow further intrauterine growth and improve neonatal outcomes depends largely on the tests used to diagnose preterm labour.

Uterine EMG can identify true preterm labour more accurately than the currently used methods (see above). Consequently, this methodology could be extremely important for research of new preterm labour treatments, because it allows the inclusion of only



Fig. 3. — Uterine electromyography propagation velocity increases as the measurement-to-delivery interval decreases. (Adapted from Lucovnik et al., 2011).

women in true preterm labour into studies. To evaluate the potential impact of uterine EMG on investigation of new preterm labour treatments, we calculated sample sizes required for studies using EMG vs. various current methods to enrol women.

The following diagnostic methods have been considered: uterine EMG, digital cervical examination, cervical length measurement, TOCO, fetal fibronectin test, and a combination of currently used methods in the clinics. We utilized the cut-offs for which positive predictive values (PPVs) have previously been reported in the literature: uterine EMG (rescaled sum of propagation velocity and PS peak frequency > 84.48) (PPV = 100%), Bishop score ≥ 4 for digital cervical examination (PPV = 42%), ≥ 4 contractions per hour on TOCO (PPV = 25%),

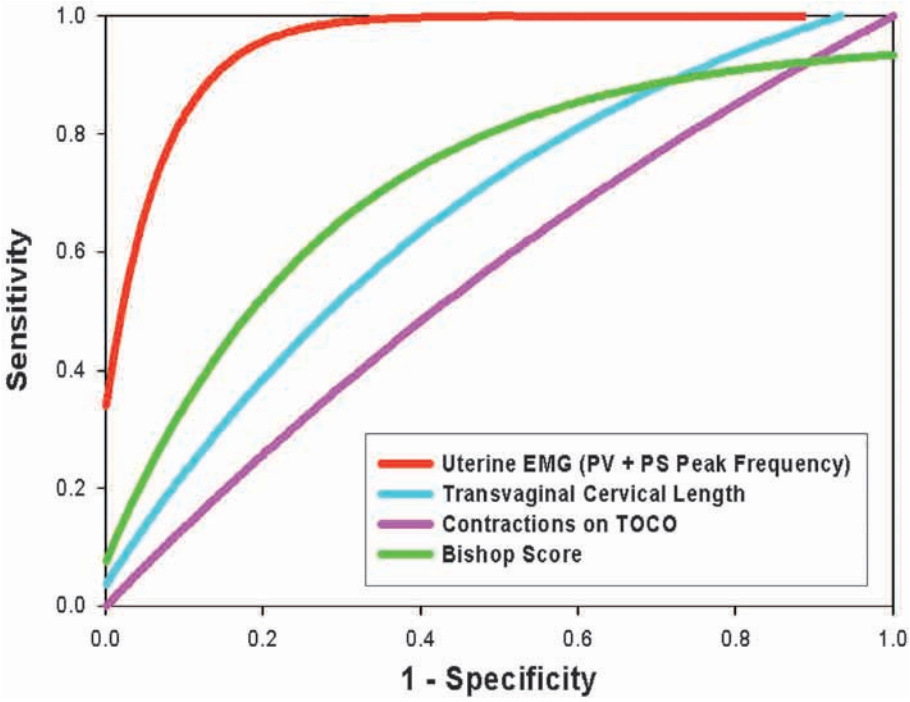

Fig. 4. — Comparison of receiver-operating-characteristics (ROC) curves for uterine electromyography (EMG) parameters (rescaled sum of propagation velocity [PV] and power spectrum [PS] peak frequency) and currently used clinical methods to predict preterm delivery within 7 days. (Adapted from Lucovnik et al., 2011).

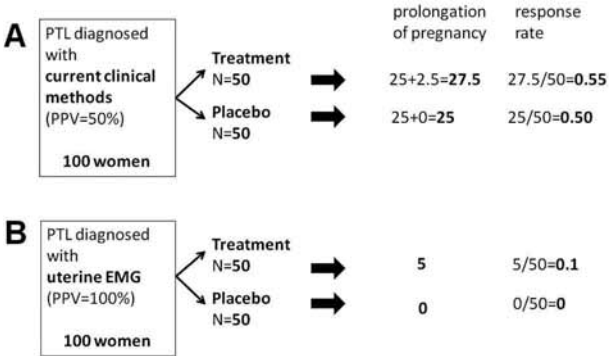

Fig. 5. — Method used to determine response rates used for sample size calculation. Figure illustrates two randomized studies of 10% effective treatment for preterm labour (PTL). Study A utilizes currently available methods to diagnose PTL and include women in the study; study B utilizes uterine electromyography (EMG). *PPV positive predictive value*.

concentration of fetal fibronectin ≥ 50 ng/mL (PPV = 43%), and cervical length of both < 30 mm and < 15 mm (PPV = 23% and PPV = 57%, respectively) (Gomez et al., 1994; Iams, 2003; Lucovnik M et al., 2011). As 50% of patients diagnosed with preterm labour do not deliver prematurely, we alleged the PPV of the combination of all methods used to diagnose preterm labour in the clinics today to be 50% (McPheeters et al., 2005). We then used these predictive values to determine the proportions of women who will not deliver preterm (response rates) in the groups treated with hypothetical treatments of various efficacies (10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, and 100% effective) vs. placebo.

We used sample size calculation based on proportions to determine how many case (treated) and control (placebo) women would be needed to reject the null hypothesis that the preterm delivery rates for cases and controls are equal with probability (power) 0.8 (80%) ($\beta = 0.8$). Type I error probability was 0.05 (5%) ($\alpha = 0.05$). Uncorrected chi-squared statistic was used to evaluate the null hypothesis. The software used for statistical analysis was PS: Power and Sample Size Calculation version 3.0 (Vanderbilt Medical Center, Nashville, TN, USA).

The following example illustrates the method of sample size calculation. Using the currently available combination of diagnostic tests, 50 of 100 women included in the study because diagnosed as being in preterm labour will not deliver preterm regardless of whether they will be treated or not (McPheeters et al., 2005). On the other hand, 50 of these 100 women will deliver preterm if not treated. If treated with a 10% effective treatment, 55 of them will not deliver preterm, while 45 will. In order to test for the efficacy of such treatment one would need to compare a group of women treated with the drug to a group of women treated with a placebo. With equal randomization to treatment groups, 50 women will receive the 10% effective treatment. 25 of these will not be in true preterm labour, and 25 will be in true preterm labour. Theoretically, 2.5 women in true labour will not deliver preterm due to treatment. Consequently, of the 50 treated women diagnosed clinically as being in preterm labour, 27.5 (25 who were not at risk in the first place and 2.5 at risk women who responded to treatment) women will not deliver preterm. The response rate will, therefore, be 55% (27.5 / 50 = 0.55). In the placebo group, 25 women will not deliver preterm because they were not in true labour in the first place, thus 50% response rate (25/50 = 0.50). Utilizing a calculator for sample size based on proportions, one can now determine how many women would have to be recruited to the study to find this small difference with an α of 0.05 and 0.8 power (β). When using the uterine EMG all 100 women will theoretically be in true labour (PPV = 100% based on our previously published data) (Lucovnik M et al., 2011). If women are randomized equally, 5 women (10%) in the treatment group will not deliver preterm (10% response rate; 5/50 = 0.1), and 0 in the placebo group (0% response rate; 0/50 = 0). Again, utilizing sample size calculator based on proportions, one can determine how many women would need to be included in the study to find this significantly larger difference (with an α of 0.05 and 0.8 power) (Fig. 5).

Table I shows that significantly smaller numbers of women would be needed to demonstrate the efficacy of treatment (or to reliably refute its effectiveness) using uterine EMG as compared to other diagnostic methods.

In the case of a hypothetically 10% effective treatment, for example, one would need to include 10134 women in the study, if the inclusion criteria would be a cervical length < 30 mm. 9096 women would need to be recruited with ≥ 4 contractions on TOCO per hour as the inclusion criteria. 5686 women would be the needed sample size with the increased concentration (≥ 50 ng/mL) of fetal fibronectin in the cervicovaginal secretions. With a Bishop score ≥ 4 one would need to include 4266 women, and with a cervical length < 15 mm, 2398 women. 3130 women would need to be included in the study using the combination of these methods. If uterine EMG would be utilized, on the other hand, the same effectiveness could be demonstrated in a study including only 148 women.

With more effective treatments the number of women needed for studies are lower. However, these numbers are always significantly lower for studies utilizing uterine EMG, regardless of how effective the treatment would be (Table I).

Table I. — Sample sizes needed to demonstrate effectiveness of treatment (with $\alpha = 0.05$ and $\beta = 0.8$) using different methods to include women into studies.

Method used to include women	Number of women needed to demonstrate effectiveness of treatment									
	10%	20%	30%	40%	50%	60%	70%	80%	90%	100%
Uterine EMG	148	68	42	28	22	16	12	8	6	4
Combination of Currently Used Methods	3130	774	340	186	116	76	54	38	28	22
Digital Cervical Examination (Bishop Score ≥ 4)	4266	1044	452	246	152	100	70	50	36	28
Transvaginal Cervical Length										
< 15 mm	2398	602	342	148	92	58	44	32	24	18
< 30 mm	10134	2434	1034	552	336	236	152	108	78	58
Contractions on TOCO ≥ 4/h	9096	2188	932	500	304	200	138	98	72	52
Fetal Fibronectin ≥ 50 ng/mL	5686	1380	592	320	196	130	90	64	46	34

Legend: EMG electromyography; TOCO tocodynamometry.

Conclusions

Non-invasive measurement of uterine EMG can identify true preterm labour more accurately than the currently used methods. It can identify patients who will 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, uterine EMG also identifies patients who, although presenting with signs and symptoms of preterm labour, are not going to deliver preterm. This can help to avoid substantial economic costs associated with unnecessary hospitalization and transport, the maternal risks associated with tocolytics, and the potential fetal risks associated with steroids.

In addition, use of uterine EMG to diagnose preterm labour and include women in studies of preterm labour treatments would significantly reduce the sample sizes required for such studies to have an adequate statistical power. This is true, regardless of how effective the treatment is. As a result, uterine EMG can lead to significant savings of time, effort, and money, when researching new methods or drugs to prevent preterm delivery.

Disclosure

Drs Lucovnik and Novak-Antolic have no financial interest in the technology described in the manuscript and therefore have no conflict of interest.

References

Balducci J, Risek B, Gilula NB et al. Gap junction formation in human myometrium: a key to preterm labor. Am J Obstet Gynecol. 1993;168:1609-15.

Bennett TA, Kotelchuck M, Cox CE et al. Pregnancy-associated hospitalizations in the United States in 1991 and 1992: a comprehensive view of maternal morbidity. Am J Obstet Gynecol. 1998;178:346-54.

Buhimschi C, Boyle MB, Garfield RE. Electrical activity of the human uterus during pregnancy as recorded from the abdominal surface. Obstet Gynecol. 1997;90:102-11.

Buhimschi C, Boyle MB, Saade GR et al. Uterine activity during pregnancy and labor assessed by simultaneous recordings from the myometrium and abdominal surface in the rat. Am J Obstet Gynecol. 1998;178:811-22.

Buhimschi C, Garfield RE. Uterine contractility as assessed by abdominal surface recording of electromyographic activity in rats during pregnancy. Am J Obstet Gynecol. 1996;174: 744-53.

Devedeux D, Marque C, Mansour S et al. Uterine electromyography: A critical review. Am J Obstet Gynecol. 1993;169(6): 1636-53.

Figueroa JP, Honnebier MB, Jenkins S et al. Alteration of 24-hour rhythms in myometrial activity in the chronically catheterized pregnant rhesus monkey after a 6-hour shift in the light-dark cycle. Am J Obstet Gynecol. 1990;163(2):648-54.

Freeman RK. Problems with intrapartum fetal heart rate monitoring interpretation and patient management. Obstet Gynecol. 2002;100:813-26.

Fuchs AR, Fuchs F, Husslein P et al. Oxytocin receptors in the human uterus during pregnancy and parturition. Am J Obstet Gynecol. 1984;150:734-9.

Fuchs I, Tsoi E, Henrich W et al. Sonographic measurement of cervical length in twin pregnancies in threatened preterm labor. Ultrasound Obstet Gynecol. 2004;23:42-5.

Garfield RE, Blennerhassett MG, Miller SM. Control of myometrial contractility: role and regulation of gap junctions. Oxf Rev Reprod Biol. 1988;10:436-90.

Garfield RE, Saade G, Buhimschi C et al. Control and assessment of the uterus and cervix during pregnancy and labour. Hum Reprod Update. 1998;4:673-95.
Giles W, Bisits A. Preterm labour. The present and future of tocolysis. Best Pract Res Clin Obstet Gynaecol. 2007;21:857-68.
Gomez R, Galasso M, Romero R et al. Ultrasonographic examination of the uterine cervix is better than cervical digital examination as a predictor of the likelihood of premature delivery in patients with preterm labor and intact membranes. Am J Obstet Gynecol. 1994;171:956-64.
Gyetvai K, Hannah ME, Hodnett ED et al. Tocolytics for preterm labor: a systematic review. Obstet Gynecol. 1999;94: 869-77.
Hack M, Fanaroff AA. Outcomes of children of extremely low birthweights and gestational age in the 1990's. Early Hum Dev. 1999;53:193-218.
Honest H, Bachmann LM, Gupta JK et al. Accuracy of cervicovaginal fetal fibronectin test in predicting risk of spontaneous preterm birth: systematic review. BMJ. 2002; 325:301.
Iams JD. Prediction and Early Detection of Preterm Labor. Obstet Gynecol. 2003;101:402-12.
Iams JD, Newman RB, Thom EA et al. Frequency of uterine contractions and the risk of spontaneous preterm delivery. N Engl J Med. 2002;346:250-5.
Iams JD, Goldenberg RL, Meis PJ et al. The length of the cervix and the risk of spontaneous premature delivery. N Engl J Med. 1996;334:567-72.
Jackson GM, Ludmir J, Bader TJ. The accuracy of digital examination and ultrasound in the evaluation of cervical length. Obstet Gynecol. 1992;79:214-8.
Leitich H, Egarter C, Kaider A et al. Cervicovaginal fetal fibronectin as a marker for preterm delivery: A meta-analysis. Am J Obstet Gynecol. 1999;180:1169-76.
Leman H, Marque C, Gondry J. Use of electrohysterogram signal for characterization of contractions during pregnancy. IEEE Trans Biomed Eng. 1999;46:1222-9.
Lucovnik M, Maner WL, Chambliss LR et al. Noninvasive uterine electromyography for prediction of preterm delivery. Am J Obstet Gynecol. 2011;204:228.e1-10.
Maner WL. Garfield RE, Maul H et al. Predicting term and preterm delivery with transabdominal uterine electromyography. Obstet Gynecol. 2003;101:1254-60.
Maner WL, Garfield RE. Identification of human term and preterm labor using artificial neural networks on uterine electromyography data. Ann Biomed Eng. 2007;35:465-73.
Marque C, Terrien J, Rihana S et al. Preterm labour detection by use of a biophysical marker: the uterine electrical activity. BMC Pregnancy Childbirth. 2007;7(Suppl 1):S5.
Maul H, Maner WL, Olson G et al. Non-invasive transabdominal uterine electromyography correlates with the strength of intrauterine pressure and is predictive of labor and delivery. J Matern Fetal Neonatal Med. 2004;15(5):297-301.
McPheeters ML, Miller WC, Hartmann KE et al. The epidemiology of threatened preterm labor: a prospective cohort study. Am J Obstet Gynecol. 2005;192:1325-30.
Peaceman AM, Andrews WW, Thorp JM et al. Fetal fibronectin as a predictor of preterm birth in patients with symptoms: A multicenter trial. Am J Obstet Gynecol. 1997;177:13-8.
Rabotti C, Mischi M, Oei SG et al. Noninvasive estimation of the electrohysterographic action-potential conduction velocity. IEEE Trans Biomed Eng. 2010;57:2178-87.
Tezuka N, Ali M, Chwalisz K et al. Changes in transcripts encoding calcium channel subunits of rat myometrium during pregnancy. Am J Physiol. 1995;269:1008–17.
Tsoi E, Fuchs IB, Rane S et al. Sonographic measurement of cervical length in threatened preterm labor in singleton pregnancies with intact membranes. Ultrasound Obstet Gynecol. 2005;25:353-6.
Wolfs GM, van Leeuwen M. Electromyographic observations on the human uterus during labour. Acta Obstet Gynecol Scand. 1979;90:1-61.
Yuan W, Lopez Bernal A. Cyclic AMP signalling pathways in the regulation of uterine relaxation. BMC Pregnancy Childbirth. 2007;7(Suppl1):S10.

Preterm Cervical Ripening in humans

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Abstract

Preterm birth (PTB) is the leading cause of neonatal mortality and morbidity. Despite the current treatment procedures, the incidence of PTB has not changed in the past thirty years. Incomplete understanding of the biological and patophysiological mechanisms underlying preterm delivery is the major obstacle to prevent PTB. Cervical ripening is necessary for vaginal delivery and understanding of preterm cervical ripening is required for developing new treatment strategies. Several important substances such as HMGB1 and its receptors, CRH and its receptors and numerous cytokines are localized in the cervix and undergo distinct changes in labour. Other important molecules, such as CRH, CRH-BP, CRH-R1, CRH-R2, HMGB1, TLR2, TLR4, IL-10, IL-12, are localized in the cervical epithelium, also indicating their role in the process of cervical ripening during labour. Furthermore, CRH stimulates IL-8 secretion from both preterm and term cervical fibroblasts. Recent studies from our group show that major inflammatory changes occur in the cervix at labour irrespective of gestational age. This indicates that cervical ripening at both term and preterm is an inflammatory process even if no infection is present. However, preterm cervical ripening still entails some differences from term cervical ripening, for example in the down-regulation of mRNA expression of Toll-like receptors (TLR-2 and TLR-4) and IL-12, higher levels of IL-10 in cervical epithelium, and presents different secretion patterns of cervical fibroblasts. Moreover, preterm cervical ripening, like preterm delivery itself, is a multifactorial disorder with pathways which are partly different from those involved in PPROM and infected preterm labour.

Key words: **Cervical ripening, cytokines, CRH, cervical fibroblasts, HMGB1, preterm labour.**

Introduction

Preterm birth can be divided into elective deliveries due to maternal or foetal indications, and spontaneous preterm birth with or without preterm premature rupture of membranes (PPROM) (Goldenberg et al., 2008). Multiple causes i.e. infection, uteroplacental ischemia or haemorrhage, stress, endocrine factors, immunologically mediated processes may be associated with preterm birth (Challis et al., 2009) (Fig. 1).

Current tocolytic therapies have not reduced the incidence of preterm birth. Thus there is a need for new strategies for treatment and prevention of preterm birth. The current treatment strategies focus upon tocolytic agents and upon diminishing the uterine contractility. However, a preterm vaginal birth first requires cervical ripening after which myometrial contractions follow. Indeed, even painfully strong contractions in combination with stiff and closed cervix do not result in preterm delivery. In order to develop new strategies to diminish the preterm birth rate, it is therefore of major importance to improve our understanding of the physiology of preterm cervical ripening.

Composition of Cervix uteri

Danforth et al. (1947) stated that the human cervix consists mainly of fibrous connective tissue. The non-pregnant cervix is composed of of 85% extracellular matrix (ECM) and 6-10% of muscle fibers (Rorie and Newton, 1967). The cervical ECM consists of collagens, proteoglycans, hyaluronan, and glycoproteins. Collagen I and collagen III are the major types and the dominating proteoglycan in

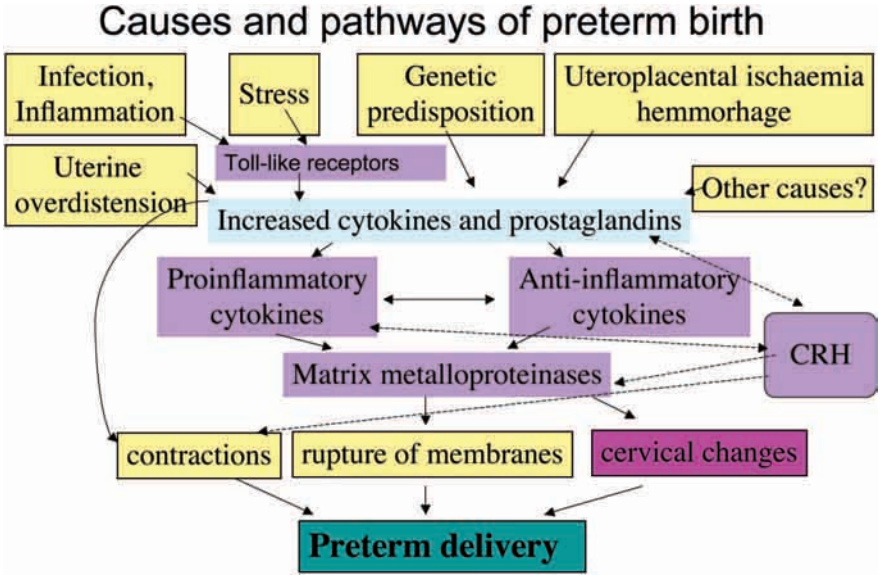

Fig. 1. — Causes and pathways of preterm birth

non-pregnant cervix uteri is decorin. Fibromodulin and biglycan, and also large proteoglycans such as versican and heparan sulfate proteoglycan are present (Uldbjerg et al., 1983b; Norman et al., 1991; Westergren-Thorsson et al., 1998).

Cervical ripening at term

Cervical softening during pregnancy is cahracterized by a gradual decrease in the collagen concentration. Simultaneously the collagen extractability increases, suggesting changes in the organization of collagen fibrils (Uldbjerg et al., 1983a; Granstrom et al., 1989). The duration of cervical dilatation during labour correlates well with the collagen concentration and its solubility (Ekman et al., 1986). Immediately after vaginal delivery at term the mRNA levels for collagen I and III decrease by up to 60% compared to those in the non- pregnant cervix, but during involution (2-4 days after delivery) the levels are increased 2.5-fold and 3.5-fold respectively (Westergren-Thorsson et al., 1998).

The decorin concentration is decreasing with 50% until final ripening while that of versican, biglycan and the heparan sulfate proteoglycans increases (Norman et al. 1993; Westergren-Thorsson et al. 1998). Versican can attract water and bind hyaluronan (Wu et al., 2005), resulting in disintegration of the collagen bundles and a change in the physical properties to produce a soft and elastic tissue, thus facilitating cervical dilatation.

Gonadal steroids

Estrogen, progesterone and insulin-like growth factor-I (IGF-I) are involved in cervical ripening (Stjernholm et al., 1996; Stjernholm et al., 1997; Wang et al., 2001). ERα mRNA decreases in the ripe cervix at delivery, while ERβ mRNA levels are increased in the term pregnant cervix not in labour (Wang et al., 2001). The ERβ antigen is also co-localized with leukocyte markers in cervix (Stygar et al., 2001).

Cervical ripening – an inflammatory reaction

Further, the process of cervical ripening at labour can be regarded as an inflammatory reaction since the levels of IL-6, IL-8 increase at term labour (Sennstrom et al., 2000). This process is also associated with cervical leukocyte invasion (Young et al., 2002; Osman et al., 2003).

Cytokines recruit activated cells, which in turn secrete degradative enzymes such as matrix metalloproteinases (MMPs). Thus, increased levels of MMP-1, MMP-2, MMP-3, MMP-8 and MMP-9 have been observed during pregnancy and at the final cervical ripening (Stygar et al., 2002; Sennstrom et al., 2003).

Fibroblasts

Fibroblasts play a crucial role in the remodelling of the extracellular matrix (Larsen et al. 2006). Activated fibroblasts produce ECM components, cytokines and matrix metalloproteinases (Malmstrom et al., 2007; Akerud et al., 2008). Cervical fibroblast cultures established from the biopsies from non-pregnant women, term pregnant women at caesarean section before the onset of labour or parturient women, present different and stable phenotypes (Malmstrom et al., 2007). There is a decrease in pro-

teoglycan secretion and an increase in IL-6, IL-8, MMP-1, MMP-3 production in the cultures from parturient donors (Malmstrom et al., 2007; Akerud et al., 2008).

Preterm cervical ripening

Prostaglandins

Prostaglandins, synthesized in foetal membranes and deciduas play an important role in parturition and cervical ripening. Local application of prostaglandin-E_2 (PGE_2) has become a routine treatment in inducing cervical ripening and labour both at term and at preterm (Ekman et al., 1983; Abelin Tornblom et al., 2002). Cervical ripening at preterm as well as at term is associated with decreased degradation of prostaglandins (Tornblom et al., 2004).

NO

Nitric oxide (NO) has been suggested as an active mediator in cervical ripening (Chwalisz and Garfield, 1998), and NO-donors induce ripening of the human cervix (Thomson et al., 1997). The expression of nitric oxide synthase (NOS) isoforms increases in the cervix in late pregnancy and parturition (Ledingham et al., 2000). Further, preterm labour is associated with higher mRNA expression of NOS isoforms in the cervix (Tornblom et al., 2005b). Cervical fluid nitric oxide metabolite (NOx) levels rise during labour, nitric oxide donor administration or cervical manipulation and are significantly related to cervical ripening. (Vaisanen-Tommiska et al., 2003).

Corticotropin-releasing hormone, CRH

CRH, also termed corticotropin-releasing factor (CRF) is the principal regulator of the hypothalamic-pituitary-adrenal (HPA) axis (Hillhouse and Grammatopoulos 2006).

CRH, CRH-BP, CRH-R1 and CRH-R2 have been identified at both mRNA and protein level in human placenta, deciduas, foetal membranes, endometrium and myometrium (Petraglia et al., 1992; Petraglia et al., 1993; Hillhouse and Grammatopoulos, 2002; Sehringer et al., 2004). Furthermore, CRH increases MMP-9 protein secretion by cultured cells from placenta and foetal membranes (Li and Challis, 2005). In addition, several studies have shown that CRH can stimulate the production of cytokines in different types of cells (Wang et al., 2007).

Klimaviciute et al (2006) found differences in CRH-BP, CRH-R1 and CRH-R2 mRNA expression in cervical tissue and myometrium.These changes seem to be related to pregnancy and labour but not to gestational age. CRH-BP, CRH-R1, CRH-R2 are down regulated during pregnancy. CRH-R2 in cervix and myometrium and CRH-BP in cervix are even more down regulated during labour.With immunohistochemistry CRH was localized in cervical epithelium with the highest concentration at term, while CRH-BP is decreased at labour, which shows possible involvement of CRH in cervical ripening (Klimaviciute et al., 2006).

In vitro CRH stimulates IL-8 production in the cultures of preterm and term cervical fibroblasts, but doesn't seem to have effect on the secretion of MMP-1 and MMP-3 (Dubicke et al., 2008) (Fig. 2).

High-mobility group box protein 1 (HMGB1)

HMGB1 is expressed by almost all cells (Bianchi and Manfredi, 2007). In 1999 it was discovered that activated macrophages secrete HMGB1 as a delayed mediator of inflammation (Wang et al., 1999). HMGB1 has also important extracellular cytokine-like functions mediating the late response to infection, injury and inflammation (Lotze and Tracey, 2005). HMGB1 induces NF-kB activation (Lotze and Tracey, 2005) and stimulates pro-inflammatory cytokine synthesis (Andersson et al., 2000).

Human term placenta expresses HMGB1. Labour does not influence its placental expression, although a tendency towards higher expression of extranuclear HMGB1 in placentas with preeclampsia has been observed (Holmlund et al., 2007). HMGB1 is expressed in human foetal membranes at term pregnancy (Ticconi et al., 2007).

A receptor for advanced glycation end-products (RAGE) and Toll-like receptor 2 (TLR2) and TLR4 are involved in HMGB1-mediated signalling (Bianchi and Manfredi, 2007).

Receptor for advanced glycation end-products (RAGE)

The receptor for HMGB1 is RAGE, a multiligand receptor of the immunoglobulin family. The soluble form of RAGE (sRAGE) receptor is considered to act as a regulator/inhibitor of HMGB1 action (Lotze and Tracey, 2005). RAGE is expressed in trophoblasts of first-trimester human chorionic villi from healthy women (Konishi et al., 2004). Human term placenta expresses RAGE, but labour does not influence this expression (Holmlund et al., 2007). RAGE staining is especially increased in the vasculature of myometrium in women with preeclampsia (Cooke et al., 2003). There are contradictory results concerning intra-amniotic infection/inflammation

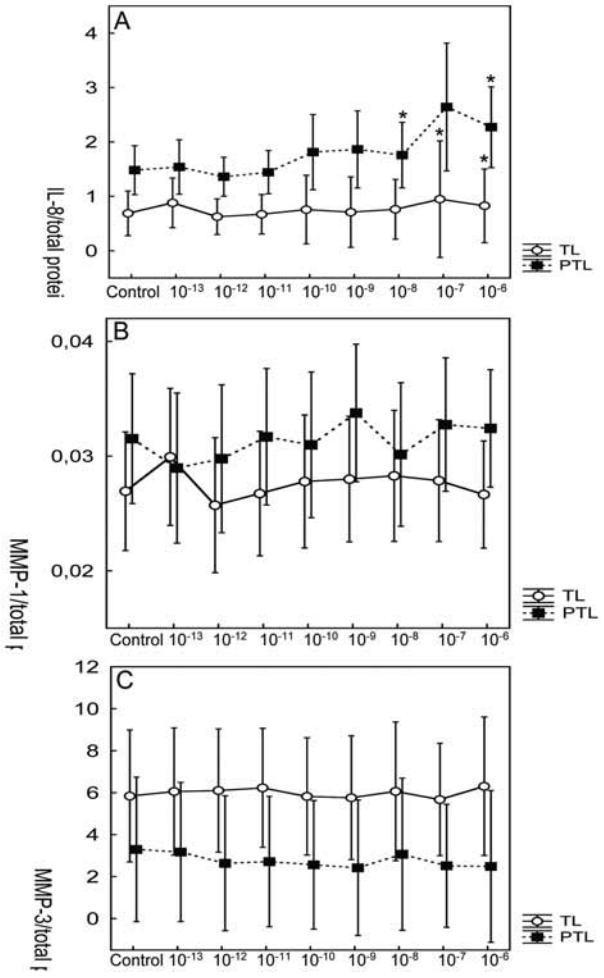

Fig. 2. — Effect of CRH on secretion of IL-8 (A), MMP-1 (B) and MMP-3 (C) by preterm (PTL) and term (TL) cervical fibroblasts. Different concentrations (M) of CRH indicated on x-axis. Data presented as mean ± SD. * $p < 0.05$ (compared with control). The secretion of IL-8 and MMP-1 was significantly higher in preterm cervical fibroblasts ($p < 0.001$ and $p < 0.05$, respectively). MMP-3 secretion was significantly higher in term cervical fibroblasts ($p < 0.001$).

and amniotic fluid concentrations of sRAGE (Buhimschi et al., 2007; Romero et al., 2008). Women with threatening preterm birth had significantly higher serum sRAGE concentrations than healthy pregnant women.

Toll-like receptors (TLRs)

These receptors are expressed on different cell types belonging to the innate, adaptive immune system and non-immunological cells. TLR2 and TLR4 were the first TLRs to be demonstrated at the protein level in human term placenta (Holmlund et al., 2002). Expression of all ten TLRs has been described in human placenta and studied in pregnancy and in labour (Patni et al., 2007). Greater mRNA expression is seen of all TLR genes in the placenta during labour (Patni et al., 2009). TLRs in the cervix have been less extensively studied. In pregnant mice, increased mRNA expression of TLR2, TLR3 and TLR4 is seen (Gonzalez et al., 2007). TLRs 1 to 6 mRNA and the protein of TLR2 and TLR4 have been found in human non-pregnant cervix (Pioli et al., 2004). Up-regulation of mRNA expression of TLR2 and TLR4, but down-regulation of TLR3 and TLR5, were observed in the cervix during labour with microarray analysis. The down-regulation of TLR3 and TLR5, but not up-regulation of TLR2 and TLR4 was confirmed with real-time RT-PCR (Hassan et al., 2006).

Dubicke et al found HMGB1, RAGE, TLR2 and TLR4 to be localized and to have an mRNA expression in the human cervix (Dubicke et al., 2010a) There was more extranuclear HMGB1 in the cervical epithelium and stroma in the preterm and term labour groups (Fig. 3). Extranuclear expression of HMGB1 during labour suggests a possible role of HMGB1 during the process of cervical ripening. There was a lower staining and tendency to lower mRNA expression for HMGB1 in the labouring groups. mRNA for RAGE was down regulated in the labouring groups, but there was higher concentration of soluble RAGE in labour. There was an up-regulation of TLR2 mRNA expression in labour. On the contrary, there were lower protein levels of TLR2 and TLR4 in labour (Fig. 4). The preterm group showed lower mRNA expression for TLR2 and TLR4 than term labour group (Fig. 4, C-D). Changes in mRNA expression of HMGB1, TLR2 and TLR4 in preterm labour, point towards possible differences in the mechanism of cervical ripening at preterm and term (Dubicke et al., 2010a).

Cytokines

Tornblom et al found in cervical biopsies obtained at preterm and term pregnancies the protein concentrations of IL-8, IL-6, and MCP-1 to be significantly increased during labour compared to non-labouring groups, whereas no changes were observed for RANTES and TNF-α. The mRNA levels of representative cytokines such as IL-8 and MCP-1 increased significantly whereas RANTES mRNA expression remained unchanged. WBC and CRP were significantly higher in the labouring groups as compared to groups not in labour. For neither of the analysed cytokines, WBC or CRP levels were there any changes between preterm and term groups (Tornblom et al., 2005a).

The role of IL-12 and IL-18 during pregnancy and parturition has attracted interest recently. IL-12 and IL-18 are important in regulating natural killer cell activities in early pregnancy, and are considered important for reproductive success. Higher IL-12 levels

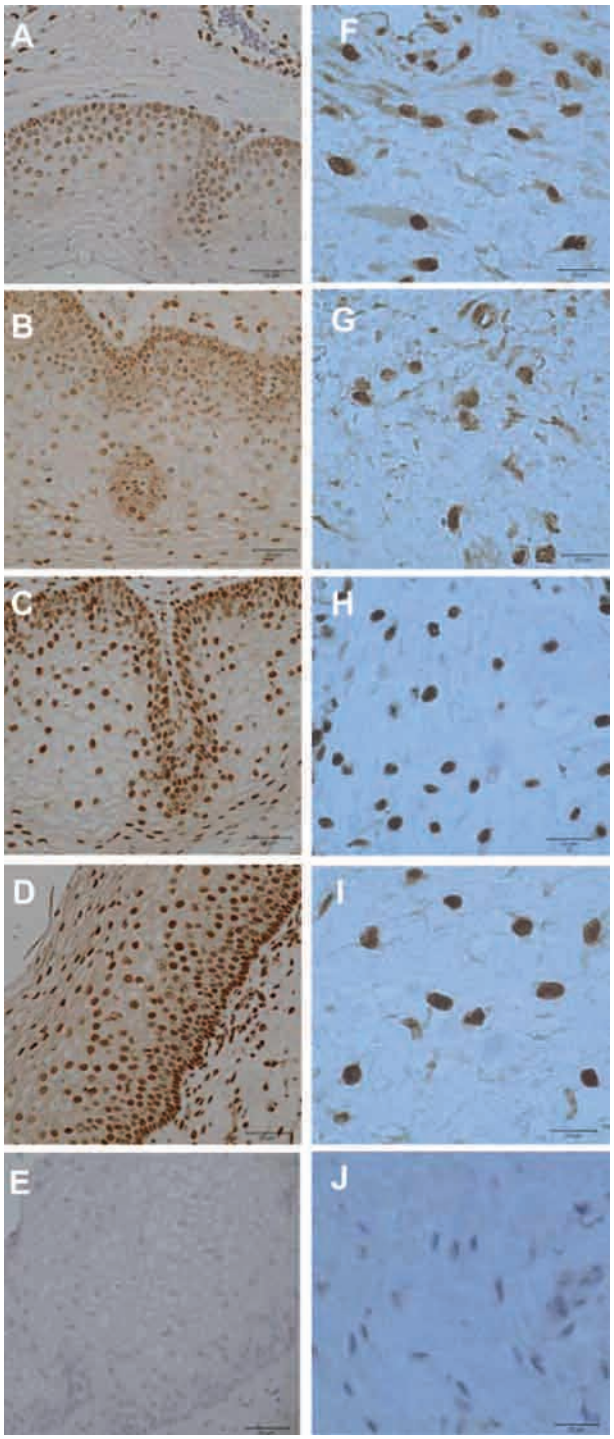

Fig. 3. — The immunohistochemical staining of HMGB1 in the squamous epithelium (column on the left) and in the stroma (column on the right) in the cervical tissue.
HMGB1 in the cervical epithelium at preterm labour **(A),** term labour **(B),** term not in labour **(C)** and non-pregnant state **(D)**. Negative control **(E).** The magnification is ×400. The mark is 50 μm.
HMGB1 in the stroma at preterm labour **(F)**, term labour **(G)**, term not in labour **(H)** and non-pregnant state **(I)**. Negative control **(J)**. The magnification is ×1000. The mark is 20 μm.

in mid-pregnancy are associated with preterm delivery with chorioamnionitis before 35 weeks of gestation (Gargano et al., 2008). Patients having low IL-18 and high IL-12 had a twofold-increased risk of delivering before 34 weeks of gestation (Ekelund et al., 2008). When infection is present in preterm labour, higher levels of IL-18 are found in amniotic fluid (Jacobsson et al., 2003). In animal studies, the frequency of foetal loss was significantly higher in IL-18 knock-out mice and in mice receiving IL-18 binding protein than in wild-type controls. IL-18 knock-out mice also present with elevated IL-12 expression in uterine tissues (Wang et al., 2006).

IL-10 is the most extensively studied of the anti-inflammatory cytokines. It decreases the production of pro-inflammatory cytokines such as IL-8, IL-6, TNF-α, IL-1β (Fortunato et al., 1998; Sato et al., 2003), matrix metalloproteinases (Fortunato et al., 2001) and prostaglandin E_2 (Brown et al., 2000) in LPS-stimulated foetal membranes. The ratio of IL-10/IL-8 decreases in cervical secretions with advancing gestational age (Mondestin-Sorrentino et al., 2007). IL-10 was significantly reduced in placental tissues in chorioamnionitis-associated preterm labour as well as in term labour, compared with second-trimester normal pregnancy samples obtained from elective terminations (Hanna et al., 2006). However, patients who delivered preterm without intra-amniotic infection, had a significantly higher median amniotic fluid IL-10 concentration than those who delivered at term (Gotsch et al., 2008).

IL-4 and IL-13 have been less studied in pregnancy and labour. IL-4 is higher in cervical secretions in women with normal pregnancies not in labour compared to women with preterm labour (Hollier et al., 2004). Higher anti-inflammatory/pro-inflammatory cytokine ratio in cervical secretions during early pregnancy is associated with a higher risk of subsequent spontaneous preterm birth (Simhan and Krohn, 2009). In addition, IL-4, IL-10 and IL-13 gene polymorphism is associated with preterm delivery (Annells et al., 2004; Kerk et al., 2006; Heinzmann et al., 2009).

Dubicke et al. (2010b) reported an up-regulation of mRNA of IL-10, IL-1α, IL-1β, but down-regulation of IL-12 and IL-18 in the cervix of the labouring groups compared to the non- labouring group irrespective of gestational age. IL-4 mRNA was detected more frequently in the preterm than in the term labour group. IL-13 was detected more frequently in the labouring groups. IL-12 mRNA expression was lower in the preterm labour group than in the term labour group. The protein levels of IL-10 remained the same in all the groups, IL-4 and IL-12 decreased, while IL-18 increased in the labouring groups. IL-4 protein levels were significantly higher in the preterm subgroup with bacterial infection compared to non-infected group. IL-10 had higher expression in squamous epithelium at preterm labour than at term (Fig. 5) (Dubicke et al., 2010b).

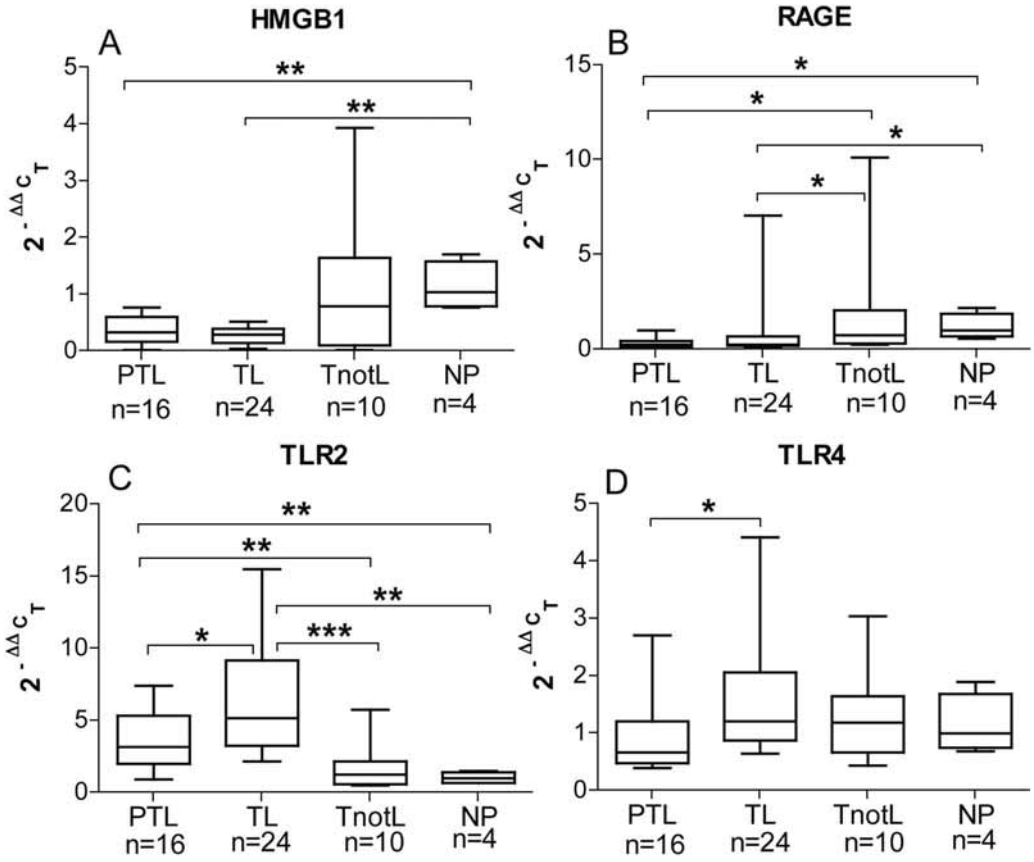

Fig. 4. — mRNA expression of HMGB1 (A), RAGE (B), TLR2 (C) and TLR4 (D) in cervical tissue. Preterm labour (PTL), term labour (TL), term not in labour (TnotL), non-pregnant (NP). The box represents 25-75% of all data with the line through the box representing the median value. The whiskers extend to the range (min/max). *p < 0.05, **p < 0.01, ***p < 0.001.

Matrix metalloproteinases (MMPs)

The MMPs have broad and diverse substrate specificity: collagenases (MMP-1, -8 and -13) break down fibrillar and non-fibrillar collagens; stromelysins (MMP-3, -7 and -10) cleave proteoglycans, fibronectin, collagens IV, V and gelatins; gelatinases (MMP-2 and -9) target collagen IV, V, elastin, proteoglycan and fibronectin (Hulboy et al., 1997). Four tissue inhibitors of MMPs (TIMPs) have been described: TIMP-1, TIMP-2, TIMP-3 and TIMP-4. The second group of MMP inhibitors are plasma α-macroglobulins (Hulboy et al., 1997). The levels of MMPs in cervix, lower uterine segment, amniotic fluid, placenta, foetal membranes and maternal plasma increase during labour (Osmers et al., 1995; Stygar et al., 2002; Sennstrom et al., 2003). Also, polymorphism in MMP-1 and MMP-9 genes is associated with PPROM (Ferrand et al., 2002).

In preterm cervical ripening MMP-1, MMP-3 and MMP-9 mRNA expression was up-regulated in labour and a tendency towards higher protein levels of MMP-8 and MMP-9 in labour has been found (Dubicke et al., 2008). A different secretion pattern at preterm and term was registered in vitro in cervical cell culture raised from cervical samples. The secretion of IL-8 and MMP-1 was significantly higher ($p < 0.001$ and $p < 0.05$, respectively), but MMP-3 secretion significantly lower in preterm cervical fibroblasts compared to term fibroblasts ($p < 0.001$) (Dubicke et al., 2008) (Fig. 2).

Preterm cervical ripening

Although it is well established that intrauterine infection can lead to preterm labour, this does not appear to be the major cause of prematurity, since infection has been demonstrated in only 25-40% of preterm births (Slattery and Morrison, 2002; Goldenberg et al., 2008). Parturition itself is an inflammatory process. Inflammatory events can be observed in the myometrium, cervix, foetal membranes and peripheral blood (Tornblom et al., 2005a; Norman et al., 2007; Challis et al., 2009). Recent studies from our group indicate that cervical ripening at both term and preterm is an inflammatory process even if no infection is present (Tornblom et al., 2004; Tornblom et al., 2005a; Dubicke et al., 2008; Dubicke et al., 2010a; Dubicke et al., 2010b). The human cervix is dominated by ECM undergoing a pronounced remodelling both during preterm and term cervical ripening. Thus preterm cervical

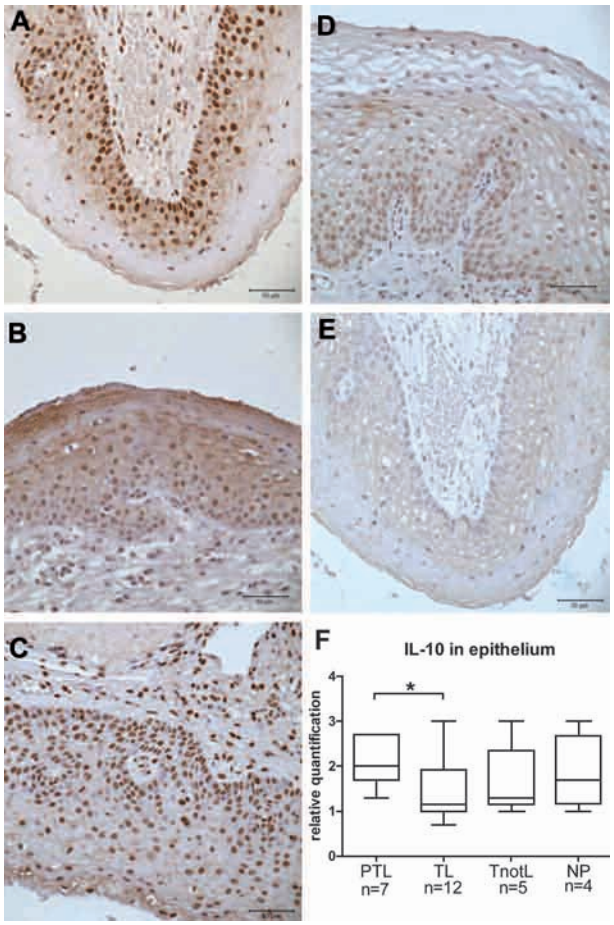

Fig. 5. — Immunohistochemical staining of IL-10 in cervical tissue.
IL-10 in the cervical epithelium at preterm labour **(A),** term labour **(B),** term not in labour **(C)** and non-pregnant state **(D)**. Negative control **(E).** Magnification ×400. The mark is 50 μm. Box and whisker plots represent the staining of IL-10 in the squamous epithelium **(F).** Preterm labour (PTL), term labour (TL), term not in labour (TnotL) and non-pregnant (NP). The box represents median value with 25%-75% of all data falling within the box. The whiskers extend to the range. The number of samples analyzed in each group is shown under the group name. A semiquantitative scale from 0 to + + + was applied. Statistically significant differences are indicated above the plot: * $p < 0.05$.

ripening seems to be a more relevant term than cervical insufficiency involved in preterm birth. The ultimate goal must be to develop new strategies to prevent preterm cervical ripening to reduce the number of spontaneous preterm births.

References

Abelin Tornblom S, Ostlund E, Granstrom L et al. Pre-term cervical ripening and labour induction. Eur J Obstet Gynecol Reprod Biol. 2002;104:120-3.

Akerud A, Dubicke A, Sennstrom M et al. Differences in heparan sulfate production in cervical fibroblast cultures from women undergoing term and preterm delivery. Acta Obstet Gynecol Scand. 2008;87:1220-8.

Andersson U, Wang H, Palmblad K et al. High mobility group 1 protein (HMG-1) stimulates proinflammatory cytokine synthesis in human monocytes. J Exp Med. 2000;192:565-70.

Annells MF, Hart PH, Mullighan CG et al. Interleukins-1, -4, -6, -10, tumor necrosis factor, transforming growth factor-beta, FAS, and mannose-binding protein C gene polymorphisms in Australian women: Risk of preterm birth. Am J Obstet Gynecol. 2004;191: 2056-67.

Bianchi ME, Manfredi AA. High-mobility group box 1 (HMGB1) protein at the crossroads between innate and adaptive immunity. Immunol Rev. 2007;220:35-46.

Brown NL, Alvi SA, Elder MG et al. The regulation of prostaglandin output from term intact fetal membranes by anti-inflammatory cytokines. Immunology. 2000;99:124-33.

Buhimschi IA, Zhao G, Pettker CM et al. The receptor for advanced glycation end products (RAGE) system in women with intraamniotic infection and inflammation. Am J Obstet Gynecol. 2007;196:181 e1-13.

Challis JR, Lockwood CJ, Myatt L et al. Inflammation and pregnancy. Reprod Sci. 2009;16:206-15.

Chwalisz K, Garfield RE. Nitric oxide as the final metabolic mediator of cervical ripening. Hum Reprod. 1998;13:245-8.

Cooke CL, Brockelsby JC, Baker PN et al. The receptor for advanced glycation end products (RAGE) is elevated in women with preeclampsia.Hypertens Pregnancy. 2003;22: 173-84.

Danforth DN. The fibrous nature of the human cervix, and its relation to the isthmic segment in gravid and nongravid uteri. Am J Obstet Gynecol. 1947;53:541-57.

Dubicke A, Akerud A, Sennstrom M et al. Different secretion patterns of matrix metalloproteinases and IL-8 and effect of corticotropin-releasing hormone in preterm and term cervical fibroblasts. Mol Hum Reprod. 2008;14:641-7.

Dubicke A, Andersson P, Fransson E et al. High-mobility group box protein 1 and its signalling receptors in human preterm and term cervix. J Reprod Immunol. 2010a;84:86-94.

Dubicke A, Fransson E, Centini G et al. Pro-inflammatory and anti-inflammatory cytokines in human preterm and term cervical ripening. J Reprod Immunol. 2010b;84:176-85.

Ekelund CK, Vogel I, Skogstrand K et al. Interleukin-18 and interleukin-12 in maternal serum and spontaneous preterm delivery. J Reprod Immunol. 2008;77:179-85.

Ekman G, Malmstrom A, Uldbjerg N et al. Cervical collagen: an important regulator of cervical function in term labor. Obstet Gynecol. 1986;67:633-6.

Ekman G, Perssen PH, Ulmsten U et al. The impact on labor induction of intracervically applied PGE2-gel, related to gestational age in patients with an unripe cervix. Acta Obstet Gynecol Scand Suppl. 1983;113:173-5.

Ferrand PE, Parry S, Sammel M et al. A polymorphism in the matrix metalloproteinase-9 promoter is associated with increased risk of preterm premature rupture of membranes in African Americans. Mol Hum Reprod. 2002;8:494-501.

Fortunato SJ, Menon R, Lombardi SJ. The effect of transforming growth factor and interleukin-10 on interleukin-8 release by human amniochorion may regulate histologic chorioamnionitis. Am J Obstet Gynecol. 1998;179:794-9.

Fortunato SJ, Menon R, Lombardi SJ et al. Interleukin-10 inhibition of gelatinases in fetal membranes: therapeutic implications in preterm premature rupture of membranes. Obstet Gynecol. 2001;98:284-8.

Gargano JW, Holzman C, Senagore P et al. Mid-pregnancy circulating cytokine levels, histologic chorioamnionitis and spontaneous preterm birth. J Reprod Immunol. 2008;79: 100-10.

Goldenberg RL, Culhane JF, Iams JD et al. Epidemiology and causes of preterm birth. Lancet. 2008;371:75-84.

Gonzalez JM, Xu H, Ofori E et al. Toll-like receptors in the uterus, cervix, and placenta: is pregnancy an immunosuppressed state? Am J Obstet Gynecol. 2007;197:296 e1-6.

Gotsch F, Romero R, Kusanovic JP et al. The anti-inflammatory limb of the immune response in preterm labor, intra-amniotic infection/inflammation, and spontaneous parturition at term: a role for interleukin-10. J Matern Fetal Neonatal Med. 2008;21:529-47.

Granstrom L, Ekman G, Ulmsten U et al. Changes in the connective tissue of corpus and cervix uteri during ripening and labour in term pregnancy. Br J Obstet Gynaecol. 1989;96: 1198-202.
Hanna N, Bonifacio L, Weinberger B et al. Evidence for interleukin-10-mediated inhibition of cyclo- oxygenase-2 expression and prostaglandin production in preterm human placenta. Am J Reprod Immunol. 2006;55:19-27.
Hassan SS, Romero R, Haddad R et al. The transcriptome of the uterine cervix before and after spontaneous term parturition. Am J Obstet Gynecol. 2006;195:778-86.
Heinzmann A, Mailaparambil B, Mingirulli N et al. Association of Interleukin-13/-4 and Toll-Like Receptor 10 with Preterm Births. Neonatology. 2009;96:175-81.
Hillhouse EW, Grammatopoulos DK. Role of stress peptides during human pregnancy and labour. Reproduction. 2002; 124:323-9.
Hillhouse EW, Grammatopoulos DK. The molecular mechanisms underlying the regulation of the biological activity of corticotropin-releasing hormone receptors: implications for physiology and pathophysiology. Endocr Rev. 2006;27:260-86.
Hollier LM, Rivera MK, Henninger E et al. T helper cell cytokine profiles in preterm labor. Am J Reprod Immunol. 2004;52:192-6.
Holmlund U, Cebers G, Dahlfors AR et al. Expression and regulation of the pattern recognition receptors Toll-like receptor-2 and Toll-like receptor-4 in the human placenta. Immunology. 2002;107:145-51
Holmlund U, Wahamaa H, Bachmayer N et al. The novel inflammatory cytokine high mobility group box protein 1 (HMGB1) is expressed by human term placenta. Immunology. 2007;122:430-7.
Hulboy DL, Rudolph LA, Matrisian LM. Matrix metalloproteinases as mediators of reproductive function. Mol Hum Reprod. 1997;3:27-45.
Kerk J, Dordelmann M, Bartels DB et al. Multiplex measurement of cytokine/receptor gene polymorphisms and interaction between interleukin-10 (-1082) genotype and chorioamnionitis in extreme preterm delivery. J Soc Gynecol Investig. 2006;13:350-6.
Klimaviciute A, Calciolari J, Bertucci E et al. Corticotropin-releasing hormone, its binding protein and receptors in human cervical tissue at preterm and term labour in comparison to non-pregnant state. Reprod Biol Endocrinol. 2006;4:29.
Konishi H, Nakatsuka M, Chekir C et al. Advanced glycation end products induce secretion of chemokines and apoptosis in human first trimester trophoblasts. Hum Reprod. 2004; 19:2156-62.
Ledingham MA, Thomson AJ, Young A et al. Changes in the expression of nitric oxide synthase in the human uterine cervix during pregnancy and parturition. Mol Hum Reprod. 2000;6:1041-8.
Li W, Challis JR. Corticotropin-releasing hormone and urocortin induce secretion of matrix metalloproteinase-9 (MMP-9) without change in tissue inhibitors of MMP-1 by cultured cells from human placenta and fetal membranes. J Clin Endocrinol Metab. 2005;90: 6569-74.
Lotze MT, Tracey KJ. High-mobility group box 1 protein (HMGB1): nuclear weapon in the immune arsenal. Nat Rev Immunol. 2005;5:331-42.
Malmstrom E, Sennstrom M, Holmberg A et al. The importance of fibroblasts in remodeling of the human uterine cervix during pregnancy and parturition. Mol Hum Reprod. 2007.
Mondestin-Sorrentino M, Smulian JC, Vintzileos AM et al. Variations in cervical IL-
10 and IL-8 concentrations throughout gestation in normal pregnancies. Am J Reprod Immunol. 2007;57:482-7.
Norman JE, Bollapragada S, Yuan M et al. Inflammatory pathways in the mechanism of parturition. BMC Pregnancy Childbirth. 2007;7 Suppl 1:S7.
Norman M, Ekman G, Malmstrom A. Changed proteoglycan metabolism in human cervix immediately after spontaneous vaginal delivery. Obstet Gynecol. 1993;81:217-23.
Norman M, Ekman G, Ulmsten U et al. Proteoglycan metabolism in the connective tissue of pregnant and non-pregnant human cervix. An in vitro study. Biochem J. 1991;275:515-20.
Osman I, Young A, Ledingham MA et al. Leukocyte density and pro-inflammatory cytokine expression in human foetal membranes, decidua, cervix and myometrium before and during labour at term. Mol Hum Reprod. 2003;9:41-5.
Osmers RG, Adelmann-Grill BC, Rath W et al. Biochemical events in cervical ripening dilatation during pregnancy and parturition. J Obstet Gynaecol. 1995;21:185-94.
Patni S, Flynn P, Wynen LP et al. An introduction to Toll-like receptors and their possible role in the initiation of labour. BJOG. 2007;114:1326-34.
Patni S, Wynen LP, Seager AL et al. Expression and activity of toll-like receptors 1-9 in the human term placenta and changes associated with labour at term. Biol Reprod. 2009; 80: 243-8.
Petraglia F, Potter E, Cameron VA et al. Corticotropin-releasing factor-binding protein is produced by human placenta and intrauterine tissues. J Clin Endocrinol Metab. 1993;77: 919-24.
Petraglia F, Tabanelli S, Galassi MC et al. Human decidua and in vitro decidualized endometrial stromal cells at term contain immunoreactive corticotropin-releasing factor (CRF) and CRF messenger ribonucleic acid. J Clin Endocrinol Metab. 1992;74:1427-31.
Pioli PA, Amiel E, Schaefer TM et al. Differential expression of Toll-like receptors 2 and 4 in tissues of the human female reproductive tract. Infect Immun. 2004;72: 5799-806.
Romero R, Espinoza J, Hassan S et al. Soluble receptor for advanced glycation end products (sRAGE) and endogenous secretory RAGE (esRAGE) in amniotic fluid: modulation by infection and inflammation. J Perinat Med. 2008;36:388-98.
Rorie DK, Newton M. Histologic and chemical studies of the smooth muscle in the human cervix and uterus. Am J Obstet Gynecol.1967;99:466-9.
Sato TA, Keelan JA, Mitchell MD. Critical paracrine interactions between TNF-
alpha and IL-10 regulate lipopolysaccharide-stimulated human choriodecidual cytokine and prostaglandin E2 production. J Immunol. 2003;170:158-66.
Sehringer B, Zahradnik HP, Simon M et al. mRNA expression profiles for corticotrophin-releasing hormone, urocortin, CRH-binding protein and CRH receptors in human term gestational tissues determined by real-time quantitative RT-PCR. J Mol Endocrinol. 2004;32:339-48.
Sennstrom MB, Brauner A, Bystrom B et al. Matrix metalloproteinase-8 correlates with the cervical ripening process in humans. Acta Obstet Gynecol Scand. 2003;82:904-11.
Sennstrom MB, Ekman G, Westergren-Thorsson G et al. Human cervical ripening, an inflammatory process mediated by cytokines. Mol Hum Reprod. 2000;6:375-81.
Simhan HN, Krohn MA. First-trimester cervical inflammatory milieu and subsequent early preterm birth. Am J Obstet Gynecol. 2009;200(4):377.e1-4.
Slattery MM, Morrison JJ. Preterm delivery. Lancet. 2002; 360:1489-97.
Stjernholm Y, Sahlin L, Akerberg S et al. Cervical ripening in humans: potential roles of estrogen, progesterone, and insulin-like growth factor-I. Am J Obstet Gynecol. 1996;174: 1065-71.
Stjernholm Y, Sahlin L, Malmstrom A et al. Potential roles for gonadal steroids and insulin-like growth factor I during final cervical ripening. Obstet Gynecol. 1997;90:375-80.
Stygar D, Wang H, Vladic YS et al. Co-localization of oestrogen receptor beta and leukocyte markers in the human cervix. Mol Hum Reprod. 2001;7:881-6.

Stygar D, Wang H, Vladic YS et al. Increased level of matrix metalloproteinases 2 and 9 in the ripening process of the human cervix. Biol Reprod. 2002;67:889-94.
Thomson AJ, Lunan CB, Cameron AD et al. Nitric oxide donors induce ripening of the human uterine cervix: a randomised controlled trial. BJOG. 1997;104: 1054-7.
Ticconi C, Zicari A, Belmonte A et al. Pregnancy-promoting actions of HCG in human myometrium and fetal membranes. Placenta. 2007;28:137-43.
Tornblom SA, Klimaviciute A, Bystrom B et al. Non-infected preterm parturition is related to increased concentrations of IL-6, IL-8 and MCP-1 in human cervix. Reprod Biol Endocrinol. 2005a;3:39.
Tornblom SA, Maul H, Klimaviciute A et al. mRNA expression and localization of bNOS, eNOS and iNOS in human cervix at preterm and term labour. Reprod Biol Endocrinol. 2005b;3:33.
Tornblom SA, Patel FA, Bystrom B et al. 15-hydroxyprostaglandin dehydrogenase and cyclooxygenase 2 messenger ribonucleic acid expression and immunohistochemical localization in human cervical tissue during term and preterm labor. J Clin Endocrinol Metab. 2004;89:2909-15.
Uldbjerg N, Ekman G, Malmstrom A et al. Ripening of the human uterine cervix related to changes in collagen, glycosaminoglycans, and collagenolytic activity. Am J Obstet Gynecol. 1983a;147:662-6.
Uldbjerg N, Malmstrom A, Ekman G et al. Isolation and characterization of dermatan sulphate proteoglycan from human uterine cervix. Biochem J. 1983b;209:497-503.
Vaisanen-Tommiska M, Nuutila M, Aittomaki K et al. Nitric oxide metabolites in cervical fluid during pregnancy: further evidence for the role of cervical nitric oxide in cervical ripening. Am J Obstet Gynecol. 2003;188:779-85.
Wang H, Bloom O, Zhang M et al. HMG-1 as a late mediator of endotoxin lethality in mice. Science. 1999;285:248-51.
Wang H, Stjernholm Y, Ekman G et al. Different regulation of oestrogen receptors alpha and beta in the human cervix at term pregnancy. Mol Hum Reprod. 2001;7:293-300.
Wang W, Nan X, Ji P et al. Corticotropin releasing hormone modulates endotoxin-induced inflammatory cytokine expression in human trophoblast cells. Placenta. 2007;28: 1032-8.
Wang X, Hagberg H, Mallard C et al. Disruption of interleukin-18, but not interleukin-1, increases vulnerability to preterm delivery and fetal mortality after intrauterine inflammation. Am J Pathol. 2006;169:967-76.
Westergren-Thorsson G, Norman M, Bjornsson S et al. Differential expressions of mRNA for proteoglycans, collagens and transforming growth factor-beta in the human cervix during pregnancy and involution. Biochim Biophys Acta. 1998;1406:203-13.
Wu YJ, La Pierre DP, Wu J et al. The interaction of versican with its binding partners. Cell Res. 2005;15:483-94.
Young A, Thomson AJ, Ledingham M et al. Immunolocalization of proinflammatory cytokines in myometrium, cervix, and fetal membranes during human parturition at term. Biol Reprod. 2002;66:445-9.

Progesterone Action in the Myometrium and Decidua in Preterm Birth

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Abstract

Progesterone is central to many reproductive processes and is critical in regulating the menstrual cycle and maintaining pregnancy. We discuss here similarities in the molecular mechanisms that regulate the process of decidualisation in endometrial stromal cells and uterine quiescence in myometrial smooth muscle cells. We discuss recent evidence that the decidua may be an important mediator of progesterone actions in the onset of labor in mammalian species lacking progesterone withdrawal. These observations have relevance to recent clinical observations of the effect of progesterone administration in the prevention of preterm labor. We suggest that further research is required to understand the role of progesterone in maintaining the decidua in late pregnancy and particular focus should be given to the mechanisms that increase prostaglandin production in the uterus at term.

Key words: **Decidua, myometrium, parturition, preterm labor, progesterone, uterus.**

Introduction

Progesterone (P4) exerts a broad spectrum of physiological actions in the cardiovascular and respiratory systems, kidney, adipose tissue, bone, testis and the brain (Graham and Clarke, 1997; Gellersen et al., 2009; 2010). The primary target, however, is the female reproductive tract where P4 has facilitatory roles in modulating the contractile waves of the junctional myometrial zone, tubal transport, and cervical secretion. These processes are superseded by indispensible P4 functions in follicular growth, ovulation and luteinization, embryo implantation, decidualization and maintenance of pregnancy during gestation. We summarise here the actions of P4 in the gravid uterus, focusing on common mechanisms that operate in the myometrium and decidua that may be relevant to term and preterm labor.

Progesterone and decidualisation

During the menstrual cycle, the luminal epithelium and underlying endometrial stroma undergo substantial transformation that renders the uterus receptive to embryo implantation (Dey et al., 2004; Brosens et al., 2009). This transformation is a highly coordinated and sequential response to the postovulatory rise in P4 levels, commencing with arrest of estrogen-dependent epithelial cell proliferation, followed by the secretory transformation of the glands, recruitment of various bone marrow-derived immune cells, and angiogenesis (Brosens et al., 1999; Gellersen and Brosens 2003; Gellersen et al., 2007). The actions of P4 are primarily mediated by differentiating stromal cells (Simon et al., 2009). This process, termed 'decidualization', is characterized by a mesenchymal-epithelial transition that transforms endometrial stromal cells into specialized secretory decidual cells (Dey et al., 2004; Gellersen et al., 2007; Cloke et al., 2008). Once decidualized the endometrium relies on a constant supply of P4 to maintain the integrity of the tissue. In the absence of successful implantation, the corpus luteum involutes and declining P4 levels trigger a switch in the secretory phenotype of the decidualizing stroma. This change in phenotype entails release of pro-inflammatory cytokines, chemokines and matrix metalloproteinases, leading to breakdown of the superficial endometrial layer, focal bleeding and menstrual shedding (Marbaix et al., 1995; Kokorine

et al., 1996; Brosens and Gellersen, 2006; Brosens et al., 2009; Brun et al., 2009; Gaide Chevronnay et al., 2009). In addition to the ability to undergo apoptosis upon P4 withdrawal, decidualized stromal cells display a number of unique properties commensurate with their function to safeguard the early conceptus, including resistance to oxidative stress induced cell death, the ability to regulate local immune responses and to coordinate trophoblast invasion (Labied et al., 2006; Gellersen et al., 2010; Leitao et al., 2010; 2011).

Endometrial responses to P4 are primarily transduced through binding to, and activation of, the nuclear receptors PR-A and -B, members of the superfamily of ligand-activated transcription factors (Misrahi et al., 1987). In addition to the primary genomic response, it is recognised that there are more rapid short-term actions of P4 that are independent of the transcriptional machinery. In contrast to genomic mechanisms the precise non-genomic actions of P4 are not well defined and are reviewed in detail elsewhere (Gellersen et al., 2009).

The nuclear receptors PR-A and PR-B are members of the nuclear steroid receptor family that share structure with the estrogen, androgen, glucocorticoid and mineralocorticoid receptors. This class of transcription factors has a modular structure of distinct functional domains that can be swapped experimentally without significant loss of function (Kastner et al., 1990; Brosens et al., 2004). PR-A and -B are transcribed from the same gene on chromosome 11 by alternative promoter usage (Kastner et al., 1990). The resultant transcripts generate two proteins that differ in size with PR-B containing an additional 164 amino acids at the amino terminus. Close analysis of the PR gene has revealed the potential for multiple protein products generated by alternative transcription, translation, or splicing. There has been speculation about the functional relevance of alternative transcripts such as PR-C, PR-M and PR-S, although the existence and physiological relevance of these isoforms remain controversial (Samalecos and Gellersen, 2008).

While the DNA and hormone binding affinities of PR-A and -B are indistinguishable, their transcriptional actions are remarkably divergent. Early experiments on reporter constructs of simple or complex progesterone response elements (PREs), suggested that PR-A displays very little intrinsic transcriptional activity and acts primarily as a dominant inhibitor of PR-B and other steroid hormone receptors (Vegeto et al., 1993). It is now clear that PR-A and PR-B govern distinct networks of target genes in a cell-specific context (Richer et al., 2002). Unequivocal support for this notion came from selective gene knockout studies in mice, demonstrating that PR-A is indispensible for ovarian and uterine functions whilst PR-B is obligatory for mammary gland development (Conneely et al., 2002; Mulac-Jericevic et al., 2003). Thus PR-A is likely to be the dominant receptor isoform in both endometrium and myometrium.

Ligand binding is thought to occur at PRs anchored in cytoplasmic multi-subunit protein complexes consisting of variable heat shock proteins (p23, HSP70, HSP40 and HSP90) and the immuophilins FKBP51 and FKB52 (Kosano et al., 1998; Tranguch et al., 2007). The assembly of PR with these macromolecular complexes plays a key role in both the dynamic trafficking of the receptor and the maintenance of an active pool of protein ready to receive freely diffusing P4 from the circulating plasma binding protein transcortin. Once bound to P4, the subsequent conformational change in PR promotes dissociation from the chaperone scaffold, followed by homologous dimerization prior to translocation to the nucleus. In the nucleus, activated PR binds to specific nucleotide recognition sequences in promoters of target genes, leading to recruitment of chromatin-modifying co-repressor or -activator complexes, and finally transcriptional repression or activation, respectively (Brosens et al., 2004).

This 'classical' model of action predicts that response to P4 signaling should be proportional to the cellular abundance of PR and associated binding proteins. Evidence in endometrial cells during the decidualization process demonstrates that this is emphatically not the case. Despite the presence of abundant PR in primary endometrial cells, exposure to P4 triggers the expression of few, if any, genes (Aghajanova et al., 2011). Although initially difficult to reconcile with the fact that decidualization is a P4 dependent process *in vivo*, subsequent experiments demonstrated that sustained activation of the protein kinase A (PKA) pathway is required to sensitize endometrial cells to P4 (Brosens et al., 1999; Gellersen and Brosens, 2003; Jones et al., 2006). The endometrial response to rising P4 during the secretory phase of the cycle is therefore preceded by the rising intracellular cAMP levels and PKA activation, which in turn induces a diverse array of transcription factors including C/EBPα, STAT5 and FOXO1 (Gellersen and Brosens, 2003). It seems likely that the recruitment of these transcription factors into the PR dependent transcriptional complex is necessary for the classical P4 decidualisation response. Importantly, because cAMP levels are under the control of exogenous factors such as prostaglandin E2, corticotropin releasing factor and relaxin, the P4 response in decidualizing endometrium is both cell and environment specific.

As previously alluded to, nuclear receptors such as PR do not have the intrinsic ability to modify chromatin structure to allow access of the transcriptional machinery to DNA (Lonard and O'Malley, 2006; Han et al., 2009; Thakur and Paramanik, 2009). The required modifications are made by recruitment of co-regulators that possess histone and DNA modifying activity. The number of known co-regulator proteins, which can either promote or inhibit transcription now exceeds 300 (Onate et al., 1995; Thakur and Paramanik, 2009), and their defined roles in regulating PR transcription in the endometrium requires further investigation.

Once formed, assembled transcription factor complexes containing PR are capable of initiating gene transcription at other, sequence-specific, transcription factor sites. Such cross-talk is critical to the cAMP dependent decidualisation response and is dependent on the binding partners p53, FOXO1, HOXA10, HOXA11, STAT5 and C/EBPβ (Christian et al., 2002a; 2002b; 2002c; Mak et al., 2002; Pohnke et al., 2004; Schneider-Merck et al., 2006; Lynch et al., 2009; Christian et al., 2011). Since PR-A is essential for decidualization it seems likely that it acts as a critical scaffold protein upon which transcription factor complexes are assembled to transcribe a cohort of decidua specific genes. Such complexes are not limited to genes containing PR binding elements (PREs) or to ligand bound PR. The PR has been demonstrated to modulate Activator Protein 1 (AP1), Nuclear Factor-KappaB (NF-κB) and Specificity Protein 1 (SP1) transcriptional activity thus expanding the transcriptional network beyond PRE containing genes (Bamberger et al., 1996; Kalkhoven et al., 1996; Owen et al., 1998) and independent of P4 (Cloke et al., 2008).

A final layer of complexity in PR signalling in endometrium comes from posttranslational modifications of the receptor. These modifications (phosphorylation, sumoylation, ubiquitination, and acetylation) are rapid and dynamic and provide a means to fine tune PR signalling in the context of complex environmental signals (Brosens et al., 1999; Lange et al., 2000; Abdel-Hafiz et al., 2002; Jones et al., 2006; Daniel et al., 2010; Leitao et al., 2010). The consequences of modification are many-fold and can involve changes in sub-cellular localisation, protein stability, targeted degradation in the proteasome, altered interactions with co-factors and/or target gene expression. A well-defined example of a physiologically important modification in the reproductive tract is the suppressive effect of sumoylation on transcriptional activity of PR-A (Jones et al., 2006). This sumoylation dependent suppression is potently stimulated by oxidative stress in endometrial cells, but is selectively disabled during the process of decidualisation, thus emphasising the context dependence of posttranslational modifications (Leitao et al., 2010; 2011).

Much of the evidence of PR action in the decidua is provided from studies on decidualisation during the menstrual cycle, implantation and early support for pregnancy prior to establishing the placenta. Relatively less well studied is the role of decidua during late pregnancy and in particular potential roles for the decidua in initiating parturition.

The role of the decidua in the onset of labor

There is abundant evidence from different species that the decidua is a major source of prostaglandins at term (Keelan et al., 2003; Olson, 2003). Consequently, the decidua may play a role in determining the length of gestation. Recent genetic studies in mice demonstrated that increased decidual prostanoid production precipitates preterm labor in the absence of P4 withdrawal, a prerequisite for term parturition in this species. Interestingly this effect could be triggered by genetic alteration of two distinct pathways. Uterine-specific deletion of p53 was sufficient to induce decidual senescence, increased Akt signalling, prostaglandin-endoperoxide synthase 2 (Ptgs2), prostaglandin F synthase and consequently greater production of the uterotonin PGF2α (Hirota et al., 2010). This effect is mediated by mammalian target of rapamycin complex 1 (mTORC1) and is reversed by low doses of the mTORC1 inhibitor rapamycin (Hirota et al., 2011). The same phenotype (*i.e.* preterm birth in the absence of progesterone withdrawal) is induced in mice hypomorphic for the prostaglandin-degrading enzyme 15-hydroxyprostaglandin dehydrogenase (15-HPGD) (Roizen et al., 2008). Thus, increased prostanoid synthesis, or decreased degradation, in the decidua is sufficient to trigger preterm birth in a species normally reliant on progesterone withdrawal for parturition. This effect can be replicated upon administration of other agonists, such as oxytocin (OT), in either wild-type or OT-deficient mice where the threshold for stimulus is lower in KO mice (Imamura et al., 2000).

These observations suggest that a complex relationship between uterine sensitivity and the magnitude of stimulation determines the timing of labor. It is clear that P4 regulates uterine sensitivity to stimulation (see infra) but it is much less obvious if it also regulates the level of stimulation *per se*. (Roizen et al., 2008). The question that arises is how this change in sensitivity and stimulation is achieved in species such as humans that do not depend on falling P4 levels to initiate labor. There is a set of experimental observations that potentially shed light

on the role of PR in modulating these thresholds in different species.

There is a clear difference in the efficacy of PR antagonists to induce labor between species that normally deliver in the presence of high P4 and those that exhibit systemic P4 withdrawal. In the latter, administration of RU486 (mifepristone), a mixed PR/GR antagonist, is sufficient to trigger preterm parturition (Chwalisz, 1994). This contrasts to species that normally deliver in the presence of high circulating P4 levels. Administration of RU486 here leads to an increased uterine sensitivity and cervical ripening but induction of labor requires co-administration of oxytocics (e.g. oxytocin or prostaglandins). In contrast, the pure PR antagonist onapristone precipitates labor without the need for oxytocics, although only when administered during mid-to-late gestation but not earlier (Elger et al., 1986; 1987). The effect of onapristone is PR-specific and reversible by co-administration of the PR agonists R5020 or gestodene (Chwalisz et al., 1995). The reason for the discrepancy in the actions of onapristone and RU486 is not understood but may be related to the high cAMP/PKA activity in the decidua, which converts RU486 into a partial PR agonist (Nordeen et al., 1993). If so, these observations suggest that the gestation-dependent increase in oxytocic drive may emanate from the decidua/fetal membranes in species lacking systemic P4 withdrawal. For example, it is possible that decidual senescence, associated with increased prostaglandins production and/or loss of prostaglandin dehydrogenase activity, could be a predetermined process that is timed relative to the implantation process.

The myometrium and onset of labor

The majority of gestation is characterized by a dominance of uterine quiescence, whereby the growing fetus develops in a safe uterine environment until a point sufficient for extra-uterine survival. It is generally accepted that prior to the onset of labor the myometrium undergoes a process of 'activation' (Challis et al., 2000) whereby the muscle becomes more electrically excitable and susceptible to stimulation by pro-contractile hormones. This process is mediated by the increase in expression of certain contraction associated protein (CAP) genes (e.g. oxytocin receptor (OXTR), prostaglandin endoperoxidase synthase 2 (PTGS2), connexin 43 (GJA1) *etc*.), concomitant changes is resting membrane potential (Parkington, Tonta et al. 1999), and a decrease in cAMP/PKA activity (Dodge et al., 1999).

Cumulative evidence from different mammalian species indicates that only some labor-associated myometrial changes are mediated directly by P4. As mentioned, administration of RU486 or onapristone leads to increased myometrial responsiveness in all species tested so far, irrespective of the time in gestation (Chwalisz and Garfield, 1994). The observed increase in uterine responsiveness occurs for both OT and prostaglandins and is not mediated by an increase is receptor number (Elger et al., 1986; Chwalisz et al., 1991; Chwalisz, 1994). The fact that increased uterine responsiveness prior to parturition is not accounted for by an increase in either ligand or receptor suggests that a more fundamental change in electrophysiological properties of the myometrium may underpin this phenomenon.

The central process that governs uterine contractions is the generation of electrical activity in the form of complex action potentials that mediate voltage-gated calcium entry and hence contractions (Blanks et al., 2007). The spread of electrical activity throughout the uterine smooth muscle is critically dependent on the formation of electrical synapses by gap junction proteins between cells (Garfield et al., 1977; 1978; 1988). An increase in cell coupling would render the uterus much more sensitive to stimulation by dramatically increasing the efficacy of oxytocics to trigger membrane depolarization (Blanks et al., 2007). This is certainly true for OT. While the uterus is sensitive to picomolar concentrations of OT *in vivo*, the binding affinity of OT for its receptor is much higher (1nM) (Blanks, 2003). Thus, coupling intracellular calcium release to a tissue level action potential and voltage gated calcium entry enables OT to elicit a full agonist response with comparatively low receptor occupancy. Consistent with this hypothesis, administration of anti-progestins in rats and guinea pigs dramatically increases gap junction proteins at the plasma membrane of uterine myocytes (Garfield et al., 1987; Chwalisz et al., 1991). In addition to gap junction proteins, PR regulates the expression of the main pore forming subunit of the voltage-gated L-type calcium channel, further intimating that P4 modulates uterine excitability (Chwalisz et al., 1995).

The observation that most mammalian species initiate parturition in response to falling circulating progesterone levels combined with the fact that PR antagonists universally increase myometrial responsiveness to uterotonics underpin the widely held view that local P4 withdrawal must trigger the onset of labor in humans. In fact, numerous mechanisms of local P4 withdrawal have been proposed, focusing either on modulation of PR function, P4 metabolism, and/or P4-dependent suppression of inflammation (Mendelson, 2009; Mesiano et al., 2011). None of these mechanisms are necessarily mutually exclusive and yet - in our view –conclusive proof that local P4

withdrawal is obligatory for labor is as yet lacking. It is indeed striking that P4 therapy is effective in the prevention of preterm labor in some but not all women (da Fonseca et al., 2003; Meis et al., 2003; Fonseca et al., 2007).

A popular concept is that a change in the ratio of PR isoforms accounts for local P4 withdrawal in the myometrium. This is based on the observation that myometrial biopsies taken during labor express relatively more PR-A than PR-B when compared to samples obtained prior to labor (Merlino et al., 2007). Further, an increase in PR-A/PR-B ratio in an immortalized myometrial cell line has been shown to activate pro-inflammatory genes (Tan et al., 2012). While attractive, the *in vivo* relevance of these observations remains difficult to test as a change in PR-A/B ratio cannot underpin labor in mice (the usual *in vivo* model) as gestation and parturition are unperturbed upon PR-B silencing. The hypothesis is pertinent to those species that do not exhibit systemic P4 withdrawal, although the model fails to explain the requirement for oxytocics to induce labor upon treatment with PR antagonists.

A related hypothesis is predicated on the observation that P4 and inflammatory signalling pathways are closely intertwined and converge on the reciprocal inhibitory interaction between PR and the NF-κB transcription factor complex. For example, P4 has been shown to inhibit binding of the NF-κB-p65 complex to response elements in the *PTGS2* promoter (Hardy et al., 2006), a process that may be mediated through physical interaction between p65 and the activated PR (Kalkhoven et al., 1996). P4 also stimulates the expression of the binding protein IκBα responsible for maintaining NF-κB in a transcriptionally inactive state in the cytosol (Hardy et al., 2006). This model assumes that the inhibitory effects of P4 are overridden in response to increased NF-κB activation, which in turn establishes a positive feedback mechanism by decreasing P4-mediated repression. In support of this notion, increased NF-κB activity has been shown to decrease the expression of PR co-activators, thus diminishing receptor activity (Condon et al., 2003). However, a recent study using primary human myocytes indicated that NF-κB activation interferes only with the ability of the PR to activate but not repress target genes (Lee et al., 2012). Further, and in contrast to observations in an immortalized cell line, P4 did not inhibit the inflammatory response in primary cultures.

A final proposed pathway for P4 withdrawal, which may be complimentary to the mechanisms proposed for PR, is a local metabolism of P4. The onset of labor in mice is associated with striking non-labor phenotypes in knockouts of the P4 metabolizing enzyme 20α-hydroxysteroid dehydrogenase (20α-HSD) and 5α-reductase type 1 (Mahendroo et al., 1996; 1999; Piekorz et al., 2005; Ishida et al., 2007). In a species that normally experiences systemic P4 withdrawal these interesting phenotypes suggest that P4 clearance from uterine tissues is also important. Interestingly, recent evidence suggests that 20α-HSD may be regulated in the uterus by STAT5b, which itself is under the regulation of miR200a(Williams et al., 2012). Furthermore, miR-200a is up regulated at term in mice and humans and is also capable of regulating the E-box binding homeobox proteins ZEB1 and ZEB2 (Renthal et al., 2010). These transcription factors also regulate the oxytocin receptor (OXTR) and connexin-43 (CX43) in a PR dependent manner. Thus, P4 metabolism and PR transcriptional activity may be co-regulated to create a concerted alteration in the P4 response.

Perspective

It is clear that there is much work still to be done before we can establish exact mechanisms of P4 and PR action in the myometrium throughout gestation and prior to parturition. Of particular importance is the need to reconcile data obtained from various *in vitro* systems and cell lines with *in vivo* observations, for example in response to PR antagonists. Furthermore, the role of PR action in late gestation in the decidua requires greater focus as relatively little is known compared to our understanding of the role of this nuclear receptor during menstrual cycle. It seems highly probable that a focus on the juxtacrine interactions between uterine compartments may yield a better understanding of the role of P4 in both term and preterm labor.

References

Abdel-Hafiz H, Takimoto GS, Tung L et al. The inhibitory function in human progesterone receptor N termini binds SUMO-1 protein to regulate autoinhibition and transrepression. J Biol Chem. 2002; 277: 33950-6.

Aghajanova L, Tatsumi K, Horcajadas JA et al. Unique transcriptome, pathways, and networks in the human endometrial fibroblast response to progesterone in endometriosis. Biol Reprod. 2011; 84: 801-15.

Bamberger AM, Bamberger CM, Gellersen B et al. Modulation of AP-1 activity by the human progesterone receptor in endometrial adenocarcinoma cells. Proc Natl Acad Sci USA. 1996; 93: 6169-74.

Blanks A. The role of oxytocin in parturition. BJOG. 2003; 110: 46-51.

Blanks AM, Shmygol A, Thornton S. Preterm labour. Myometrial function in prematurity. Best Pract Res Clin Obstet Gynaecol. 2007; 21: 807-19.

Blanks AM, Shmygol A, Thornton S. Regulation of oxytocin receptors and oxytocin receptor signaling. Semin Reprod Med. 2007; 25: 52-9.

Brosens JJ, Gellersen B. Death or survival – progesterone-dependent cell fate decisions in the human endometrial stroma. J Mol Endocrinol. 2006; 36: 389-98.

Brosens JJ, Hayashi N, White JO. Progesterone receptor regulates decidual prolactin expression in differentiating human endometrial stromal cells. Endocrinol. 1999; 140: 4809-20.
Brosens JJ, Hodgetts A, Feroze-Zaidi F et al. Proteomic analysis of endometrium from fertile and infertile patients suggests a role for apolipoprotein A-I in embryo implantation failure and endometriosis. Mol Hum Reprod. 2010; 16: 273-85.
Brosens JJ, Tullet J, Varshochi R et al. Steroid receptor action. Best Prac Res Clin Obstet & Gynaecol. 2004; 18: 265-83.
Brosens JJ, Wilson MS, Lam EW. FOXO transcription factors: from cell fate decisions to regulation of human female reproduction. Adv Exp Med Biol. 2009; 665: 227-41.
Brun JL, Galant C, Delvaux D et al. Menstrual activity of matrix metalloproteinases is decreased in endometrium regenerating after thermal ablation. Hum Reprod. 2009; 24: 333-40.
Challis JRG, Matthews SG, Gibb W et al. Endocrine and paracrine regulation of birth at term and preterm. Endocr Rev. 2000; 21: 514-50.
Christian M, Lam EW, Wilson MS et al. FOXO transcription factors and their role in disorders of the female reproductive tract. Curr Drug Targets. 2011; 12: 1291-302.
Christian M, Mak I, White JO et al. Mechanisms of decidualization. Reprod Biomed Online. 2002a; 4 Suppl 3: 24-30.
Christian M, Pohnke Y, Kempf R et al. Functional association of PR and CCAAT/enhancer-binding protein beta isoforms: promoter-dependent cooperation between PR-B and liver-enriched inhibitory protein, or liver-enriched activatory protein and PR-A in human endometrial stromal cells. Mol Endocrinol. 2002b; 16: 141-54.
Christian M, Zhang X, Schneider-Merck T et al. Cyclic AMP-induced forkhead transcription factor, FKHR, cooperates with CCAAT/enhancer-binding protein beta in differentiating human endometrial stromal cells. J Biol Chem. 2002c; 277: 20825-32.
Chwalisz K. The use of progesterone antagonists for cervical ripening and as an adjunct to labour and delivery. Human Reprod. 1994; 9 Suppl 1: 131-61.
Chwalisz K, Fahrenholz F, Hackenberg M et al. The progesterone antagonist onapristone increases the effectiveness of oxytocin to produce delivery without changing the myometrial oxytocin receptor concentrations. Am J Obstet Gynecol. 1991; 165: 1760-70.
Chwalisz K, Garfield RE. Antiprogestins in the induction of labor. Ann New York Acad Sci. 1994; 734: 387-413.
Chwalisz K, Stockemann K, Fuhrmann U et al. Mechanism of action of antiprogestins in the pregnant uterus. Ann New York Acad Sci. 1995; 761: 202-23.
Cloke B, Huhtinen K, Fusi L et al. The androgen and progesterone receptors regulate distinct gene networks and cellular functions in decidualizing endometrium. Endocrinol. 2008; 149: 4462-74.
Condon JC, Jeyasuria P, Faust JM et al. A decline in the levels of progesterone receptor coactivators in the pregnant uterus at term may antagonize progesterone receptor function and contribute to the initiation of parturition. Proc Natl Acad Sci USA. 2003; 100: 9518-23.
Conneely OM, Mulac-Jericevic B, DeMayo F et al. Reproductive functions of progesterone receptors. Recent Prog Horm Res. 2002; 57: 339-55.
da Fonseca EB, Bittar RE, Carvalho MH et al. Prophylactic administration of progesterone by vaginal suppository to reduce the incidence of spontaneous preterm birth in women at increased risk: a randomized placebo-controlled double-blind study. Am J Obstet and Gynecol. 2003; 188: 419-24.
Daniel AR, Gaviglio AL, Czaplicki LM et al. The progesterone receptor hinge region regulates the kinetics of transcriptional responses through acetylation, phosphorylation, and nuclear retention. Mol Endocrinol. 2010; 24: 2126-38.
Dey SK, Lim H, Das SK et al. Molecular cues to implantation. Endocr Rev. 2004; 25: 341-73.
Dodge KL, Carr DW, Yue C et al. A role for AKAP (A kinase anchoring protein) scaffolding in the loss of a cyclic adenosine 3',5'-monophosphate inhibitory response in late pregnant rat myometrium. Mol Endocrinol. 1999; 13: 1977-87.
Elger W, Beier S, Chwalisz K et al. Studies on the mechanisms of action of progesterone antagonists. J Steroid Biochem. 1986; 25: 835-45.
Elger W, Fahnrich M, Beier S et al. Endometrial and myometrial effects of progesterone antagonists in pregnant guinea pigs. Am J Obstet Gynecol. 1987; 157: 1065-74.
Fonseca EB, Celik E, Parra M et al. Progesterone and the risk of preterm birth among women with a short cervix. N Engl J Med. 2007; 357: 462-9.
Gaide Chevronnay HP, Galant C, Lemoine P et al. Spatiotemporal coupling of focal extracellular matrix degradation and reconstruction in the menstrual human endometrium. Endocrinol. 2009; 150: 5094-105.
Garfield RE, Blennerhassett MG, Miller SM. Control of myometrial contractility: role and regulation of gap junctions. Oxf Rev Reprod Biol. 1988; 10: 436-90.
Garfield RE, Gasc JM, Baulieu EE. Effects of the antiprogesterone RU 486 on preterm birth in the rat. Am J Obstet Gynecol. 1987; 157: 1281-5.
Garfield RE, Sims S, Daniel EE. Gap junctions: their presence and necessity in myometrium during parturition. Science. 1977; 198: 958-60.
Garfield RE, Sims SM, Kannan MS et al. Possible role of gap junctions in activation of myometrium during parturition. Am J Physiol. 1978; 235: C168-79.
Gellersen B, Brosens IA, Brosens JJ. Decidualization of the human endometrium: mechanisms, functions, and clinical perspectives. Semin Reprod Med. 2007; 25: 445-53.
Gellersen B, Brosens J. Cyclic AMP and progesterone receptor cross-talk in human endometrium: a decidualizing affair. J Endocrinol. 2003; 178: 357-72.
Gellersen B, Fernandes MS, Brosens JJ. Non-genomic progesterone actions in female reproduction. Hum Reprod Update. 2009; 15: 119-38.
Gellersen B, Reimann K, Samalecos A et al. Invasiveness of human endometrial stromal cells is promoted by decidualization and by trophoblast-derived signals. Hum Reprod. 2010; 25: 862-73.
Graham JD, Clarke CL. Physiological action of progesterone in target tissues. Endocr Rev. 1997; 18: 502-19.
Han SJ, Lonard DM, O'Malley BW. Multi-modulation of nuclear receptor coactivators through posttranslational modifications. Trends Endocrinol Metab. 2009; 20: 8-15.
Hardy DB, Janowski BA, Corey DR et al. Progesterone receptor plays a major antiinflammatory role in human myometrial cells by antagonism of nuclear factor-kappaB activation of cyclooxygenase 2 expression. Mol Endocrinol. 2006; 20: 2724-33.
Hirota Y, Cha J, Yoshie M et al. Heightened uterine mammalian target of rapamycin complex 1 (mTORC1) signaling provokes preterm birth in mice. Proc Natl Acad Sci USA. 2011; 108: 18073-8.
Hirota Y, Daikoku T, Tranguch S et al. Uterine-specific p53 deficiency confers premature uterine senescence and promotes preterm birth in mice. The J Clin Invest. 2010; 120: 803-15.
Imamura T, Luedke CE, Vogt SK et al. Oxytocin modulates the onset of murine parturition by competing ovarian and uterine effects. Am J Physiol Regul Integr Comp Physiol. 2000; 279: R1061-7.
Ishida M, Choi JH, Hirabayashi K et al. Reproductive phenotypes in mice with targeted disruption of the 20alpha-hydroxysteroid dehydrogenase gene. J Reprod Dev. 2007; 53: 499-508.
Jones MC, Fusi L, Higham JH et al. Regulation of the SUMO pathway sensitizes differentiating human endometrial stromal cells to progesterone. Proc Natl Acad Sci USA. 2006; 103: 16272-7.
Kalkhoven E, Wissink S, van der Saag PT et al. Negative interaction between the RelA(p65) subunit of NF-kappaB and the progesterone receptor. J Biol Chem. 1996; 271: 6217-24.

Kastner P, Krust A, Turcotte B et al. Two distinct estrogen-regulated promoters generate transcripts encoding the two functionally different human progesterone receptor forms A and B. EMBO J. 1990; 9: 1603-14.
Keelan JA, Blumenstein M, Helliwell RJ et al. Cytokines, prostaglandins and parturition--a review. Placenta. 2003; 24 Suppl A: S33-46.
Kokorine I, Marbaix E, Henriet P et al. Focal cellular origin and regulation of interstitial collagenase (matrix metalloproteinase-1) are related to menstrual breakdown in the human endometrium. J Cell Sci. 1996; 109 (Pt 8): 2151-60.
Kosano H, Stensgard B, Charlesworth MC et al. The assembly of progesterone receptor-hsp90 complexes using purified proteins. J Biol Chem. 1998; 273: 32973-9.
Labied S, Kajihara T, Madureira PA et al. Progestins regulate the expression and activity of the forkhead transcription factor FOXO1 in differentiating human endometrium. Mol Endocrinol. 2006; 20: 35-44.
Lange CA, Shen T, Horwitz KB. Phosphorylation of human progesterone receptors at serine-294 by mitogen-activated protein kinase signals their degradation by the 26S proteasome. Proc Natl Acad Sci USA. 2000; 97: 1032-7.
Lee Y, Sooranna SR, Terzidou V et al. Interactions between inflammatory signals and the progesterone receptor in regulating gene expression in pregnant human uterine myocytes. J Cell Mol Med. 2012; doi: 10.1111/j.1582-4934.2012.01567.x.
Leitao B, Jones MC, Fusi L et al. Silencing of the JNK pathway maintains progesterone receptor activity in decidualizing human endometrial stromal cells exposed to oxidative stress signals. FASEB. 2010; 24: 1541-51.
Leitao BB, Jones MC, Brosens JJ. The SUMO E3-ligase PIAS1 couples reactive oxygen species-dependent JNK activation to oxidative cell death. FASEB. 2011; 25: 3416-25.
Lonard DM, O'Malley BW. The expanding cosmos of nuclear receptor coactivators. Cell. 2006; 125: 411-4.
Lynch VJ, Brayer K, Gellersen B et al. HoxA-11 and FOXO1A cooperate to regulate decidual prolactin expression: towards inferring the core transcriptional regulators of decidual genes. PLoS One. 2009; 4: e6845.
Mahendroo MS, Cala KM, Russell DW. 5 alpha-reduced androgens play a key role in murine parturition. Mol Endocrinol. 1996; 10: 380-92.
Mahendroo MS, Porter A, Russell DW et al. The parturition defect in steroid 5alpha-reductase type 1 knockout mice is due to impaired cervical ripening. Mol Endocrinol. 1999; 13: 981-92.
Mak IY, Brosens JJ, Christian M et al. Regulated expression of signal transducer and activator of transcription, Stat5, and its enhancement of PRL expression in human endometrial stromal cells in vitro. J Clinical Endocrinol Metab. 2002; 87: 2581-8.
Marbaix E, Kokorine I, Henriet P et al. The expression of interstitial collagenase in human endometrium is controlled by progesterone and by oestradiol and is related to menstruation. Biochem J. 1995; 305 (Pt 3): 1027-30.
Meis PJ, Klebanoff M, Thom E et al. Prevention of recurrent preterm delivery by 17 alpha-hydroxyprogesterone caproate. N Engl J Med. 2003; 348: 2379-85.
Mendelson CR. Minireview: fetal-maternal hormonal signaling in pregnancy and labor. Mol Endocrinol. 2009; 23: 947-54.
Merlino AA, Welsh TN, Tan H et al. Nuclear progesterone receptors in the human pregnancy myometrium: evidence that parturition involves functional progesterone withdrawal mediated by increased expression of progesterone receptor-A. J Clin Endocrinol Metab. 2007; 92: 1927-33.
Mesiano S, Wang Y, Norwitz ER. Progesterone receptors in the human pregnancy uterus: do they hold the key to birth timing? Reprod Sci. 2011; 18: 6-19.
Misrahi M, Atger M, d'Auriol L et al. Complete amino acid sequence of the human progesterone receptor deduced from cloned cDNA. Biochem Biophys Res Comm. 1987; 143: 740-8.
Mulac-Jericevic B, Lydon JP, DeMayo FJ et al. Defective mammary gland morphogenesis in mice lacking the progesterone receptor B isoform. Proc Natl Acad Sci USA. 2003; 100: 9744-9.
Nordeen SK, Bona BJ, Moyer ML. Latent agonist activity of the steroid antagonist, RU486, is unmasked in cells treated with activators of protein kinase A. Mol Endocrinol. 1993; 7: 731-42.
Olson DM. The role of prostaglandins in the initiation of parturition. Best Prac Res Clin Obstet Gynaecol. 2003; 17: 717-30.
Onate SA, Tsai SY, Tsai MJ et al. Sequence and characterization of a coactivator for the steroid hormone receptor superfamily. Science. 1995; 270: 1354-7.
Owen GI, Richer JK, Tung L et al. Progesterone regulates transcription of the p21(WAF1) cyclin- dependent kinase inhibitor gene through Sp1 and CBP/p300. J Biol Chem. 1998; 273: 10696-701.
Parkington HC, Tonta MA, Brennecke SP et al. Contractile activity, membrane potential, and cytoplasmic calcium in human uterine smooth muscle in the third trimester of pregnancy and during labor. Am J Obstet Gynecol. 1999; 181: 1445-51.
Piekorz RP, Gingras S, Hoffmeyer A et al. Regulation of progesterone levels during pregnancy and parturition by signal transducer and activator of transcription 5 and 20alpha-hydroxysteroid dehydrogenase. Mol Endocrinol. 2005; 19: 431-40.
Pohnke Y, Schneider-Merck T, Fahnenstich J et al. Wild-type p53 protein is up-regulated upon cyclic adenosine monophosphate-induced differentiation of human endometrial stromal cells. J Clin Endocrinol Metab. 2004; 89: 5233-44.
Renthal NE, Chen CC, Williams KC et al. miR-200 family and targets, ZEB1 and ZEB2, modulate uterine quiescence and contractility during pregnancy and labor. Proc Natl Acad Sci USA. 2010; 107: 20828-33.
Richer JK, Jacobsen BM, Manning NG et al. Differential gene regulation by the two progesterone receptor isoforms in human breast cancer cells. J Biol Chem. 2002; 277: 5209-18.
Roizen JD, Asada M, Tong M et al. Preterm birth without progesterone withdrawal in 15-hydroxyprostaglandin dehydrogenase hypomorphic mice. Mol Endocrinol. 2008; 22: 105-12.
Samalecos A, Gellersen B. Systematic expression analysis and antibody screening do not support the existence of naturally occurring progesterone receptor (PR)-C, PR-M, or other truncated PR isoforms. Endocrinol. 2008; 149: 5872-87.
Schneider-Merck T, Pohnke Y, Kempf R et al. Physical interaction and mutual transrepression between CCAAT/enhancer-binding protein beta and the p53 tumor suppressor. J Biol Chem. 2006; 281: 269-78.
Simon L, Spiewak KA, Ekman GC et al. Stromal progesterone receptors mediate induction of Indian Hedgehog (IHH) in uterine epithelium and its downstream targets in uterine stroma. Endocrinol. 2009; 150: 3871-6.
Tan H, Yi L, Rote NS et al. Progesterone receptor-a and -B have opposite effects on proinflammatory gene expression in human myometrial cells: implications for progesterone actions in human pregnancy and parturition. J Clin Endocrinol Metab. 2012; 97: E719-30.
Thakur MK, Paramanik V. Role of steroid hormone coregulators in health and disease. Horm Res. 2009; 71: 194-200.
Tranguch S, Wang H, Daikoku T et al. FKBP52 deficiency-conferred uterine progesterone resistance is genetic background and pregnancy stage specific. J Clin Invest. 2007; 117: 1824-34.
Vegeto E, Shahbaz MM, Wen DX et al. Human progesterone receptor A form is a cell- and promoter-specific repressor of human progesterone receptor B function. Mol Endocrinol. 1993; 7: 1244-55.
Williams KC, Renthal NE, Condon JC et al. MicroRNA-200a serves a key role in the decline of progesterone receptor function leading to term and preterm labor. Proc Natl Acad Sci USA. 2012; 109: 7529-34.

Use of progestagens during early pregnancy

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Abstract

The term "progestagens" covers a group of molecules including both the natural female sex hormones Progesterone and 17-hydroxy Progesterone as well as several synthetic forms, all displaying the ability to bind Progesterone receptors.
Several studies have used Progesterone and related steroids in the attempt to prevent spontaneous miscarriage, and treat recurrent miscarriage.
The present paper aims to provide a comprehensive review of the literature on progestagens effects during early pregnancy. We looked only at the results from randomized controlled trials. We found and analyzed 15 trials on the prevention of recurrent miscarriage and 2 trials on the treatment of miscarriage. The results demonstrated that there is no evidence to support the routine use of progestagens for the treatment of threatened miscarriage.

Key words: **Miscarriage, progestagens, Progesterone, 17 α OH Progesterone, 17 α OH Progesterone Caproate, recurrent miscarriage.**

Introduction

Progesterone (Fig. 1) is a steroid hormone which plays a crucial role in each step of human pregnancy. In early pregnancy Progesterone is produced by the corpus luteum whose duration has been estimated 12 ± 2 days. This organ is fundamental for pregnancy maintenance until the placenta (syncytiotrophoblast) takes over its function at 7-9th week of gestation, just after the expression of major histocompatiblity complex antigens is suppressed in extra-embryonic fetal tissue.

Progesterone is an essential hormone in the process of reproduction. Indeed, it induces secretory changes in the lining of the uterus and is essential for a successful implantation of the embryo. Moreover, Progesterone modulates the immune response of the mother to prevent rejection of the embryo, and enhances uterine quiescence and suppresses uterine contractions. Therefore it is theoretically plausible that P supplementation may reduce the risk of miscarriage in women with a history of recurrent miscarriages. Several studies have used Progesterone and related steroids (progestagens- Fig. 1) in the attempt to prevent spontaneous miscarriage and to increase the embryo implantation rates in assisted reproduction programmes. The term "progestagens" cover a group of molecules including both the natural female sex hormones Progesterone and 17-hydroxy Progesterone (17P) as well as several synthetic forms, all displaying the ability to bind Progesterone receptors.

Although pharmacokinetics and pharmacodynamics features of progestagens have been studied, their use in human pregnancy remains controversial, i.e. the way of administration. Indeed, progestagens could be administered by three routes: orally, vaginally or intramuscularly. Oral administration guarantees optimal compliance by patients but shows many disadvantages; this route also results in side effects such as nausea, headache and sleepiness. The vaginal route results in higher concentrations in the uterus but does not reach high and constant blood levels. The drug administered intramuscularly occasionally induces non-septic abscesses, although it is the only route which results in optimal blood levels (Whitehead et al., 1990; Szabo and Szilagyi, 1996; Cunningham, 2001; Di Renzo et al., 2005; Christian et al., 2007). Several reports hypothesized an association between intrauterine exposure to

Progesterone **17 α OH Progesterone** **17 α OH Progesterone Caproate**

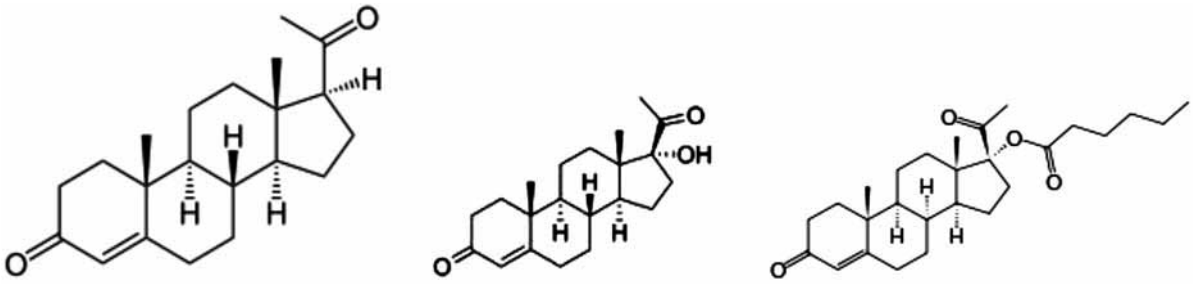

Fig. 1. — Progesterone and Progestagens

progestagens in the first trimester of pregnancy and genital abnormalities in male and female fetuses. This was due to the possible up-regulation of androgen receptor operated by pharmacological doses of these steroids. However, maternal safety of progestagens has been reported in different trials. Neonatal safety has been evaluated in only one trial where mothers have been treated with 17 Progesterone. No effects of general health status, external genitalia, and psychomotor development have been reported at follow-up. Since the paucity of data, ongoing trials are encouraged to include the follow-up of neonates in their study design (Mosby, 2001).

The present paper aims to provide a comprehensive view of the literature on the effects of progestagens during early pregnancy. We describe the effects of progestagens for preventing recurrent miscarriages and managing threatened miscarriage.

Miscarriage is defined as pregnancy loss before 23 weeks' gestation, based on the first day of the last menstrual period. Miscarriage is associated with considerable physical and psychological morbidity, particularly in developing countries (World Health Organization, 1992). Haemorrhage into the decidua basalis and necrotic changes in the tissues adjacent to the bleeding usually accompany abortion; the ovum becomes detached and stimulates uterine contractions that result in expulsion (Cunningham, 2001).

Recurrent miscarriage has been defined as 3 or more consecutive episodes of spontaneous pregnancy losses with the same biological father (World Health Organization, 1992). Extensive investigation of women involved will fail to find a recognisable cause in up to half of the cases. Luteal phase defects, immunotolerance derangements, chromosomal anomalies and endocrine disorders are the most common recognisable causes. Accepting an independent risk of miscarriage to be 15%, a second loss could be calculated to occur at a rate of 2-3% while a third loss is expected in 0.34% of women.

More than 80% of miscarriages occur before the 12th week, and the rate decreases rapidly thereafter. It is a common complication of pregnancy occurring in 15% to 20% of all clinically recognized pregnancies with 1% to 2% of couples suffering a recurrent early loss. It is thought, however, that the true incidence of early spontaneous miscarriage may be much higher. Indeed, after implementation of assisted reproduction techniques we are able to detect biochemical pregnancies and therefore to conclude that the rate of early spontaneous miscarriage in the first trimester is higher than expected (Everett, 1997).

Chromosomal anomalies cause at least half of these early miscarriages. The risk increases with parity and at the extremes of maternal age (>34 years and <20 years old). Other risk factors for miscarriage include maternal infections (such as vaginitis, urinary tract infections), endocrine abnormalities (diabetes mellitus, hypothyroidism, pituitary adenoma), insufficient production of Progesterone by the corpus luteum (short and/or abnormal luteal phase), polycystic ovary syndrome, drug use, environmental factors, maternal autoimmune disorders (such as anti-phospholipids antibodies), previous history of two or more miscarriages and acquired or inherited uterine malformations (Szabo and Szilagyi, 1996). Despite all the above recognised or supposed factors, the cause of miscarriage cannot be identified in almost 50% of cases. Threatened miscarriage manifests itself through vaginal bleeding, with or without abdominal pain, while the cervix is closed and the fetus is viable inside the uterine cavity. The introduction of ultrasound scans in the management of bleeding in early pregnancy has improved the diagnosis tremendously (Hemminki, 1998).

During the past 50 years several trials investigated the use of progestagens for the prevention of miscarriage. Actually the therapeutic value of progestagens remains to be established. This might be due to the poor design of the studies which evaluated hormone

effectiveness. Moreover, a lot of different aetiologies are associated with threatened miscarriages and heterogeneity of studies has not been accounted for.

Methods

Literature searches were performed in The Cochrane Library, only randomized controlled trials (RCTs) were considered. The search terms were as follows: "progestagens", "miscarriage", "recurrent miscarriage". We performed a search about all types of progestagens, their applications and potential effects for preventing recurrent miscarriage and for managing miscarriage, regardless of the dose, duration or route of administration compared with placebo, no treatment or other intervention.

All sources of information were read and evaluated by one of the authors (GD), and were later independentely checked by another author (VV).

Results

Two recent Cochrane Reviews (Haas and Ramsey, 2009; Wahabi et al., 2011) selected the randomized controlled trials (RCT) or quasi-randomized controlled trials published between 1966 and 2006. They included all types of progestagens in the prevention of miscarriage (Haas and Ramsey, 2009) and for the treatment of threatened miscarriage (Wahabi et al., 2011), regardless of the dose, duration or route of administration and compared with placebo, no treatment or other interventions. No further RCTs were published after those included in the Cochrane Review.

Wahabi et al. (2011) selected the trials published in 7 databases and references from relevant articles. The authors included only the trials where the administration of progestagens started before pregnancy and continued during pregnancy. Twenty-nine studies were potentially eligible for inclusion, of these, 15 studies were included after applying the inclusion criteria. The characteristics of these RCTs are described in Table 1.

Haas and Ramsey (2009) selected the RCTs or quasi-randomized controlled trials that appeared in 30 journals and the proceedings of major conferences describing all types of progestagens in the treatment of threatened miscarriage. Participants were all pregnant women, with a history of threatened miscarriage and a confirmed viable pregnancy.

Table 1. — Characteristics of randomized controlled trials analized for preventing recurrent miscarriage (RCT: Randomized Controlled Trial, PTB: preterm birth).

Authors/year	Population studied	Intervention	Comparison	Outcome measured
El Zibdeh et al. 2005	Pregnant women (aged <35 years) with at least 3 consecutive unexplained abortions with same partner N = 180/180	- Dihydrogesterone - Oral - Until miscarriage or 12 weeks - 10 mg twice daily	RCT *vs* no treatment	Miscarriage, PTB, fetal malformations
Goldzieher et al. 1964	Women who never had a term pregnancy and with a history of 2 or more miscarriages or with a history of 1 or more term pregnancy followed by a minimum number of 2 consecutive miscarriages N = 54/54	- Medroxy Progesterone acetate - Oral - Not stated - 10 mg daily	Double blind, RCT *vs* placebo	Miscarriage, PTB
Klopper et al. 1965	Women who had 2 or more miscarriages, no pregnancy beyond 28 weeks' gestation, were less than 10 weeks into the current pregnancy and with no other obvious causes of miscarriage	- Cyclopentyl onol ether of Progesterone - Oral - Not stated - 50 mg twice daily	Double blind, RCT *vs* placebo	Miscarriage
Moller et al. 1965	Women with a positive pregnancy test. N = 40/40	- Medroxy Progesterone acetate - Oral - 20 mg/day for 3 days, followed by 10 mg/day for 11 days	Double blind, RCT *vs* placebo	Miscarriage

Moller et al. 1965	Women with a positive pregnancy test. N = 63/63	- Medroxy Progesterone acetate - Oral - 40 mg/day for 3 days, followed by 20 mg/day for 11 days	Double blind, RCT *vs* placebo	Miscarriage
Moller et al. 1965	Women with a positive pregnancy test. N = 153/153	- Medroxy Progesterone acetate - Oral - 80 mg/day for 3 days, followed by 40 mg/day for 11 days	Double blind, RCT *vs* placebo	Miscarriage
Levine et al. 1964	Women with 3 consecutive miscarriages of less than 16 weeks' gestation and with no signs of threatened miscarriage in the current pregnancy N = 56/30	- 17OHPc - IM - until miscarriage or 36th week - 500 mg weekly	Double blind, RCT *vs* placebo	Miscarriage, PTB
Anderson et al. 2002	Women having undergone IVF or ICSI with a positive pregnancy test 14 days after transfer N = 303/303	- Progesterone vaginally - 3 weeks - 200 mg 3 times daily	RCT *vs* no treatment	Miscarriage
Reijnders et al. 1988	One or more of the following criteria: pregnancy after ovulation induction; 2 or more previous miscarriages; period of infertility for more than 12 months. Evidence of a viable fetus at 6 weeks of pregnancy N = 64/64	- 17OHPc - i.m. - from 7th to 12th week - 500 mg/week	Double blind, RCT *vs* placebo	Miscarriage, PTB
Shearman et al. 1963	Women with 2 or more consecutive abortions and low or falling pregnanediol levels N = 50/50	- 17OHPc - i.m. - Up to 8th week 250 mg/weekly; then increasing dose to 500 mg till 16th week; then reducing doses to 250 mg/weekly until 24th week	Double blind, RCT *vs* placebo	Miscarriage
Berle et al. 1980	Women up to 20 weeks gestation with bleeding N° = 300/300	- 90% received oral allylestrenol 15-20 mg/day - 10% received 250 mg of i.m. 17OHPc daily or every 2 days - Duration not stated	Double blind, RCT *vs* placebo	Miscarriage
Tognoni et al. 1980	Women with threatened miscarriage up to 14 weeks' gestation N° =145/139	- Oral allylestrenol 10 mg/d - 25 mg 17HP im every 5 d - 8 weeks	RCT *vs* placebo	Miscarriage
Gerhard et al. 1987	Women with vaginal bleeding in pregnancy and a closed cervical os N° = 64/35	- Progesterone - Vaginal suppositories - 25 mg twice/day	Double blind, RCT *vs* placebo	Miscarriage, birth weight, preterm labour
Swyer & Daley 1953	Women with 2 or more consecutive miscarriages before 12 weeks' gestation N = 113/113	- 6 × 25 mg Progesterone pellets inserted in the gluteal muscle	RCT *vs* control group	Miscarriage, birth weight, PTB, stillbirth
Corrado et al. 2002	Women undergoing mid-second trimester amniocentesis N = 616/584	- Progesterone IM. 200 mg/day for 3 days followed by 17OHPc IM 340 mg twice/week - Duration unclear	RCT *vs* control group	Miscarriage, birth weight, preterm delivery

Table 2. — Characteristics of included studies for treating miscariages.

Authors/year	Population studied	Intervention	Comparison	Outcomes measured
Gerhard et al. 1987	Women in the first trimester of pregnancy with confirmation of fetal viability by ultrasound scan N = 64/35.	Progesterone - Vaginal suppositories - 14 days after bleeding stopped - 25 mg twice/day	Double blind, RCT *vs* placebo	Miscarriage, birth-weight, preterm labour
Palagiano et al. 2004	50 women with previous diagnosis of inadequate luteal phase, threatened miscarriage and confirmed fetal viability	Progesterone - Vaginal suppositories - 5 days - 90 mg/day	RCT *vs* placebo	Pain relief uterine contractions, blood loss

Thirty-four studies were potentially eligible for inclusion. Only 2 were included after applying the inclusion criteria (Gerhard et al., 1987; Palagiano et al., 2004). The characteristics of these RCTs are described in Table 2.

These two trials met the inclusion criteria and involved 85 participants. Due to a paucity of data, subgroup analysis for early and late miscarriage, effect of progestagens by type, dose, and route of administration could not be carried out.

Discussion

The overall meta-analysis conducted on the use of progestagens for the treatment of recurrent miscarriages (Haas and Ramsey, 2009) showed no statistically significant difference in miscarriage rates between progestagens and placebo groups (Peto OR 0.98; 95% CI 0.78 -1.24). The analysis regarding the method of administration also showed no statistically significant difference between progestagens and placebo groups.

No evidence for an effect favouring the use of progestagens in women with recurrent miscarriage was found when compared to placebo (Peto OR 1.11, 95% CI 0.79-1.56, P = 0.54). Four studies (Shearman and Garrett, 1963; Levine, 1964; Reijnders et al., 1998; Corrado et al., 2002) compared intramuscular progestagens with placebo, the results showed no differences in miscarriages between the two groups (Peto OR 0.77; 95% CI 0.36-1.68, P = 0.52). Only one study compared vaginally administered progestagens with placebo, a second one comparing it with no treatment. The incidence of recurrent miscarriage was similar in both groups (Peto OR 0.74; 95% CI0.40-1.35, P = 0.32).

The analysis concerning recurrent miscarriage considered 4 trials, of these 2 trials (Levine, 1964; El-Zibdeh, 2005) enrolled only women who had suffered three or more miscarriages, 2 others (Swyer and Daley, 1953; Goldzieher, 1964) provided separate pregnancy outcome data by number of previous consecutive pregnancy losses. The results showed a reduction in miscarriage in favour of those in the progestagens group (Peto OR 0.38; 95% CI 0.20 to 0.70, P = 0.002). This finding however should be approached with caution as numbers are small.

Meta-analysis of the effect of vaginal Progesterone on miscarriage when compared to placebo (Wahabi et al., 2011) showed a point estimate which suggests a reduction of miscarriage rate with the use of Progesterone (risk ratio 0.47; 95% confidence interval 0.17 to 1.30), but the uncertainty about the outcome of this study is due to the small sample size.

Conclusion

Based on scarce data there is no evidence to support the routine use of progestagens for the treatment of threatened miscarriage. Information about potential harms to the mother or child, or both, with the use of progestagens is lacking. There has been much speculation about progestagens but the results of these meta-analysis show no statistically significant difference between women receiving progestagens for preventing threatened miscarriage or miscarriage when compared to placebo, other treatments or no treatment. Further, larger RCTs investigating potential harms as well as benefits, are urgently needed. A finding of a significantly reduced miscarriage rate in women with a history of recurrent miscarriage was found only in studies that included women with at least three miscarriages. This finding was obtained with different types of progestagens and therefore deserves further study before entering in clinical practice.

References

Anderson NA, Popovic-Todorovic B, Schmidt K et al. Progesterone supplement during early gestations after IVF or ICSI has no effect on the delivery rates: a randomized controlled trial. Hum Reprod 2002;17:357-61.

Berle P, Budenz M, Michaelis J. Is hormonal therapy still justified in imminent abortion? Z Geburtshilfe Perinatol 1980;184:353-8.

Christian MS, Brent RL, Calda P. Embryo-fetal toxicity signals for 17alpha-hydroxyprogesterone caproate in high-risk pregnancies: a review of the non-clinical literature for embryo-fetal toxicity with progestins. J Matern Fetal Neonatal Med. 2007;20:89-112.

Corrado F, Dugo C, Cannata M et al. A randomised trial of progesterone prophylaxis after midtrimester amniocentesis. Eur J Obstet Gynecol Reprod Biol 2002;100:196-8.

Cunningham FG. Reproductive success and failure. *Williams obstetrics*. Section IX. 21st Edition. New York: McGraw-Hill, 2001.

Di Renzo GC, Mattei A, Gojnic M et al. Progesterone and Pregnancy. Current opinion Obstet Gynecol. 2005;17:598-600.

El-Zibdeh MY. Dydrogesterone in the reduction of recurrent spontaneous abortion. J Steroid Biochem Mol Biol 2005; 97:431-4.

Everett C. Incident and outcome of bleeding before the 20th week of pregnancy: prospective study from general practice. BMJ 1997;315:32-4.

Gerhard I, Gwinner B, Eggert-Kruse W et al. Double-blind controlled trial of progesterone substitution in threatened abortion. Biol Res in Pregnancy Perinatol 1987;8:26-34.

Goldzieher JW. Double-blind trial of a progestin in habitual abortion. JAMA 1964;188:651-4.

Haas DM, Ramsey PS. Progestogen for preventing miscarriage. Cochrane Database Syst Rev. 2009: CD003511.

Hemminki E. Treatment of miscarriage: current practice and rationale. Obstet Gynecol 1998;91:247-53.

Klopper A, MacNaughton. M. Hormones in recurrent abortion. J Obstet Gynaecol Br Commonw 1965;72:1022-8.

Levine L. Habitual abortion. A controlled clinical study of progestational therapy. West J Surg Obstet Gynecol 1964; 72:30-6.

Moller K, Fuchs F. Double blind controlled trial of 6- methyl-17-acetoxyprogesterone in threatened abortion. J Obstet Gynaecol Br Commonw1965;72:1042-4.

Mosby Incorporated. Medroxyprogesterone acetate. Harcourt Health Sciences Incorporated, 2001.

Palagiano A, Bulletti C, Pace MC et al. Effects of vaginal progesterone on pain and uterine contractility in patients with threatened abortion before twelve weeks of pregnancy. Ann N Y Acad Sci 2004;1034:200-10.

Reijnders FJL, Thomas CMG, Doesburg WH et al. Endocrine effects of 17 alphahydroxyprogesterone caproate during early pregnancy: a double-blind clinical trial. Br J Obstet Gynaecol 1988;95:462-8.

Shearman R, Garrett W. Double-blind study of effect of 17-hydroxyprogesterone caproate on abortion rate. BMJ 1963;1:292-5.

Swyer GIM, Daley D. Progesterone implantation in habitual abortion. BMJ 1953;1:1073-86.

Szabo I, Szilagyi A. Management of threatened abortion. Early Pregnancy 1996;2:233-40.

Tognoni G, Ferrario L, Inzalaco M et al. Progestogens in threatened abortion. Lancet 1980;2: 1242-3.

Wahabi HA, Abed Althagafi NF, Elawad M, Al Zeidan RA. Progestogen for treating threatened miscarriage. Cochrane Database Syst Rev 2011;(3):CD005943.

Whitehead MI, Hillard TC, Crook D. The role and use of progestogens. Obstet Gynecol 1990;75(4 Suppl):59S-76S; discussion 81S-83S.

World Health Organization. International Statistical Classification of Diseases and Related Health Problems, 10th Edition.,Vol. 1, Geneva, 1992.

Progestagens for preventing preterm birth

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Abstract

The term "progestagens" covers a group of molecules including both the natural female sex hormones Progesterone and 17-hydroxy Progesterone as well as several synthetic forms, all displaying the ability to bind Progesterone receptors. Several studies have used Progesterone and related steroids in an attempt to prevent spontaneous preterm birth (PTB).

The present paper aims to provide a comprehensive review of the literature on the effects of progestagens effects in preventing spontaneous PTB. We consider only the information derived from randomized controlled trials (RCTs). Results are reported in 4 sections according to the clinical risk factor: 1- history of preterm delivery, 2- short cervix, either symptomatic or asymptomatic, 3- multiple pregnancies, 4- threatened preterm labour.

For the clinicians, the back home message is that a) 17 OH Progesterone caproate (17OHPc) is able to prevent PTB in women at risk because of their obstetric history, and b) vaginal Progesterone prevents PTB in women with an asymptomatic short cervix. The conclusion that progestagens are useless in multiple gestations has recently been challenged by an individual participant data (IPD) meta-analysis. No firm indications on the use of progestagens for PTB prophylaxis in women presenting with other risk factors such as symptomatic short cervix or threatened preterm labour could be found with the available data.

Key words: **Progestagens, Progesterone, 17 α OH Progesterone, 17 α OH Progesterone Caproate, preterm labour, preterm birth.**

Introduction

The syndromic nature of spontaneous preterm labour was a concept first proposed in 1994 (Romero et al., 1994). In 2009, Romero et al., have reinforced that preterm parturition should not be considered a monolithic entity, but rather a syndrome. The term ''syndrome'' is defined in the Oxford Medical Dictionary as ''a combination of symptoms and signs that form a distinct clinical picture indicative of a particular disorder''. The following mechanisms of disease have been implicated: 1) intrauterine infection/inflammation; 2) uterine ischemia; 3) uterine over-distension; 4) abnormal allogenic recognition; 5) allergic-like reaction; 6) cervical disease; and 7) endocrine disorders. This view controverts the concept that preterm parturition is simply "labor before its time" and that all causes of preterm labor are the same. The implicit consequence that preterm parturition is a syndrome with multiple etiologies is that there would not be a single treatment, diagnostic method or preventive strategy that will work for all preterm births.

So the 'cause' of preterm labour is multifactorial in origin, and it is important to consider the role of any identifiable risk factor in a woman's pregnancy.

The knowledge that an increased activity of endogenous Progesterone was a necessary event for the development and the maintenance of pregnancy dates back to the first half of the last century. Around the 60s the idea was acquired that a withdrawal of endogenous Progesterone was related to the onset of labour, even preterm. Since then, Progesterone and related synthetic compounds such as 17OHPc as well as other progestagens have been tested in clinical trials to prevent the challenging phenomenon

of PTB (Romero et al., 1994). In one of the first meta-analyses ever published about perinatal interventions, it was demonstrated that 17OHPc treatment was associated with a reduced rate of PTB in respect with placebo or no intervention. Surprisingly, such achievement was not implemented into clinical practice, nor did scientific societies endorse such conclusions producing recommendations. The Williams Obstetrics textbook 21st edition released in 2001 did not even mention Progesterone among interventions to prevent PTB (Cunningham, 2001). Clinical and experimental studies re-started in the year 2000. Now, an almost equal number of RCTs and meta-analyses are available while several trials are still being planned or are ongoing. In such clinical trials Progesterone has been administered through daily vaginal route by using both 8% gel and 100-400 micronized hormones. On the other hand, 17OHPc has been administered through IM injections, by using doses ranging from 250 to 682 mg/week, with drug dissolved in castor or ethyl oil. Given the different biological actions of Progesterone and 17OHPc, considering that we still ignore the mechanism of action of such treatments, given the heterogeneity of the inclusion criteria utilized in the different studies, it seems difficult at present to put together the results of all these RCTs under the umbrella of "progesterone treatment" (Facchinetti et al., 2009; Di Renzo et al., 2011). Maternal safety of either micronized Progesterone or 17OHPc administration has been reported in different trials. Neonatal safety has been evaluated in only one trial where mothers have been treated with 17OHPc. No effects on general health status, external genitalia and psychomotor development have been reported at follow-up. However, there is concern about the increase in fetal death in mid-trimester and the higher incidence of gestational diabetes linked to 17OHPc (Di Renzo et al., 2011).

Methods

Literature searches were performed in the following electronic databases: Medline and The Cochrane Library. We performed a search over the period January 1975 to June 2012 and only RCTs were included.

We performed a search about progestagens, their applications and potential effects for preventing PTB. Results are subdivided into 4 sections according to the clinical presentation of subject. Effects of progestagens were evaluated in (1) women with history of preterm delivery, (2) patients with short cervix, (3) women with multiple pregnancies and (4) patients with symptoms of threatened preterm labour.

Results

History of preterm delivery

The most significant and consistently identified risk factor for PTB is a woman's history of previous PTB. Data suggests the rate of recurrent PTB in this group of women to be 22.5%, a 2.5 times increased risk ratio when compared with women without previous spontaneous PTB (Mercer et al., 1999; Facchinetti and Vaccaro, 2009). For women with a history of a single PTB, the recurrence risk in a subsequent pregnancy is approximately 15%, increasing to 32% where there have been two previous PTBs (Carr-Hill and Hall, 1985). Information derived from population-based cohort data suggests that for women who give birth between 20 and 31 weeks' gestation in one pregnancy, 29.3% will give birth prior to 37 weeks in a subsequent pregnancy (Adams et al., 2000). For approximately 10% of these women, the PTB will occur at a similar gestational age (Adams et al., 2000; Kistka et al., 2007). In up to 50% of cases of PTB, the cause is spontaneous onset of labour or preterm premature rupture of membranes (pPROM) (McLaughlin et al., 2002). The obstetric history is an important predictor of spontaneous preterm delivery both in singleton and twin gestations, in particular previous spontaneous preterm delivery and late miscarriage.

In a Cochrane review, Dodd et al. (2012) included 11 RCTs (2714 women and 3452 infants) and concluded that, for women with a past history of spontaneous PTB, progestagens are associated with a statistically significant reduction in the risk of PTB less than 34 weeks' gestation (142 women; risk ratio (RR) = 0.15; 95% confidence interval (CI) 0.04 to 0.64) (one study - da Fonseca et al., 2003). Progestagens are associated with a statistically significant reduction for PTB less than 37 weeks' gestation (four studies -1255 women; RR 0.80; 95% CI 0.70 to 0.92) and infant birth weight less than 2500 grams (two studies - 501 infants; RR 0.64; 95% CI 0.49 to 0.83) (Dodd et al., 2012).

After the Cochrane review publication, Berghella et al. (2010) published a planned secondary analysis of the NICHD-sponsored trial evaluating cerclage in women with singleton gestation, a history of PTB and a ultrasound-measured short cervix. In 152 women, without cerclage, 17OHPc failed to prevent PTB < 35 weeks (the main outcome) in respect with placebo. However, the intervention group reported significantly less PTB < 24 weeks and a trend toward less perinatal death when compared to placebo.

Patients with a short cervix

Other characteristics of pregnant women are associated with an increased risk for PTB. These include women with a short cervix identified by ultrasound assessment, the presence of fetal fibronectin in the vaginal secretions, and other symptoms or signs of threatened preterm labour (Goldenberg et al., 1996; Berghella et al., 2005).

The cervix has a crucial role in the etiology of PTB, as well as in its potential prevention strategies. The identification of a short cervix on ultrasound examination (the cut off is 25 mm) has been associated with a likelihood ratio of PTB before 34 weeks' gestation of 6.29 (95% confidence interval (CI) 3.29 to 12.02) (Honest et al., 2002). The likelihood ratio of PTB before 34 weeks associated with a negative ultrasound test result was 0.79 (95% CI 0.65 to 0.95). The risk for spontaneous PTB in both singleton and twin pregnancies is inversely correlated with the cervical length: the shorter the cervix the higher the risk of preterm delivery.

The cervix is composed by 80-85% connective tissue, with collagen type 1 making up 70% of whole collagen. Cervical ripening has been hypothesized as the effect of a local activation of diverse biochemical pathways sharing those of an aseptic, pro-inflammatory reaction. The increased secretion of interleukins and nitric oxide in cervical fluids has been reported to be associated with PTB. Such mediators ultimately stimulate apoptosis, activation of proteases and disaggregation of collagen fibrils allowing cervical shortening (Lockwood et al., 1994; Facchinetti and Vaccaro, 2009).

It has been suggested before that progesterone-active compounds showed immunosuppressive effects. Indeed, Progesterone receptors were found on lymphocytes and the activation of such receptors interferes with interleukins secretion through the progesterone-induced blocking factor. In human decidual cells, *in vitro* medroxyprogesterone acetate (MPA) was able to block both IL-1β and thrombin-induced release of IL-11, a cytokine which is over-expressed in samples of decidua collected from patients undergoing a PTB associated to intrauterine infection. Moreover, in a mouse model of inflammation-induced PTB, both MPA and 17OHPc pre-treatment protected animals from PTB.

Recently, the pivotal role of IL-1β in accounting for PTB was highlighted in an experiment done in non-human primates. Pregnant animals received amniotic fluid injection of the pro-inflammatory cytokines. While IL-6 and IL-8 were rather ineffective, IL-1β injection induced preterm delivery within a few days.

In humans, IL-1β levels have consistently been found elevated in cervico-vaginal and/or cervical fluids, thus predicting those women destined to undergo spontaneous PTB. IL-1β is an early marker of host-response to infection and seems to play a main role in host reaction, more particular in cases where PTB is associated with amniotic fluid infection. IL-1β initiates a cascade of paracrine events activating several cytokines. The observation that 17OHPc induce a selective inhibition of cervical IL-1β is a key phenomenon and possibly has a pivotal role in mediating the protective effect on PTB (MacIntyre et al., 2012).

In the Cochrane review from Dodd et al. (2012) progestagens were associated with a statistically significant reduction in the risk of PTB less than 34 weeks (one study - 250 women; RR 0.58; 95% CI 0.38 to 0.87) and neonatal sepsis (one study - 274 infants; RR 0.28; 95% CI 0.08 to 0.97) when compared to placebo in women with a short cervix.

In a RCT of 17OHPc treatment for PTB prevention in nulliparous women with cervical length (CL) less than 30 mm, the frequency of PTB did not differ between the drug and placebo groups (25.1% vs. 24.2%) (Grobman, 2012). There was also no difference in delivery < 35 weeks (13.5% vs 16.1%, $p = 0,35$) or < 32 weeks (8.6% vs. 9.7%, $p = 0,61$). Although the power to show a difference was limited due to sample size, subgroup analyses also failed to demonstrate benefit from 17OHPc in women with a CL < 15 mm. According to this study, weekly IM injection of 17OHPc does not reduce the frequency of PTB in nulliparous women with a short cervix < 30 mm.

Two large RCTs on vaginal Progesterone are available. In the first one published by Fonseca et al. (2007), CL was measured by transvaginal ultrasonography at a median of 22 weeks of gestation in 24,620 pregnant women seen for routine antenatal care. CL was 15 mm or less in 413 women (1.7%) and 250 (60.5%) of them were randomly assigned to receive vaginal Progesterone (200 mg each night) or placebo from 24 to 34 weeks of gestation. Spontaneous PTB before 34 weeks of gestation was less frequent in the vaginal Progesterone group than in placebo group (19.2% vs. 34.4%; RR, 0.56; 95% CI, 0.36 to 0.86). Vaginal Progesterone was associated with a non-significant reduction in neonatal morbidity (8.1% vs. 13.8%; RR, 0.59; 95% CI, 0.26 to 1.25; P = 0.17).

The other RCT by Hassan et al. (2011) was a multicenter, randomized, double-blind, placebo-controlled trial that enrolled asymptomatic women with a singleton pregnancy and a sonographic short cervix (10–20 mm) at 19 + 0 to 23 + 6 weeks of gestation. Women were allocated randomly to receive vaginal Progesterone gel or placebo starting from 20 to 23 +

6 weeks until 36 + 6 weeks, or until rupture of membranes or delivery, whichever occurred first. Out of 465 randomized women, seven were lost to follow-up and 458 were included in the analysis. Women allocated to receive vaginal Progesterone gel (90 mg) had a lower rate of PTB before 33 weeks than did those allocated to placebo (8.9% vs 16.1%; RR, 0.55; 95% CI, 0.33–0.92). Vaginal Progesterone was also associated with a significant reduction in the rate of PTB before 28 weeks (5.1% vs. 10.3%, p = 0.04) and 35 weeks (14.5% vs. 23.3%, p = 0.02). Progesterone was also effective on neonatal outcomes such as respiratory distress syndrome (3.0% vs. 7.6%, p = 0.03), any neonatal morbidity or mortality event (7.7% vs. 13.5%, p = 0.04) and a birth weight < 1500 g (6.4% vs. 13.6%, p = 0.01). There were no differences in the incidence of treatment-related adverse events between the groups.

Women with multiple pregnancies

Multiple pregnancies contribute disproportionately to PTB. Overall, 52.2% of multiple births deliver before 37 weeks and 10.7% before 32 weeks (Stock et al., 2010). In the United States, the rate of PTB and very PTB among triplets shows a 92% and 36% increase respectively (Grobman, 2012). In the USA, 57% of twins are born with low birth weight, compared to 6% of singletons (Combs et al., 2011). The mechanisms of PTB may be different to those operating in women with a singleton pregnancy. Preterm premature rupture of membranes (pPROM) is more prevalent in twin gestations and is a major contributor to PTB (Sela and Simpson, 2011).

As yet, no treatments have been identified that can prevent PTB in multiple pregnancy. Six RCTs of progestagens to prevent PTB in twin pregnancies have now been published i.e. (Fonseca et al., 2007; Rouse et al., 2007; Briery et al., 2009; Cetingoz et al., 2011; Norman et al., 2009; Combs et al., 2011).

These studies randomized women to either 17OHPc or vaginal Progesterone pessary, and placebo. In one study, which included 67 women with twin pregnancies, 100 mg of vaginal Progesterone was found to reduce delivery before 37 weeks gestation (OR 3.48 [1.2-10.5]) (Cetingoz et al., 2011). In all the remnant studies treatment with 17OHPc or vaginal Progesterone did not lead to any significant reduction in PTB or fetal loss. In the two largest trials, however, a non-significant increase in intrauterine death was seen in the treatment group (Rouse et al., 2007; Norman et al., 2009). Furthermore, a significant difference in median gestational age favoring placebo was found in the other large trial (Combs et al., 2011).

We are aware of five other trials of progestagens in multiple pregnancies that are nearing completion or publication. In total these trials have included 3,522 women and more than 7,000 infants. An ongoing individual participant data (IPD) meta-analysis of randomized trials is combining data from these high-quality clinical trials in order to provide valuable information regarding the benefits and potential harms of progestagens. The primary outcome is adverse perinatal outcome i.e. a composite measure of perinatal mortality and significant neonatal morbidity.

In the only published meta-analysis, Romero et al. (2012) have shown that among twin gestations the administration of Progesterone did not significantly reduce the risk of PTB < 33 weeks of pregnancy (RR, 0.70; 95% CI, 0.34 –1.44). However, the intervention significantly decreased the risk of composite neonatal morbidity and mortality (RR, 0.52; 95% CI, 0.29–0.93). There were no significant differences in other outcome measures among the vaginal Progesterone and placebo groups. Moreover in the subgroup of women with a twin gestation and a short cervix, Romero et al. (2012) found that vaginal Progesterone reduces the rate of PTB at < 33 weeks by 30% and significantly reduces the composite neonatal morbidity/mortality of twins. A confirmatory study using available progestagens is urgently needed to confirm these data since a negative effect was found by the Netherlands network: 671 women with multiple gestations were randomized and intervention did not affect the rate of adverse composite neonatal outcome when compared to placebo (Lim et al., 2011).

Patients with symptoms of threatened preterm labour

In a woman's current pregnancy another characteristic that may place her at increased risk of PTB, is a threatened preterm labour. However, symptoms of preterm labour are often aspecific, i.e. Women with preterm contraction and modified cervix deliver before 37 weeks in 10-40% of the cases (Dodd et al., 2012). The question whether 17OHPc treatment affects changes in cervical length was studied in a single centre, randomised, prospective study including women with singleton pregnancy, between 25 and 33 + 6 weeks and hospitalized for preterm labour (Facchinetti et al., 2007). Sixty undelivered patients after tocolysis were randomly allocated to either observation or to receive 341 mg of 17OHPc IM twice/week, until 36 weeks of gestation. CL was measured by transvaginal ultrasound at discharge, and at day 7 and day 21 post discharge. Shortening of the cervix in the observation group was higher than in the 17OHPc group, both at day 7 (2.37 ± 2.0

Table 1. — Effects of progestagens in reducing preterm birth (PTB = preterm birth, IPD = individual patient data).

PROGESTAGEN RISK FACTOR	17 α Hydroxy Progesterone Caproate	Vaginal P suppository	Vaginal P gel
History (At least one previous spontaneous PTB)	**PREVENTS PTB** 2 RCTs (250 mg/weekly) (Johnson, 1975; Meis 2003)	**PREVENTS PTB** 1 RCT (100 mg/day) (da Fonseca, 2003)	**INEFFECTIVE** 1 RCT (90 mg/day) (O'Brien, 2007)
Hystory plus short cervix	**PREVENTS Previable Birth** 1 RCT (250 mg/weekly) (Berghella, 2010)		
Short cervix - Asymptomatic	**INEFFECTIVE** 1 RCT (250 mg/weekly) (Grobman, 2012)	**PREVENTS PTB** 1 RCT (200 mg/day) (Fonseca, 2007)	**PREVENTS PTB** 1 RCT (90 mg/day) (Hassan, 2011)
		PREVENTS PTB 1 IPD (Romero, 2012)	
Short cervix - Symptomatic	**PREVENTS PTB** 1 RCT (open) (341 mg/twice week) (Facchinetti, 2007)		-
	INEFFECTIVE 1 RCT (open) (500 mg/twice week) (Rozenberg, 2012)	**DELAY PARTURITION** 1 RCT (open) (400 mg/day) (Borna, 2008)	-
Multiple Pregnancy	**INEFFECTIVE** 3 RCTs (250 mg/weekly) (Rouse, 2007; Combs, 2011; Lim, 2011)	-	**INEFFECTIVE** 1 RCT (Norman, 2009)
Multiple Pregnancy plus short cervix		**INEFFECTIVE** (REDUCES COMPOSITE NEONATAL MORBIDITY/MORTALITY) 1 IPD (Romero, 2012)	

vs. 0.83 ± 1.74 mm, p = 0.002) and day 21 (4.60 ± 2.73 vs. 2.40 ± 2.46 mm, p = 0.002). Treatment with 17OHPc was associated with both a reduction in the risk of cervical shortening ≥ 4 mm (OR: 0.18; 95% C.I.: 0.04-0.66) and in the risk of PTB (OR: 0.15; 95% C.I.: 0.04-0.58) (Facchinetti et al., 2007).

An open-label, multi-centre, RCT included 184 women with singleton pregnancies and short cervix (cervical length less than 25 mm) admitted at 24-31 weeks' gestation for preterm labour successfully arrested by tocolytic treatment (Rozenberg et al., 2012). Randomization assigned them to receive (or not) 500 mg of i.m. 17OHPc after tocolysis ended, repeated semi-weekly until 36 weeks or PTB. The primary outcome was the time from randomization to delivery. The 17OHPc and control groups did not differ significantly for median time to delivery (64 [42–79] and 67 [46–83] days, respectively) or rates of delivery before 37, 34, or 32 weeks of gestation or adverse perinatal outcomes. According to this study, biweekly injections of 17OHPc did not significantly prolong pregnancy. In this study the latency from randomization to delivery was much longer than in other trials. Such difference is probably due to inclusion criteria, which selected a population with less severe threatened preterm labour (Rozenberg et al., 2012).

Another single centre, randomized study performed in Australia including 70 women, demonstrated that vaginal Progesterone (400 mg daily) was associated with a PTB < 37 weeks reduction (OR = 0.52; CI95%: 028-0.98) (Borna and Sahabi, 2008).

A recent controlled trial also involving a single centre was performed in 70 women presenting with

symptoms of threatened preterm labour. After the arrest of uterine activity with Magnesium Sulphate they were randomized to vaginal Progesterone (400 mg daily until delivery) or no treatment. The use of vaginal Progesterone was associated with a longer latency preceding delivery, although no figures on PTB rate were reported (Saleh Gargari et al., 2012).

Conclusion

The knowledge about the clinical use of progestagens is expanding very rapidly, as witnessed by the number of studies published during the last year. Moreover, not less than 10 RCTs are ongoing according to their registration at clinicaltrials.gov website. Such an increased interest on hormone intervention in the prevention of PTB is so dynamic that one cannot write firm conclusions.

Anyway, at present, the clinical evidences of progestagens efficacy are summarised in Table 1. According to the listed risk factors, either Progesterone or 17OHPc seem to be useful, in definite circumstances. However, it has to be underlined that the number of trials supporting or disconfirming progestagens efficacy is limited and included only a few hundreds of women.

For the clinicians, the back home message is that a) 17OHPc is able to prevent PTB in women at risk because of their obstetric history, and b) vaginal Progesterone prevents PTB in women with an asymptomatic short cervix. The conclusion that progestagens are useless in multiple gestations reached by several trials has recently been challenged by an IPD meta-analyses. Romero et al. (2012) found a reduction in the risk of composite neonatal mortality/morbidity.

Furthermore, no firm indications on the use of progestagens for PTB prophylaxis in women presenting with other risk factors (symptomatic short cervix, threatened preterm labour) could be reached with the available data.

The policy to universally screen asymptomatic women at their morphological ultrasound examination, detecting those who are risk for short cervix for treating them with progestagens is actually debated (Romero et al., 2012). Interestingly, a cost-effectiveness analysis of such policy is ongoing in the Netherlands (van Os et al., 2011).

References

Adams MM, Elam-Evans LD, Wilson HG et al. Rates of and factors associated with recurrence of preterm delivery. JAMA. 2000;283:1591-6.

Berghella V, Figueroa D, Szychowski JM et al. 17-α-hydroxyprogesterone caproate for the prevention of preterm birth in women with prior preterm birth and a short cervical length. Am J Obstet Gynecol. 2010;202:351. e1-6.

Berghella V, Ness A, Bega G et al. Cervical sonography in women with symptoms of preterm labour. Obstet Gynecol Clin North Am. 2005;32:383-96.

Borna S, Sahabi N. Progesterone for maintenance tocolytic therapy after threatened preterm labour: a randomized controlled trial. Aust N Z J Obstet Gynaecol. 2008;48:58-63.

Briery CM, Veillon EW, Klauser CK et al. Progesterone does not prevent preterm births in women with twins. South Med J. 2009;102:900-4.

Carr-Hill RA, Hall MH. The repetition of spontaneous preterm labour. Br J Obstet Gynaecol. 1985;92:921-8.

Cetingoz E, Cam C, Sakallı M et al. Progesterone effects on preterm birth in high-risk pregnancies: a randomized placebo-controlled trial. Arch Gynecol Obstet. 2011;283: 423-9.

Combs CA, Garite T, Maurel K et al. 17-hydroxyprogesterone caproate for twins pregnancy: a double bind, randomized clinical trial. Am J Obstet Gynecol. 2011;204:221-8.

Cunningham FG. Reproductive success and failure. *Williams's obstetrics*. Section IX. 21st Edition. New York: McGraw-Hill, 2001.

da Fonseca EB, Bittar RE, Carvalho MH et al. Prophylactic administration of progesterone by vaginal suppository to reduce the incidence of spontaneous preterm birth in women at increate risk: a randomized placebo-controlled double-blind study. Am J Obstet Gynecol. 2003;188:419-24.

Di Renzo GC, Roura LC, Facchinetti F and European association of perinatal medicine study group on "Preterm Birth". Guidelines for the management of spontaneous preterm labor: identification of spontaneous preterm labour, diagnosis of preterm premature rupture of membranes, and preventive tools for preterm birth. J Matern Fetal Neonatal Med. 2011;24: 659-67.

Dodd JM, Flenady V, Cincotta R et al. Prenatal administration of progesterone for preventing preterm birth in women considered to be at risk of preterm birth. Cochrane Database Syst Rev. 2012:CD004947.

Facchinetti F, Venturini P, Blasi I et al. Changes in cervical competence in preterm labour. Br J Obstet Gynaecol. 2005; 112:23-7.

Facchinetti F, Paganelli S, Comitini G et al. Cervical length changes during preterm cervical ripening: effects of 17-alpha-hydroxyprogesterone caproate. Am J Obstet Gynecol. 2007;194:453-4.

Facchinetti F, Vaccaro V. Pharmacological use of progesterone and 17-α-hydroxyprogesterone caproate in the prevention of preterm delivery. Minerva Ginecol. 2009; 61:401-9.

Fonseca EB, Celik E, Parra M et al. Fetal Medicine Foundation Second Trimester Screening Group. Progesterone and the risk of preterm birth among women with a short cervix. N Engl J Med. 2007;357:462-9.

Goldenberg RL, Iams JD, Miodovnik M et al. The preterm prediction study: risk factors in twin gestations. National Institute of Child Health and Human Development Maternal–Fetal Medicine Units Network. Am J Obstet Gynecol. 1996; 175:1047–53.

Grobman W. Randomized controlled trial of progesterone treatment for PTB prevention in nulliparous women with cervical length less than 30 mm. Am J Obstet Gynecol. Suppl 2012; S367.

Hassan SS, Romero R, Vidyadhari D et al. Vaginal progesterone reduces the rate of preterm birth in women with a sonographic short cervix: a multicenter randomized, double-blind, placebo-controlled trial. Ultrasound Obstet Gynecol. 2011; 38:18-31.

Honest H, Bachmann LM, Gupta JK et al. Accuracy of cervicovaginal fetal fibronectin test in predicting risk of spontaneous preterm birth: systematic review. Brit Med J. 2002;325:301.

Johnson JW, Austin KL, Jones GS et al. Efficacy of 17α-hydroxyprogesterone caproate in the prevention of premature labor. N Eng J Med. 1975;293:675-80.

Kistka ZA, Palomar L, Boslaugh SE et al. Risk for postterm delivery after prevoius postterm delivery. Am J Obset Gynecol. 2007;196:241. e1-6.

Lim AC, Schuit E, Bloemenkamp K et al. 17α- Hydroxyprogesterone Caproate for the Prevention of Adevrse Neonatal Outcome in Multiple Pregnancies. A Randomized Controlled Trial. Obstet Gynecol. 2011;118:513-20.

Lockwood CJ, Ghidini A, Wein R et al. Increased interleukin-6 concentrations in cervical secretions are associated with preterm delivery. Am J Obst Gynecol. 1994; 171:1097-102.

MacIntyre DA, Sykes L, Teoh TG et al. Prevention of preterm labour via the modulation of inflammatory pathways. J Matern Fetal Neonatal Med.. 2012;25:17-20.

McLaughlin KJ, Crowther CA, Vigneswaran P et al. Who remains undelivered more than seven days after a single course of prenatal corticosteroids and gives birth at less than 34 weeks? Aust N Z Obstet Gynaecol. 2002;42:353-7.

Meis PJ, Klebanoff M, Thom E et al. Prevention of recurrent preterm delivery by 17α-hydroxyprogesterone caproate. N Eng J Med. 2003;348:2379-85.

Mercer BM, Goldenberg RL, Moawad AH et al. The preterm prediction study: effect of gestational age and cause of preterm birth on subsequent obstetric outcome. National Institute of Child Health and Human Development maternal-Fetal Medicine Units Network. Am J Obstet Gynecol. 1999; 181:1216-21.

Norman JE, Mackenzie F, Owen P et al. Progesterone for the prevention of preterm birth in twin pregnancy (STOPPIT): a randomised, double-blind, placebo-controlled study and meta-analysis. Lancet. 2009;373:2034-40.

O'Brien JM, Adair CD, Lewis DF et al. Progesterone vaginal gel for the reduction of recurent preterm birth: primary results from a randomized, double-blind, placebo-controlled trial. Ultrasound Obstet Gynecol. 2007;30:687-96.

Romero R, Mazor M, Munoz H et al. The preterm labor syndrome. Ann N Y Acad Sci. 1994;734:414-29.

Romero R, Nicolaides K, Conde-Agudelo A et al. Vaginal Progesterone in Women with an Asymptomatic Sonographic Short Cervix in the Midtrimester Decreases Preterm Delivery and Neonatal Morbidity: A Systematic Review and Meta-Analysis of Individual Patient Data. Am J Obstet Gynecol. 2012;206:124. e1-124.

Romero J, Rebarber A, Saltzman DH et al. The prediction of recurrent preterm birth in patient on 17-α-hydroxyprogesterone caproate using serial fetal fibronectin and cervical length. Am J Obstet Gynecol. 2012;207:51. e1-5.

Rouse DJ, Caritis SN, Peaceman AM et al. A trial of 17α-hydroxyprogesterone caproate to prevent prematurity in twins. N Engl J Med. 2007;357:454-61.

Rozenberg P, Deruelle P, Chauveaud A et al. Prevention of preterm delivery after successful tocolysis in preterm labour by 17α- hydroxyprogesterone caproate: a randomized controlled trial. Am J Obstet Gynecol. 2012;206. e1-9.

Saleh Gargari S, Habibolahi M, Zonobi Z et al. Outcome of vaginal progesterone as a tocolytics agent: randomized clinical trial. ISRN Obstet Gynecol. 2012;2012 ID607906.

Sela HY, Simpson LL. Preterm premature rupture of membranes complicating twin pregnancy: management considerations. Clin Obstet Gynecol. 2011;54:321-9.

Stock S, Norman J. Preterm and term labour in multiple pregnancies. Semin Fetal Neonatal Med. 2010;15:336-41.

van Os MA, van der Ven JA, Kleinrouweler CE et al. Preventing preterm birth with progesterone: costs and effects of screening low risk women with a singleton pregnancy for short cervical length, the Triple P study. BMC Pregnancy Childbirth. 2011;11:77. Abstract.

Actions of progestins for the inhibition of cervical ripening and uterine contractions to prevent preterm birth

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Abstract

The importance of progesterone (P4) for maintenance of pregnancy, its role in cervical ripening and uterine contractions is at least partly established and therefore, not surprisingly, the basis for the concept to use P4 as a treatment for preterm birth. Due to the complexity of the condition of preterm birth there are still questions concerning the optimal population that might benefit, timing of treatment, dosage, vehicle and route of administration. Recently vaginal P4 and intramuscular 17-alpha-hydroxyprogesterone caproate (17P) have been used to prevent preterm birth in patients with a high risk for early delivery.
The aim of this study was to assess cervical changes throughout pregnancy in rats and the timing of term and preterm delivery after various progestin treatments given by different routes and vehicles in hope of identifying better treatment regimens.
This paper presents results that suggest that there are better routes of treatment than the vaginal route (e.g. topical), that the vehicle used in many of the clinical studies (Replens®) is not appropriate due to a low release of the steroid and consequently low uptake of P4, and that inhibition of birth is primarily due to inhibition of uterine contractility that can be achieved by supplementation of P4 but not with 17P.

Key words: **17-alpha-hydroxyprogesterone, cervical ripening, preterm birth, progesterone, uterine contractions.**

Introduction

Preterm birth is the major challenge in obstetrics today. Despite all efforts and intensive research, the rate of preterm birth has risen in the last years and varies from about 5% to 11% in Europe (Euro-Peristat Project, 2008). Although only a small number of all preterm births occur before 34 weeks (approximately one sixth), these infants are especially endangered and are responsible for the majority of morbidity, mortality and costs (Werner et al., 2011). There are even higher rates of preterm birth observed in the USA, where the problem escalated in the last 25 years with an increase of the preterm birth rate of 36% (Martin et al., 2007). 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. Many biochemical and functional changes occur in the cervical connective tissue during gestation, which are summarized in the term cervical ripening. It is an active biochemical process with similarities to an inflammatorylike reaction and occurs independent of uterine contractions (Liggins, 1981; Garfield et al., 1998). Arpad Csapo was a pioneer in characterizing P4 as a key player for the maintenance of pregnancy (Csapo, 1956). Csapo's observations were mainly derived from animal studies on rabbits and rodents where, in contrast to primates and humans, a sharp P4 withdrawal occurs before the onset of labor and delivery. The biochemical and molecular actions of P4 in this specific context were illuminated by several studies. Undoubtedly, if P4 action is blocked, the cascade of cervical ripening will lead to clinical, biochemical and morphological changes (Chwalisz and Garfield, 1994). Despite the findings and the progress the exact mechanism how P4 exerts its effects on the uterus is still unrevealed. Nevertheless, supported by experimental results from animal and in vitro

studies, there is growing evidence also from large randomized placebo-controlled clinical trials that a P4 supplementation reduces the rate of preterm birth in a certain subpopulation of patients that might be identified in an asymptomatic state in midgestation by vaginal ultrasound examination and by measuring the cervical length (da Fonseca et al., 2007; Hassan et al., 2011; Romero et al., 2012).

A better understanding of the mechanisms of preterm labor, a method to diagnose endangered women and the renewed appreciation of the role of P4 in the prevention of preterm labor are key factors in the concept of pharmacological prevention of preterm birth. Nevertheless, important questions concerning the exact mechanisms of action, optimal progestin formulation, dosage, route of administration and timing of therapy are still only partly answered. We therefore performed in vitro and in vivo studies in the rat, focusing on the type of progestin, the route of administration and the vehicle used for vaginal and topical administration using rats that deliver at term withouth prior treatment with progestins. This is a well recognized bioassay of progestin and antiprogestin potency that has been used reliably in many studies. Time of delivery was determined and cervical changes were assessed by an optical method using an endoscopic camera and by measurements of light-induced fluorescence with an instrument called collascope.

Materials and Methods

Timed-pregnant Sprague-Dawley rats (N = 6/ group) were treated 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. Treatments: Injections (subcutaneous (s.c.), daily): vehicle: sesame oil; P4 (4 mg); R5020 (2 mg); 17P (10 mg); vaginal (bid): vehicle: Replens® (base for Crinone®, Columbia Laboratories) ; P4 (15 mg, Crinone®); R5020 (1 mg); oral (bid): vehicle: sesame oil or H_2O; P4 (15 mg); topical (bid): vehicle: Replens®, sesame oil or fish oil; P4 (15 mg). The control rats were treated with the appropriate vehicles. The antiprogestin, RU-486 (3 mg in 0.2 ml sesame oil), was injected s.c. once on day 16 of gestation.

For the measurements with the collascope and the endoscopic camera the animals were anaesthetized. The amount of cervical collagen (assessed by measurement of the autofluorescent properties of cross-linked collagen with an instrument, termed collascope (Reproductive Research Technologies, Houston, TX, USA) and changes of the surface area of the cervix (calculated from digitized photographs by morphometric methods using the software ImageJ 1.43) were evaluated in vivo every second day starting on day 13 of pregnancy and on postpartum days 3 and 5. Student's t-test was used to compare the results of a treatment group to its specific control group at any time in gestation and postpartum and also to determine the differences in delivery times. A two-tailed probability value of $P < 0.05$ was considered statistically significant.

For more details see the material and method section in the two publications by Kuon et al. (Kuon et al., 2010; 2011). All procedures were approved by the Animal Care and Use Committee of the St. Joseph's Hospital and Medical Center in Phoenix.

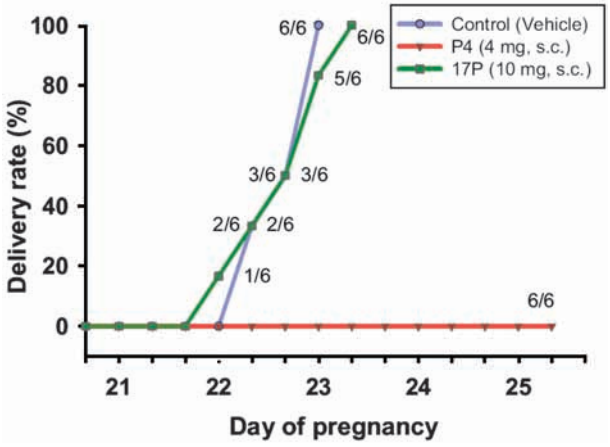

Fig. 1. — The percentage of animals (N = 6/ group) that delivered vs. day of pregnancy after various treatments. Delivery times after daily treatment (s.c.) with vehicle, P4 and 17P. Note that injections of P4 completely blocked delivery, whereas 17P had no effect on delaying term delivery (Kuon et al., 2010).

Results

Effects of treatment with P4

Subcutaneous and topical P4 with vehicle sesame oil, partially, or fish oil, completely blocked delivery. Vaginal, oral and topical P4 in Replens® failed to inhibit delivery (Fig. 1, 6).

Light-induced fluorescence (LIF) is significantly ($P < 0.05$) higher in the P4 injection group and increases in surface area (SA) of the cervix are lower correspondingly compared with vehicle controls for any day of gestation (Fig. 3A, 3B). There is no difference ($P > 0.05$) between day 25 (delayed delivery) in the P4 injection group (Fig. 1) compared to control animals at day 21 of gestation. LIF and SA before treatment at day 13 show no significant

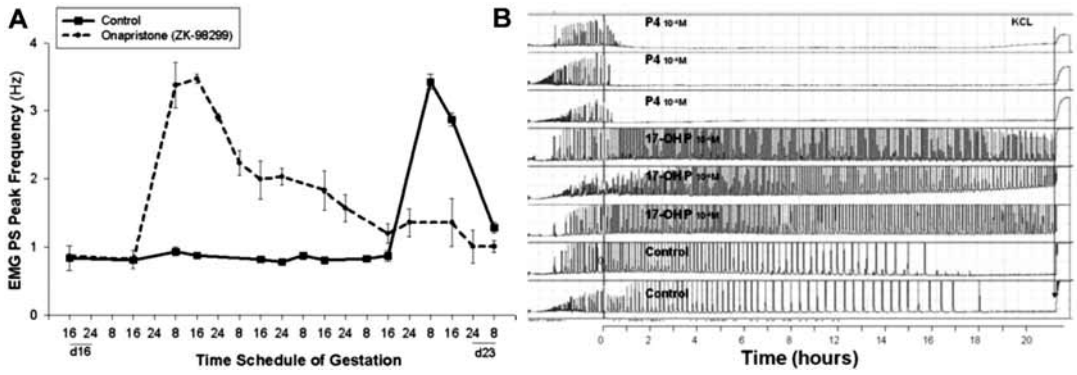

Fig. 2. — **A.** EMG activity in animals delivering at term and preterm after onapristone. Treatment with onapristone (ZK-98299) on day 16 of pregnancy induces preterm delivery and an increase of the electromyographic power spectrum (PS) peak frequency in rats (Modified from figures in Shi et al., 2008) ; **B.** The effects of P4 (top three traces) and 17P (= 17-OHP); traces 4–6 from top) on myometrial contractility. Potassium chloride was added to the baths just prior to termination of the experiment. Note the inhibition of myometrial contractility with P4, but not with 17P (Ruddock et al., 2008).

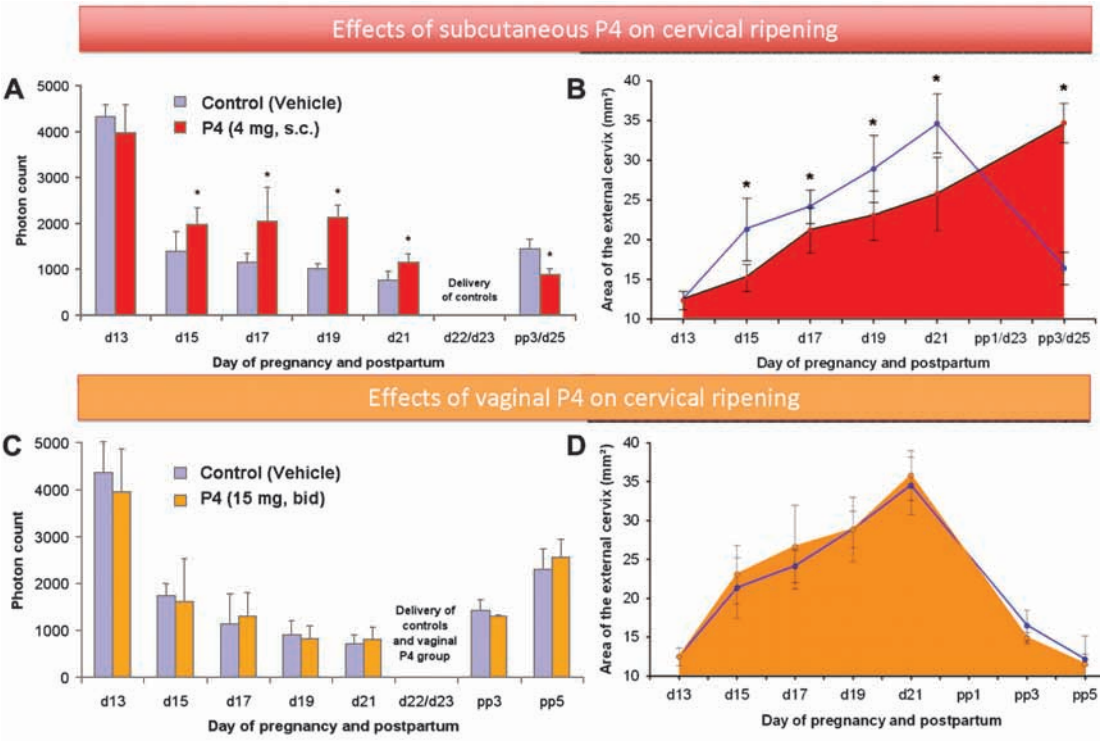

Fig. 3. — **A + C.** Bar graphs showing means ± SD of cervical light-induced fluorescence (LIF) obtained in vivo from pregnant rats at different days of pregnancy and postpartum (N = 6/ group) treated with P4 or vehicle. Figure 3A: Daily treatment with vehicle (controls) or P4 (4 mg, s.c.). Note that delivery is inhibited in the treatment group. Figure 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 (From Kuon et al., 2010) ; **B + D.** Figure showing surface area (SA) of cervix (means ± SD of the surface area of the cervix) obtained in vivo from pregnant rats (N = 6/ group) at different days of pregnancy and postpartum treated with P4 or vehicle (same treatment regime as for Figure 3 A + C) (From Kuon et al., 2011).
Asterisks indicate $P < 0.05$ compared with controls.

differences ($P > 0.05$) between the treatment and the control group and this is similar for all treatment groups mentioned with other treatments (Figs. 3, 4).

There are no significant differences ($P > 0.05$) between the vaginal P4 group and vehicle controls at any time in gestation (Fig. 3C, 3D).

Effects of treatment with 17P

17P failed to inhibit delivery in rats (Fig. 1, 6). LIF is significantly higher ($P < 0.05$) and increases in SA are lower in the 17P treated group (until day 19 only) compared with vehicle controls (Fig. 4).

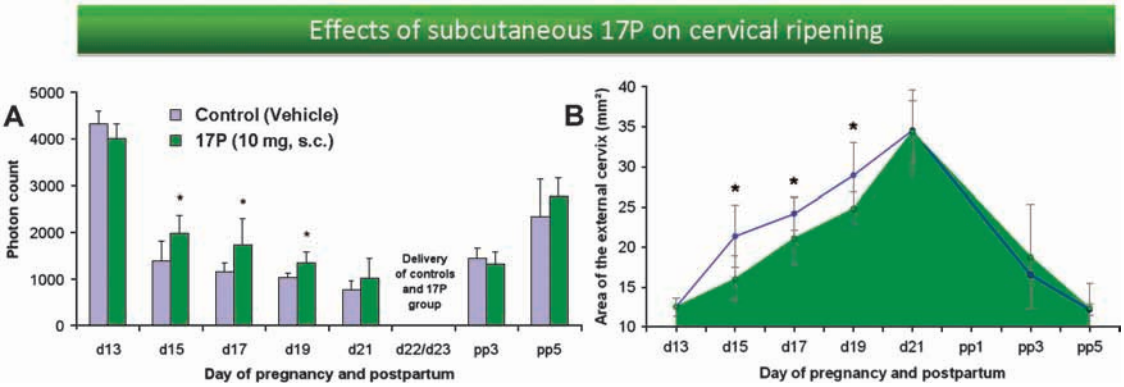

Fig. 4. — Daily treatment of pregnant rats (N = 6/ group) with vehicle (controls) or 17P (10 mg, s.c.). Note that significant differences are only observed until day 19 of gestation (From Kuon et al., 2010; 2011).

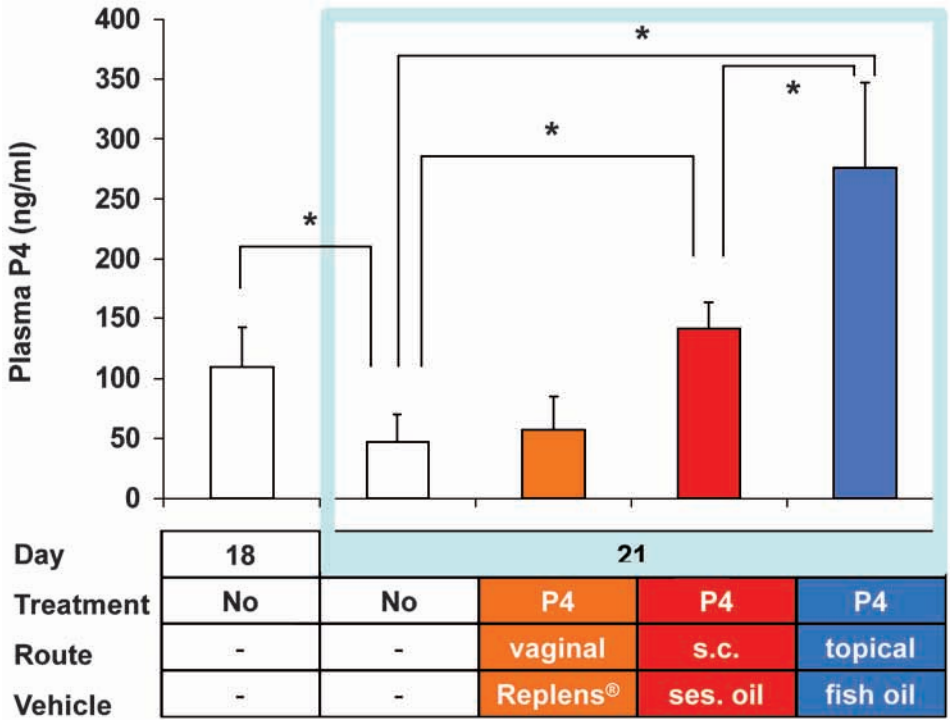

Fig. 5. — Plasma P4 levels in pregnant rats (N = 6/ group) on day 18 and 21 without any treatment (control) and on day 21 after treatment from day 18 until delivery with vaginal P4 (15 mg, bid), s.c. P4 (4 mg), or topical P4 in fish oil (15 mg, bid). Asterisks indicate $P < 0.05$ compared with controls. Note the physiological P4 withdrawal from day 18–21 in untreated rats, which is prevented by s.c. and topical P4, but not by vaginal P4 (From Kuon et al., 2010).

Effects of treatment with R5020

Subcutaneous injections (s.c.) and vaginal R5020 completely blocked delivery (Fig. 6).

Effects of treatment with RU-486

RU-486 treatment increases the SA and LIF are lower ($P < 0.05$) during preterm delivery (rats were delivering 24-48 hours after injection) compared to controls (results not shown).

Comment

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, vehicle and the route of administration. An optical evaluation of the exterior of the cervix and the use of light-induced fluorescence (LIF) of cross-linked collagen with an instrument called collascope can be useful to assess quantitative changes in cervical ripening in vivo. Throughout

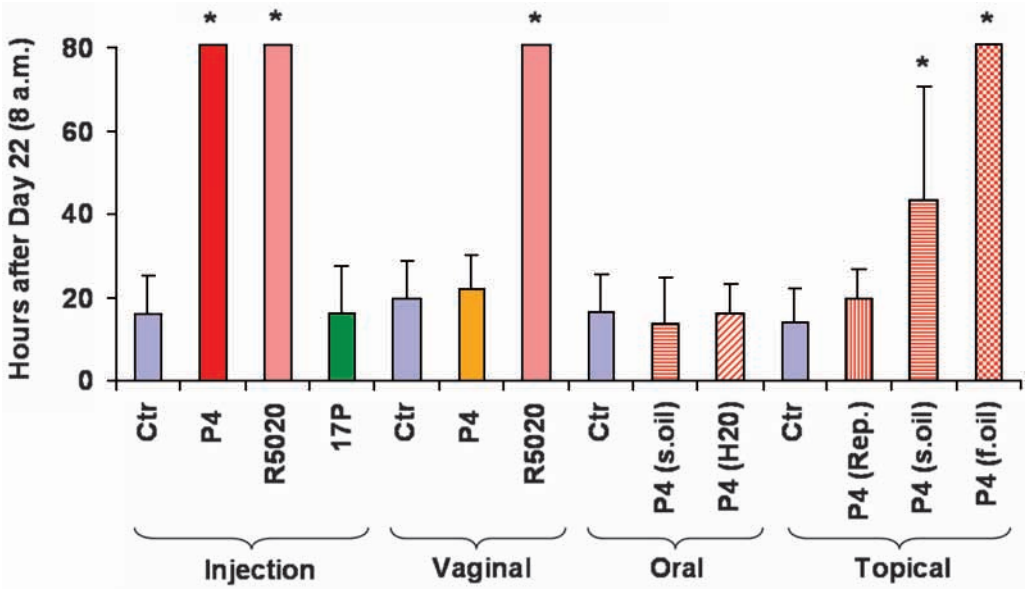

Fig. 6. — Time of delivery (= hours after 8 a.m. of day 22 of gestation) of pregnant rats (N = 6/ group) treated (daily from day 13 of gestation) with vehicles (Ctr) and various progestins by different routes of administration – injections (s.c.; daily): vehicle: sesame oil; P4 (4 mg); R5020 (2 mg); 17P (10 mg); vaginal (bid): vehicle: Replens®; P4 (15 mg, Crinone®); R5020 (1 mg); oral (bid): vehicle: sesame oil or H_2O; P4 (15 mg); topical (bid): vehicle: Replens®, sesame oil or fish oil; P4 (15 mg). Asterisks indicate $P < 0.05$ compared with controls (From Kuon et al., 2010).

gestation the collascope indicates a decreasing photon count which describes the remodelling of the extracellular matrix including a decrease in collagen concentration and switch from insoluble to more soluble collagen (Kuon et al., 2010). This decrease of collagen results in the softening of the cervix which is assessed by the endoscopic camera with an increase in SA of the cervix as a consequence. We hypothesize that the increase of the surface area of the external cervix during cervical ripening is due to the rearrangement of the extracellular matrix, the dilatation of blood vessels, the increase of hyaluronan and the influx of water causing the cervical tissue to swell. As anticipated in the postpartum period the LIF increases progressively indicating a reversal of ripening, whereas the SA of the cervix decreases. As the decrease of LIF and the increase in SA of the cervix are decelerated in the parenteral (s.c. injection) P4 (Fig. 3A, 3B) and 17P (Fig. 4A, 4B) group we conclude that these treatments delay cervical ripening but do not entirely prevent it, indicating the involvement of other control pathways. Vaginal P4, even at 7.5 X the parenteral dose, does neither inhibit ripening, as indicated in this study by changes in the LIF or the SA of the cervix, nor delivery possibly because of reduced uptake (Fig. 3C, 3D). P4, but not 17P, inhibits delivery and may be more effective for treatment of preterm labor (Fig. 1, 6). However this depends crucially on the route of administration and the vehicle, as only s.c. and topical P4 in sesame oil (partially) and fish oil (completely), but not vaginal, oral or topical P4 in Replens® blocked delivery (Fig. 6). As the cervix manages to ripen also in the parenteral P4 treated group at the end of gestation we conclude that the block of delivery is not due to an unripe cervix but must be due to an inhibition of uterine contractions.

The present study does demonstrate significant differences in cervical ripening in early pregnancy with some progestin treatments and this may correspond to differences in cervical length estimated in some of the clinical studies of progestin treatment (Facchinetti et al., 2007; O'Brien et al., 2009).

Current studies for prevention of preterm birth with P4

P4 has long been considered as a candidate to regulate uterine contractility and cervical function and consequently the onset and progression of labor. Early studies also discussed the potential benefit of 17P for the treatment or prevention of preterm labor (Johnson et al., 1975; Keirse, 1990). Several clinical studies investigated in the last few years the effects of a treatment with intramuscular 17P and P4 given orally and vaginally on the reduction of preterm birth and cervical changes (Durnwald et al., 2009; Facchinetti et al., 2007; Fonseca et al., 2003, 2007; de Franco et al., 2007; Hassan et al., 2011; O' Brien et al., 2007, 2009; Rai et al., 2009). These studies and their conclusions raise questions about the ability of P4 and 17P to inhibit preterm birth and also

were difficult to compare, as the treatments and study populations included were significantly different.

As recommended in a current report of the American College of Obstetrics and Gynecology (ACOG) Committee and the Society for Maternal Fetal Medicine (SMFM), P4 should only be used for the prevention of preterm delivery in a "singleton pregnancy with a prior spontaneous preterm birth due to spontaneous preterm labor or premature rupture of membranes" (Society for Maternal Fetal Medicine Publications Committee. ACOG Committee Opinion number 419 October 2008 (replaces no. 291, 2008)). The Committee pointed out that the ideal P4 formulation still is unidentified, as well as the dosage and route of administration.

In a systematic review, utilizing individual patient data of five trials using vaginal P4 for prevention of preterm birth in women with an asymptomatic sonographic short cervix, Romero et al.(2012) tried to address this problem and concluded that the treatment was associated with a significant reduction in the rate of preterm birth before 28 (number needed to treat (NNT) 18), 33 and 35 weeks and also an improved neonatal outcome. The study showed a 42% reduction of the rate of preterm birth before 33 weeks of gestation in singleton pregnancies that received vaginal P4 (Prochieve®, 8% micronized progesterone in Replens base, Columbia Laboratories---the same product we used in this study for vaginal P4), which was the primary outcome (NNT 11). A significant reduction was shown for women with a previous history of preterm birth as well as for women without. The study also pointed out that there is no difference in efficacy of 90-100 mg vaginal P4 administered as a gel compared to 200 mg P4 capsules. However in twin gestations vaginal P4 failed to reduce the rate of preterm birth significantly before gestational week 33. As the optimal cut-off of a short cervix is still not determined, Romero et al. (2012) chose a cervical length below 25 mm in midtrimester, as this length represents the 10th percentile for the cervical length in this period of gestation. Romero also suggested P4 supplementation as an alternative therapy to cerclage in women with a singleton gestation, previous spontaneous preterm birth and a short cervix < 25 mm, as the cerclage reduces preterm birth before 35 weeks by 30 % (Berghella et al., 2011) whereas the non-invasive P4 shows a reduction before 33 weeks by 46% (Berghella et al., 2011; Romero et al., 2012). Despite some controversial results, the U.S. Food and Drug Administration (FDA) in 2011 approved Makena® (17 alpha hydroxyprogesterone caproate) to reduce the risk of preterm delivery before 37 weeks of pregnancy, in pregnant women with a history of at least one spontaneous preterm birth. In February 2012, due to inadequate data, the FDA disapproved the P4 vaginal gel Crinone® for the reduction of risk of preterm birth in women with short uterine cervical length at the midtrimester of pregnancy, saying that "Crinone® was not more effective than the placebo". The FDA panel especially was sceptical about foreign data as analyses indicated that P4 gel was not associated with a reduction in preterm birth in the U.S. subpopulation at any gestational age. Our studies reviewed in this paper predicted the outcome of the FDA trial for Crinone®. However the members of the advisory board of the FDA agreed at least that the use of P4 vaginal gel in pregnancy is safe. This indicates that the time of a universal recommendation to treat patients with a short cervix in midtrimester with vaginal P4 gel has not yet come. Additionally this demonstrates the importance of intense further research and also the need to investigate alternative ways of P4 supplementation concerning the choice of vehicle, the route of administration, the timing of treatment and the selection of the population.

Proposed mechanism of actions

There is a great body of evidence about the role and effects of P4 on cervical ripening that can be induced with antiprogestins in all species examined so far, including humans and primates (Chwalisz and Garfield, 1994). Supported by investigations of our group, there is growing evidence of direct actions of P4 on uterine contractility.

In the rat model we showed that P4 treatment delayed cervical ripening and completely blocked parturition (Figs. 1, 3, 6) (Kuon et al., 2010;2011). However, cervical ripening only was decelerated and not blocked and the cascade was finalized despite continual supplementation of P4. Assuming that both a ripe cervix and uterine contractions are required for successful delivery, it may be concluded that a block (i.e. inhibition) of uterine contractions is the responsible mechanism for delay of parturition in the rat model.

Furthermore treatment with onapristone, which is a pure antiprogestin, induces preterm delivery and increases the uterine electromyographic activity in rats (Fig. 2A) (Shi et al., 2008)). It has been suggested that P4 regulates parturition through genomic actions mediated by the nuclear P4 receptor that function as ligand-activated transcription factors and modulates various proteins that are thought to be involved in maintenance of pregnancy and initiation of labor (Garfield et al., 1980;1998). Moreover, recent studies indicate a direct suppression of uterine contractility by P4, but not 17P, by binding to membrane receptors through a non-genomic, rapid

mechanism and direct affection of cell function by modulating intracellular signal transduction pathways (Fig. 2B) (Ruddock et al., 2008). This inability of 17P to inhibit uterine activity has also been reported by others (Sexton et al., 2004). Recently different types of P4 receptors on the plasma membrane have been identified, and are thought to be responsible for these fast actions of P4 (Mesiano, 2004).

Our group investigated in in vitro studies the inhibition of uterine contractility with various tocolytics with and without P4 (Baumbach et al., 2012). P4 alone had little effect to inhibit contractility in uterine tissues from women undergoing cesarean section at term. However, P4 combined with the tocolytic drug nifedipine was significantly more effective than nifedipine alone. Further studies are essential to evaluate the potential use of P4 as a tocolytic therapy especially in combination with all the different tocolytic compounds that are on the market. However the first results of the studies are promising and encouraging for further research.

The myometrium is composed of billions of smooth muscle cells, which are coupled together electrically by gap junctions that are composed of connexin proteins, the most important of which is thought to be connexin 43 (Garfield et al., 1998). During the long period of pregnancy in which uterine quiescence is required, the density and concentration of these cell to cell contacts is low, indicating poor coupling and decreased electrical conductance. Finally at term this situation changes dramatically: the cell junctions increase, the cells become coupled and form a so called electrical syncytium that is thought to be essential for effective uterine contractions (Sims et al., 1982). The density of myometrial gap junctions is downregulated by P4, whereas P4 antagonists dramatically upregulate their expression (Garfield et al., 1998). However, as several hours of P4 administration is needed to observe these genomic effects, this mechanism can not account for the acute and fast acting tocolytic effects of P4 that have been described. As mentioned before no withdrawal of P4 hormone level can be detected in humans. Nevertheless changes of the P4 receptor in the decidua, amnion, and cervix have been shown and might account for a functional P4 withdrawal that corresponds to the fall of hormone levels in rodents (Zakar and Hertelendy, 2007).

One of the established pathways that lead to preterm cervical changes and contractions of the uterus are infections, which are associated with an increase of the release of proinflammatory cytokines such as interleukin (IL)-1beta, tumor necrosis factor-alpha and IL-8 that induce cervical ripening by activating the production of matrix metalloproteinases and thereby the degradation of the cervical collagen structure (Peltier, 2003). P4 has the ability to modulate antibody production and also to reduce the production of proinflammatory cytokines by direct and indirect interaction on immune cells and with this it might interrupt the cascade of inflammation and the irreversible degradation of the matrix that lead to softening and effacement (Kelly, 1994; Word et al., 2007).

S.c. administration of 17P also delayed cervical ripening (Fig. 4)., although less effectively than did P4 which indicates the importance of the choice of the type of progestin.

Route of administration

Recent reports that studied the effects of P4 supplementation focused on the vaginal route of administration (Romero et al., 2012). The vaginal route is thought to have some extra benefits when effects focused on the uterus are desired (De Ziegler et al., 1997). Not unlike the concept of the liver first-pass effect after administration of oral drugs, De Ziegler et al. established the term "uterine first-pass effect", that might be attributed to the network of arteries and veins linking the vagina to the uterus. This effect should favour an optimal uterine exposure after transvaginal treatment with sex steroids (Cicinelli et al., 2000). There are many studies of the uptake of P4 by the vaginal route that established the phenomenon of the uterine first-pass effect, but most of these studies were accomplished on postmenopausal women. Richardson et al. showed that the absorption of drugs across the vaginal epithelium is influenced by several physiologic factors, such as the properties of the epithelium and the volume, viscosity and pH of the vaginal fluid (Richardson, 1992). Thus the uptake of vaginally administered drugs may differ in pregnancy due to changes in the vagina epithelium or other factors. In our studies with P4, given by different routes and vehicles, we measured plasma P4 level changes at the end of pregnancy (Fig. 5).

Treatment with vaginal P4 did not inhibit the physiological P4 withdrawal, had no decelerating effect on cervical ripening and also failed to inhibit delivery in our study, which questions this route of administration, at least in rats (Figs. 3C, 3D, 5, 6). Vaginal R5020 blocked delivery effectively and possibly solved the problem of low uptake by the vaginal route by its high binding affinity to the P4 receptor (Fig. 6).

In this study oral P4 failed to inhibit delivery in rats probably because of a low uptake and the liver first-pass effect (Fig. 6). The main advantage of oral administration of P4 is its non-invasiveness and consequent acceptability. However, there is a high

variation of absorption of oral P4, and the metabolism by the first-pass effect in the liver is rapid, which makes the oral administration essentially ineffective. Moreover, considerable side effects such as intrahepatic cholestasis, sleepiness, fatigue, headaches and nausea are more common when P4 is given orally (O'Brien et al., 2007; Rode et al., 2009). Even sedative and hypnotic effects or fluid retention have been described. Three randomized trials compared oral P4 to placebo for prevention of preterm birth. In the studies published in 1986 and 1991, oral P4 did not prolong gestation in patients treated for preterm labor (Erny et al., 1986; Noblot et al., 1991). In contrast, Rai et al. reported a reduction in preterm delivery and also a reduction in neonatal morbidity and mortality in women with a history of at least one preterm birth who received oral P4 from recruitment (18-24 weeks) until 36 weeks or delivery (Rai et al., 2009).

Transdermal supplementation of P4 to prevent preterm birth has not been studied in humans although it is quite common to use this route for the application of steroids in humans for other indications. Sitruk-Ware explained that this route may additionally help administering other progestins that are inactive when given orally (Sitruk-Ware, 2007). For example the progestin nestorone (a 19-norprogesterone derivative), that possess a high specificity for binding to the P4 receptor with no or little interaction with other steroid receptors, is rapidly metabolized and therefore inactive after oral administration but shows a high bioavailability after transdermal application and thus promising studies are underway to investigate transdermal gels and sprays for contraception (Sitruk-Ware and Nath, 2010). R5020, the 19-norprogesterone that we used in our animal study, blocked parturition very effectively, also in very low doses and might additionally be a candidate for transdermal application. Furthermore, depending on the carrier, the transdermal route was associated with a high uptake and plasma P4 levels and was very effective in postponing delivery in rats (Figs. 5, 6).

Holzer et al. (2005) investigated the effects and side-effects of P4 cream on the female skin. They described minimum side-effects, which also occurred in the placebo group and therefore they concluded that the vehicle used might be responsible for the skin irritations. Additionally the study reported that topical P4 improved the visco-elastic properties and firmness of the skin significantly, which might also help to reduce the appearance of stretch marks as a positive side-effect. Thus this route of administration should be considered as a non-invasive, easy to administer alternative and might become the focus of future research.

Because of the ineffectiveness of oral P4, luteal support in infertility treatments was initially given by intramuscular injections but is now abandoned due its side-effects, such as patient discomfort, inflammatory reactions and even abscesses (Kleinstein, 2005). The effectiveness of intramuscular injections of P4 to prevent preterm birth has not been evaluated in clinical trials. The reason for this is that daily intramuscular injections would be required to maintain therapeutic serum levels due to the relatively short half-life of P4. This would make this intervention very invasive, especially if P4 was to be given by prolonged prophylactic administration to women at increased risk for preterm birth.

17P is a long-acting progestin and is given exclusively intramuscularly in clinical trials Even with weekly intramuscular injections, however, side-effects such as injection-side 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 (Meis et al., 2003). Our data supports the view that 17P is a weak progestin and thus unlikely to be useful for preterm labor treatment.

More data is needed before any formulation and route of P4 administration for prevention of preterm birth can be recommended over the others. In our study only s.c. injections of P4 and transdermal administration of P4 in sesame oil (partly) and in fish oil (completely) (not in Replens®, see below) blocked delivery (Fig. 6). Notably, none of these routes has been used in clinical trials to date. On the other hand, oral P4 and vaginal P4 administration, studied in humans so far, have not had any effect on time of delivery in our study in rats. Concluding from recent studies that showed some minor effects on preterm birth after vaginal treatment with P4 it is possible that the effects of topical or s.c. P4 might be far superior.

The importance of the vehicle

As described above the favoured pathway of administrating P4 in the recent studies has been the vaginal route. Hassan et al. used a gel (Crinone®) utilizing a bioadhesive, biocompatible polymer as a base (Replens) that is believed to attach to the vaginal epithelium with proposed advantages such as facilitated diffusion of P4 across the vaginal epithelium to accomplish a sustained release effect. Another way to administer P4 vaginally is the use of capsules/tablets, which originally have been designed for oral administration. These capsules are difficult to administer and are associated with discomfort (itching, burning), local irritation of the vaginal mucosa (e.g. erythema) and vaginal discharge, adverse effects which are also seen after treatment with Crinone® (Kleinstein, 2005). Kleinstein et al.

compared the efficacy and tolerability of vaginal P4 capsules (Utrogest® 200, a soft gelatin capsule containing P4 suspended in oil) with P4 gel (Crinone® 8%). Both treatments resulted in similar outcomes with respect to implantation, ongoing pregnancy, and abortion rates. In another study, Kleinstein et al. compared the bioavailability of Utrogest® 200 to that of Crinone® in healthy young women (Kleinstein et al., 2002). The duration of peak levels and the bioavailability was higher with Utrogest® 200 which question the superiority of the vaginal gel that is often assumed. The adverse effects of the mentioned vaginal treatments might be resolved by a vaginal ring that releases P4 continuously. Vaginal rings that deliver hormones were developed for contraception and hormone therapy and have been very successful by combining many advantages that might also be important in the context of a treatment for preterm birth: high acceptability and user satisfaction as the long-acting system can be easily administered by the patient, it needs no daily attention or administration as it stays in place without being noticed. Additionally the problem of a discontinuous supply of the drug due to a system that needs replacement might be resolved with a vaginal P4 ring.

There are numerous topical P4 fabricates with different characteristics on the market to treat symptoms in postmenopausal women. The uptake of P4 is greatly influenced by the characteristics of the formulation including P4 concentration and solubility of the formulation, physical and chemical properties of ingredients, the effect on the integrity of the skin and also the site and surface area of the application (Stanczyk et al., 2005).

In our study, topical micronized P4 in oil but not in Replens®, the vehicle of the vaginal P4 gel Crinone®, was very effective to inhibit delivery and also to forestall the withdrawal of P4 at the end of gestation (Figs. 5, 6). Again this indicates the importance of the vehicle, also for the transdermal route of administration.

Conclusion

There is growing evidence for beneficial effects of P4 as a treatment of preterm birth. However present clinical studies are still controversial and pose serious questions about the effectiveness of Crinone®. Our own studies demonstrated:

1. The vaginal route is not the best route of administration. Topical (transdermal) administration is better. Topical micronized P4 in fish oil is an effective route to block delivery and might be a novel route of administration in clinical studies to treat preterm birth.
2. The base used for the vaginal gel preparation (Replens®) is not appropriate because when it is used topically it does not allow uptake and inhibit delivery.
3. The choice of the progestin is crucial. P4 and R5020, but not 17P, inhibit delivery in rats. More effective treatment of preterm birth might be achieved with a more potent progestin (as we show for R5020).
4. P4 is able to block uterine contractions. Inhibition of birth is primarily due to inhibition of uterine contractility and not to a block of cervical ripening.

The route of administration and the vehicle is critical and ongoing research should not limit the investigations to the vaginal route of administration, as the alternatives, in particular the transdermal application of P4, might be promising. Further studies are indispensable to address the effects of a P4 supplementation given by other routes and in other vehicles. Additionally, as P4 successfully suppresses uterine contractions in our experiments, its application as a tocolytic agent to treat the acute symptoms of preterm labor needs to be studied intensely.

References

Baumbach J, Shi SQ, Shi L et al. Inhibition of uterine contractility with various tocolytics with and without progesterone: in vitro studies. Am J Obstet Gynecol. 2012; 206(3):251-5.

Berghella V, Rafael TJ, Szychowski JM et al. Cerclage for short cervix on ultrasonography in women with singleton gestations and previous preterm birth: a meta-analysis. Obstet Gynecol. 2011;117:663-71.

Chwalisz K, and Garfield RE. Antiprogestins in the induction of labor. Ann N Y Acad Sci. 1994;734:387-413.

Cicinelli E, de Ziegler D, Bulletti C et al. Direct transport of progesterone from vagina to uterus. Obstet Gynecol. 2000; 95:403-6.

Csapo A. Progesterone block. Am J Anat. 1956;98:273-91.

da Fonseca EB, Bittar RE, Carvalho MH et al. Prophylactic administration of progesterone by vaginal suppository to reduce the incidence of spontaneous preterm birth in women at increased risk: a randomized placebo-controlled double-blind study. Am J Obstet Gynecol. 2003;188:419-24.

da Fonseca EB, Celik E, Parra M, et al.. Progesterone and the risk of preterm birth among women with a short cervix. N Engl J Med. 2007;357:462-9.

De Ziegler D, Bulletti C, De Monstier B et al. The first uterine pass effect. Ann N Y Acad Sci. 1997;828:2919.

DeFranco EA, O'Brien JM, Adair CD et al. Vaginal progesterone is associated with a decrease in risk for early preterm birth and improved neonatal outcome in women with a short cervix: a secondary analysis from a randomized, double-blind, placebo-controlled trial. Ultrasound Obstet Gynecol. 2007;30:697-705.

Durnwald CP, Lynch CD, Walker H et al. The effect of treatment with 17 alpha-hydroxyprogesterone caproate on changes in cervical length over time. Am J Obstet Gynecol. 2009;201: 410-5.

Erny R, Pigne A, Prouvost C et al. The effects of oral administration of progesterone for premature labor. Am J Obstet Gynecol. 1986;154:525-9.
Euro-Peristat Project, wS, EUROCAT, EURONEOSTAT. European Perinatal 4 Health ReportAvailable. 2008; from: http://www.europeristat.com
Facchinetti F, Paganelli S, Comitini G et al. Cervical length changes during preterm cervical ripening: effects of 17-alpha-hydroxyprogesterone caproate. Am J Obstet Gynecol. 2007;196:451-4.
Garfield RE, Kannan MS, Daniel, EE. Gap junction formation in myometrium: control by estrogens, progesterone, and prostaglandins. Am J Physiol. 1980;238:C81-9.
Garfield RE, Saade G, Buhimschi C et al. Control and assessment of the uterus and cervix during pregnancy and labour. Hum Reprod Update. 1998;4:673-95.
Hassan SS, Romero R, Vidyadhari D et al. Vaginal progesterone reduces the rate of preterm birth in women with a sonographic short cervix: a multicenter, randomized, double-blind, placebo-controlled trial. Ultrasound Obstet Gynecol. 2011;38:18-31.
Holzer G, Riegler E, Hoenigsmann H et al. Effects and side-effects of 2% progesterone cream on the skin of peri- and postmenopausal women: results from a double-blind, vehicle-controlled, randomized study. Br J Dermatol. 2005; 153:626-34.
Johnson JW, Austin KL, Jones GS et al. Efficacy of 17alpha-hydroxyprogesterone caproate in the prevention of premature labor. N Engl J Med. 1975;293:675-80.
Keirse MJ. Progestogen administration in pregnancy may prevent preterm delivery. Br J Obstet Gynaecol. 1990;97:149-54.
Kelly RW. Pregnancy maintenance and parturition: the role of prostaglandin in manipulating the immune and inflammatory response. Endocr Rev. 1994;15:684-706.
Kleinstein J. Efficacy and tolerability of vaginal progesterone capsules (Utrogest 200) compared with progesterone gel (Crinone 8%) for luteal phase support during assisted reproduction. Fertil Steril. 2005;83:1641-9.
Kleinstein J, Schlegelmilch R, Mazur D et al. Pharmacokinetic comparison of progesterone capsules with a progesterone gel after vaginal administration. Arzneimittelforschung. 2002; 52:615-21.
Kuon RJ, Shi SQ, Maul H et al. Pharmacologic actions of progestins to inhibit cervical ripening and prevent delivery depend on their properties, the route of administration, and the vehicle. Am J Obstet Gynecol. 2010;202:451-9.
Kuon RJ, Shi SQ, Maul H et al. A novel optical method to assess cervical changes during pregnancy and use to evaluate the effects of progestins on term and preterm labor. Am J Obstet Gynecol. 2011;205(1):82.e15-20.
Liggins CG. Cervical ripening as an inflammatory reaction. In Elwood, DA and Andersson, A.B.M. Cervix in Pregnancy and Labour.Churchill Livingstone, Edinburgh. 1981;1-9.
Martin JA, Hamilton BE, Sutton PD et al. Births: final data for 2005. Natl Vital Stat. 2007; Rep 56:1-103.
Meis PJ, Klebanoff M, Thom E et al. Prevention of recurrent preterm delivery by 17 alpha-hydroxyprogesterone caproate. N Engl J Med. 2003;348:2379-85.
Mesiano S. Myometrial progesterone responsiveness and the control of human parturition. J Soc Gynecol Investig. 2004; 11:193-202.
Noblot G, Audra P, Dargent D et al. The use of micronized progesterone in the treatment of menace of preterm delivery. Eur J Obstet Gynecol Reprod Biol. 1991;40: 203-9.
O'Brien JM, Adair CD, Lewis D et al. Progesterone vaginal gel for the reduction of recurrent preterm birth: primary results from a randomized, double-blind, placebo-controlled trial. Ultrasound Obstet Gynecol. 2007;30:687-96.
O'Brien JM, Defranco EA, Adair CD et al. Effect of progesterone on cervical shortening in women at risk for preterm birth: secondary analysis from a multinational, randomized, double-blind, placebo-controlled trial. Ultrasound Obstet Gynecol. 2009;34:653-9.
Peltier MR. Immunology of term and preterm labor. Reprod Biol Endocrinol. 2003;1: 122.
Rai P, Rajaram S, Goel N et al. Oral micronized progesterone for prevention of preterm birth. Int J Gynaecol Obstet. 2009;104:40-3.
Richardson JL, Illum L. The vaginal route of peptide and protein drug delivery. Adv Drug Deliv Rev. 1992;8:341-66.
Rode L, Klein K, Nicolaides KH et al. Prevention of preterm delivery in twin gestations (PREDICT): a multicenter, randomized, placebo-controlled trial on the effect of vaginal micronized progesterone. Ultrasound Obstet Gynecol. 2011; 38:272-80.
Rode L, Langhoff-Roos J, Andersson C et al. Systematic review of progesterone for the prevention of preterm birth in singleton pregnancies. Acta Obstet Gynecol Scand. 2009;88:1180-9.
Romero R, Nicolaides K, Conde-Agudelo A et al. Vaginal progesterone in women with an asymptomatic sonographic short cervix in the midtrimester decreases preterm delivery and neonatal morbidity: a systematic review and metaanalysis of individual patient data. Am J Obstet Gynecol. 2012;206:121-4.
Ruddock NK, Shi SQ, Jain S et al. Progesterone, but not 17-alpha-hydroxyprogesterone caproate, inhibits human myometrial contractions. Am J Obstet Gynecol. 2008;199: 391-7.
Sexton DJ, O'Reilly MW, Friel AM et al. Functional effects of 17alpha-hydroxyprogesterone caproate (17P) on human myometrial contractility in vitro. Reprod Biol Endocrinol. 2004;2:80.
Shi SQ, Maner WL, Mackay LB et al. Identification of term and preterm labor in rats using artificial neural networks on uterine electromyography signals. Am J Obstet Gynecol. 2008;198:231-4.
Sims SM, Daniel EE, Garfield RE. Improved electrical coupling in uterine smooth muscle is associated with increased numbers of gap junctions at parturition. J Gen Physiol. 1982; 80:353-75.
Sitruk-Ware R. Routes of delivery for progesterone and progestins. Maturitas. 2007;57:77-80.
Sitruk-Ware R, and Nath A. The use of newer progestins for contraception. Contraception. 2010;82:410-7.
Society for Maternal Fetal Medicine Publications Committee. ACOG Committee Opinion number 419 October 2008 (replaces no. 291). Use of progesterone to reduce preterm birth. Obstet Gynecol. 2008;112:963-5.
Stanczyk FZ, Paulson RJ, Roy S. Percutaneous administration of progesterone: blood levels and endometrial protection. Menopause. 2005;12:232-7.
Werner EF, Han CS, Pettker CM et al. Universal cervical-length screening to prevent preterm birth: a cost-effectiveness analysis. Ultrasound Obstet Gynecol. 2011;38:32-7.
Word RA, Li XH, Hnat M et al. Dynamics of cervical remodeling during pregnancy and parturition: mechanisms and current concepts. Semin Reprod Med. 2007;25:69-79.
Zakar T, Hertelendy F. Progesterone withdrawal: key to parturition. Am J Obstet Gynecol. 2007;196:289-96.

Use of progesterone and progestin analogs for inhibition of preterm birth and other uterine contractility disorders

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Abstract

In this paper we focus on preterm birth as a uterine contractility disorder caused by hypercontractility of the myometrium. We describe changes in uterine function during term and preterm labor and delivery. We also examine the usefulness of measurement of uterine electromyographic (EMG) activity, noninvasively monitored from the abdominal surface of pregnant patients. The use of progesterone treatment for preterm birth is discussed and we conclude that present therapies with progesterone could be improved by changing the route of administration. Finally we show the results of recent studies that show that progesterone injections completely inhibit uterine EMG activity when given several days to hours before normal delivery. These studies illustrate how progesterone suppresses labor at term or preterm, probably through repression of genes which control excitability and conduction of electrical activity. However, direct profusion of soluble progesterone into the uterine cavity has little immediate inhibitory action and this may demonstrate that progesterone has no direct, nongenomic effects, at least in the rat model used. Further studies are required to determine the effects of progesterone on human uterine EMG activity and whether progesterone treatments will prevent preterm birth.

Key words: **Preterm birth, preterm labor, progesterone, uterine contractility, uterine EMG, myometrial contractility.**

Introduction

Preterm birth, i.e. birth prior to 37 weeks gestation, might be considered a uterine contractility disorder. Uterine contractility disorders are major health problems resulting in deaths of large numbers of babies and women worldwide and discomfort and difficulties with reproduction in women. Uterine contractility disorders include hypercontractility such as preterm labor/birth and dysmenorrhea and hypocontractility such as prolonged labor and post partum uterine atony (lack of tonic uterine contractile activity). In addition contractility disorders may include infertility and other puzzles related to women's reproductive function. In this brief review we will outline the basis of uterine contractions and methods for the diagnosis of problems and discuss possible treatment strategies using progesterone or progestin analogs and the basis for their use. We will also demonstrate that progesterone acts mainly by suppression of uterine contractility to inhibit birth.

Csapo (1981) recognized the complex structure of the uterus and noted that it is composed of billions of small smooth muscle cells organized into bundles. Normally the uterus contracts in a phasic manner, relaxing and contracting with a certain characteristic periodicity. The fundamental basis for a uterine contraction is known to be produced by the underlying electrical activity of the muscle cells (Kuriyama, 1961; Kao, 1977; Marshall, 1982; Garfield et al., 1988). Just as in the heart the electrical activity (action potentials) which is generated in pacemaker areas produce a contraction by spreading over the heart, electrical activity of the uterus initiates and sustains contractions by conduction over the uterus. However, in the uterus a single action potential is not responsible for a strong contraction as in the heart, but rather bursts of action potentials generate contractions by changes in the influx of ions, particularly Na^+ and Ca^{2+}, into the muscle cells and relaxation is created by an efflux of ions, notably K^+ and Ca^{2+}, out of the cells. Figure 1 shows the relationship

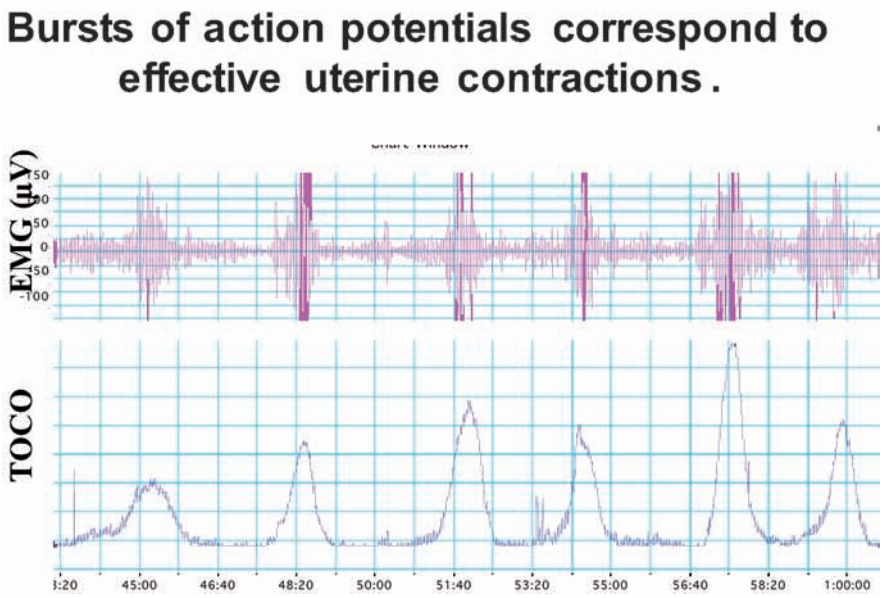


Fig. 1. — Figure shows six bursts of uterine electrical myographic activity (EMG) recorded from the abdominal surface of a pregnant patient in labor (top of figure) and the corresponding intrauterine pressure recorded with a pressure transducer. Note the correspondence between the bursts and contractions.

between electrical activity of the uterus organized into bursts and the mechanical, contraction, response of the muscle. The paper by Dr. Miyoshi in this monograph reviews studies of the electrical properties of the uterus and how tocolytics modify ion flow and contractility.

Some years ago we and others found that we could record electrical activity of the uterus by placing electrodes on the abdominal surface (Buhimschi and Garfield, 1996; Buhimschi et al., 1997; Buhimschi et al., 1998; Garfield et al., 1998; Garfield et al., 1998). This is similar to recording EKG of the heart by placing electrodes at specific locations on the body to record action potentials of cardiac muscle. We also noted that in humans contractions recorded with a tocodynamometer or intrauterine pressure device corresponded to the EMG signals recorded from the abdominal surface (Fig. 2). This technology allows one to noninvasively monitor uterine contractions and identify uterine contractility disorders. The use of abdominal surface measurements of uterine EMG activity allows for diagnosis of term and preterm labor (Garfield et al., 1998; Maner et al., 2003; Maul et al., 2004; Garfield et al., 2005; Maner and Garfield, 2007; Shi et al., 2008). The diagnosis of labor using EMG activity as recorded from the abdominal surface is discussed in more detail by Miha Lucovnik in this same issue. Lucovnik combined several parameters of uterine EMG to find high predictability for diagnosis of preterm and term labor (Lucovnik et al., 2011; Lucovnik et al., 2011). The EMG methods and analysis are powerful technology that will eventually replace uterine monitoring by the tocodynamometer and intrauterine pressure monitoring.

Preterm Labor/Birth and Progestin Treatments

Preterm birth is one of the biggest health problems in the world and a challenge in obstetrics for physicians to identify early signs of labor and impending premature birth and define an appropriate treatment that will stop birth of a premature baby and allow a patient to proceed until term. Presently there are about 15 million babies born prematurely in the world among a total of about 150 million births per year (World Health Organization, 2012). Premature birth is the largest contributor to neonatal deaths in the world. Thus, finding ways to diagnose women that will deliver early and finding appropriate treatments to arrest premature birth should be one of the highest priorities in medicine today. Use of uterine EMG measurements may help in identifying patients that will deliver early and help in determining treatment strategies.

There have been many studies in animals and humans on the use of progesterone to inhibit labor and prevent premature birth. Csapo (1981) was a champion in this field and he first suggested that progesterone acts to inhibit uterine contractility. It is noteworthy that Csapo did not really consider the cervix important in the onset and maintenance of labor and delivery. Since then there have been numerous studies and this topic emerged again when in 2003 it was demonstrated in two important studies that progestins (Meis et al., 2003) and vaginal

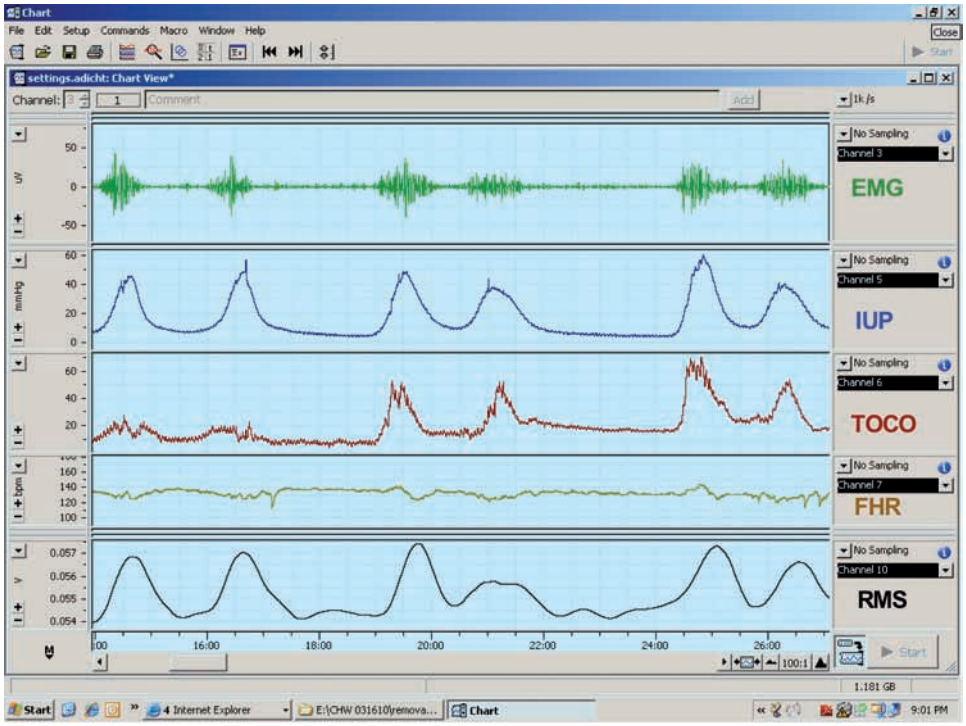


Fig. 2. — Recording of EMG activity (top tracing) recorded from the abdominal surface, intrauterine pressure (IUP), and tocodynamometer pressure (TOCO), fetal heart rate (FHR) and root mean square of the EMG signals (RMS) in a patient in labor. Note that bursts of EMG activity correspond to changes in pressure recorded with IUP and TOCO and that the conversion of EMG signals to RMS resemble signals obtained with the IUP and TOCO. This shows that recording of EMG signals and conversion of these signals to RMS can be used to replace TOCO and IUP techniques for monitoring the contractions of a pregnant patient. Monitor from Reproductive Research Technologies, LLP, Houston, Texas.

progesterone (da Fonseca et al., 2003) could reduce preterm birth in women with recurrent preterm birth or women at high risk for preterm birth. More recently these studies have been duplicated in a multicenter study with vaginal progesterone (Crinone® in Replens base, Columbia Laboratories) using patients with a short cervical length as a risk factor for preterm birth (Hassan et al., 2011). However, our studies suggested that vaginal progesterone is not the best route to administer progesterone as vaginal Crinone in rats does not inhibit birth whereas subcutaneous injections and transdermal progesterone completely block delivery (Kuon et al., 2010). Thus it was no surprise to us that the FDA recently rejected the approval of the use of vaginal progesterone for treatment of preterm birth declaring that there was no significant difference between the vaginal product and the placebo in clinical trials, but the FDA did conclude that such progesterone treatment posed no significant risk to the mother or child. The use of 17 alpha hydroxyprogesterone caproate has been approved by the US FDA and is marketed as Makena™ .

Facchinetti (2007) indicated that 17 alpha hydroxyprogesterone caproate reduces cervical ripening if given to prevent preterm birth. Our studies on the effects of progestins on the uterus show that 17 alpha hydroxyprogesterone caproate does not inhibit human uterine contractility in vitro from tissues obtained during pregnancy, while authentic progesterone rapidly and reversible suppresses contractions (Ruddock et al., 2008). In addition 17 alpha hydroxyprogesterone caproate did not prevent delivery at term in rats while progesterone either topical or parenteral, but not vaginal application (Crinone®), prevented birth (Kuon et al., 2010; 2011). We concluded from these studies that 17 alpha hydroxyprogesterone caproate may not be the best treatment for prevention of preterm birth. Similarly, vaginal progesterone is unlikely to be successful in preventing preterm birth because the vaginal route is not the best way to administer progesterone and the vehicle used for Crinone (Repens) does not release progesterone effectively. 17 alpha hydroxyprogesterone is a weak progestin and unlikely to prevent preterm birth. On the other hand topical or parenteral treatments of progesterone may be much better treatments to reduce the incidence of preterm birth. These studies are outlined in more detail in the paper by Kuon and Garfield in this issue.

It is not exactly clear how progesterone prevents term or preterm birth. It is possible that progestins affect cervical ripening or uterine contractility or both. There is strong evidence that antiprogestins ripen the cervix and initiate preterm birth or abortion at almost any state of gestation in animals and

humans (Chwalisz and Garfield 1994; Chwalisz et al., 1995; 1997; Shi et al., 2000). However, generally the cervix ripens slowly throughout pregnancy when progesterone levels are increasing in both animals and humans. Thus it is difficult to see how progesterone might regulate the cervix. On the other hand the uterus begins to contract normally only during a short time prior to birth. In other words the cervix and uterus change functionally in different time frames when progesterone and indeed estrogen levels are changing. From our early studies we found that electrical coupling between myometrial cells increases dramatically at term during birth in all species studied (Garfield et al., 1977; Garfield and Hayashi, 1981). In addition electrical coupling increases in the myometrium during treatments to initiate preterm birth (Garfield et al., 1982; Sims et al., 1982; Demianczuk et al., 1984; Cole et al., 1985; Garfield et al., 1987; 1988; Miller et al., 1989; Miyoshi et al., 1996; 1998). These observations are important because increased spread of current allows the uterus to contract more forcefully, by recruitment of large numbers of muscle cells, during term or preterm birth. Our studies included both structural studies as well as functional studies to measure coupling between myometrial cells. Recent studies indicate that progesterone treatment only slightly delays cervical ripening (Kuon et al., 2010; 2011). It was concluded that progesterone must prevent delivery by suppression of uterine contractility rather than have an action on the cervix to prevent delivery. In this paper we show that progesterone inhibits uterine electromyographic activity and thereby prevents delivery.

Progesterone inhibits uterine electromyographic activity and prevents preterm and term birth

Previously we have measured EMG activity in rats, humans and other species during term and preterm birth. In all species there is a dramatic increase in the frequency, amplitude and power of the EMG signals during birth that underlie and are responsible for uterine contractions (Garfield et al., 1998; Shi et al., 2008; Lucovnik et al., 2011). Recently we have examined whether progesterone inhibits birth at term in rats. This is a well recognized model that has been used to bioassay the effects of progestins. We administered micronized progesterone topically and parenterally (subcutaneously, s.c.) daily beginning from day 19, day 20, or day 21 of gestation and 8 hours before normal delivery on day 22. All topical or parenteral (s.c.) treatments prevented birth up to 80 hours beyond the normal time of delivery observed in control, vehicle treated, rats (Fig. 3). The significance of these observations is that proges-

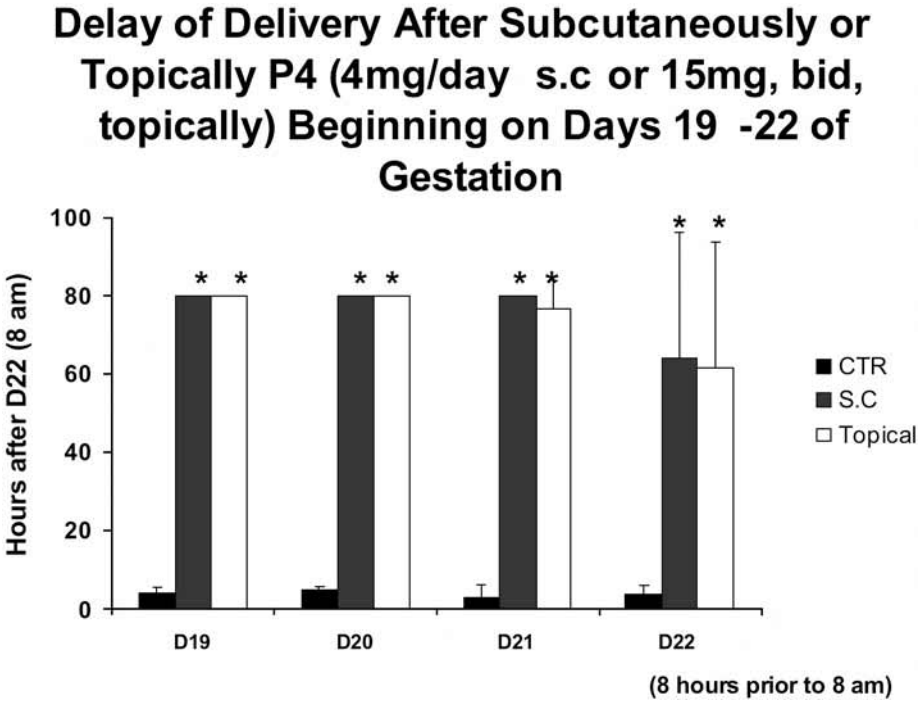


Fig. 3. — Figure 3 shows the delay in delivery in pregnant rats treated daily with parenteral (4 mg subcutaneous injection, S.C., of micronized progesterone) or topical progesterone (2 times daily, 15 mg micronized progesterone in fish oil) for 3 days (days 19-21), 2 days (days 20-21), 1 day (day 21) or 8 hours before normal delivery (day 22) on day 22 at 8 a.m. 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 a.m. 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.

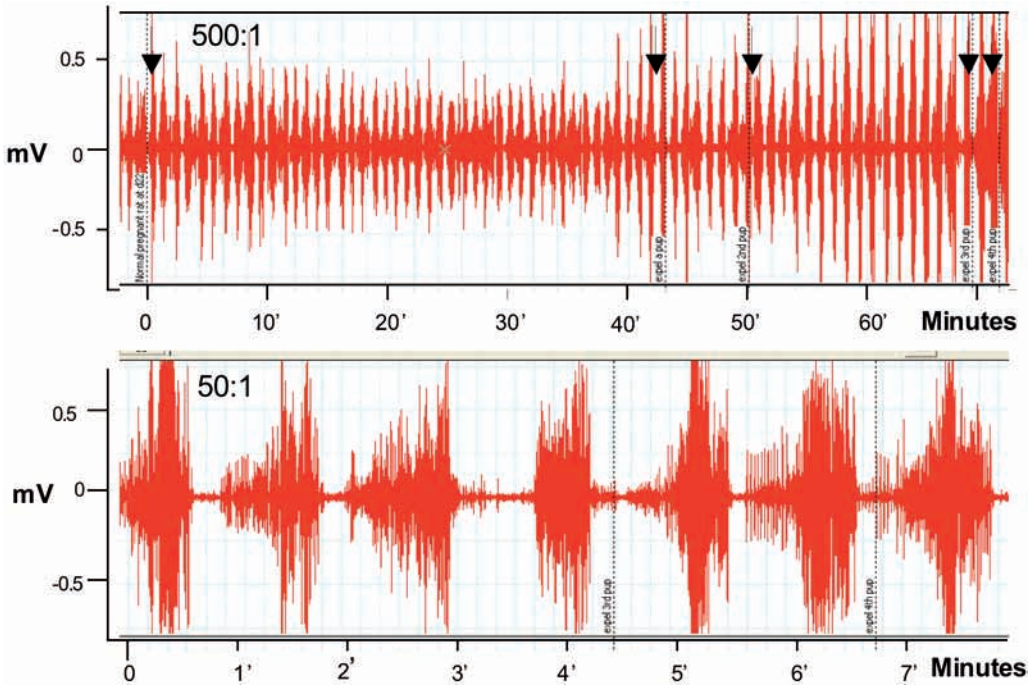


Fig. 4. — EMG recordings from a normal pregnant rat on day 22 of gestation during delivery (arrows indicate delivery of a fetus). Recordings showing > 60 minutes (top record) and for > 7 minutes (bottom). Note delivery of fetuses as marked with arrows. This indicates that the animals deliver their fetuses 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.

terone can prevent birth when given after the decline in progesterone levels in the blood and even after the cervix is already ripened and prepared for delivery. These studies suggest that topical or parenteral progesterone treatments, but not vaginal progesterone, inhibit uterine contractility to prevent birth. Why vaginal progesterone is not effective is not clear but it is possible that Replens, the base used by Columbia Laboratories for Crinone, does not release progesterone. The reason for suggesting this is because micronized progesterone in Replens base applied topically does not inhibit term birth in rats (Kuon et al., 2010; 2011). Although these early studies indicate that topical or parenteral progesterone, but not vaginal progesterone, can partially delay cervical ripening it does not prevent it from softening. Successful delay in delivery with only a few hours treatment with progesterone suggests that progesterone treatment might be used to prevent acute preterm birth.

To examine the effects of progesterone on uterine 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. 4) and very similar what we had observed previously in animals fitted with internal EMG devices to measure activity without anesthesia (Shi et al., 2008). Animals treated with progesterone 1 to 2 days prior to normal delivery had very low levels of EMG activity (Fig. 5). We repeated these experiments in many studies and noted the following: Progesterone (s.c.) significantly reduces the EMG burst frequency (bursts/30 min ± SEM: 1 day treatment =11.1 ± 1.7 vs. controls 23.2 ± 3.5, $P < 0.01$; 2 day 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 day treatment = 127 ± 31 vs. controls 230 ± 14, $P < 0.02$). Thus the mean burst integrals (V^2) are suppressed at 1 ($P < 0.001$) and 2 ($P < 0.002$) days after P4 treatment vs. controls, but not the burst duration ($P > 0.05$, ca. 30 seconds). This indicates that progesterone treatment suppresses uterine EMG activity and thereby inhibits birth. We also examined whether progesterone treatments given by infusion would inhibit uterine electrical activity and thereby prevent contractions and delivery at term. If so this could indicate that progesterone has a nongenomic action to inhibit uterine contractions and thus it might be used in case

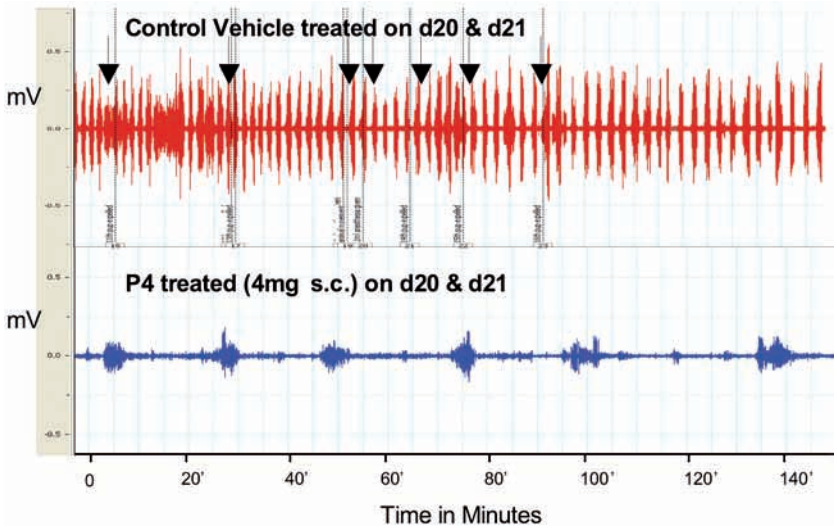


Fig. 5. — Examples of EMG recordings from pregnant rats on day 22 of gestation from control rat (vehicle treated during delivery, top tracing, arrows indicate delivery of a fetus) and rat after two days treatment with P4 (4 mg micronized progesterone, P4, given subcutaneously for 2 days on day 20 and 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.

of acute preterm labor where a patient is already in established preterm labor that is likely to lead to preterm birth. Infusion of bolus injections of soluble progesterone through a catheter inserted into the uterine cavity in close proximity to the EMG recording electrodes did not produce an observable inhibition of contractions even at high progesterone doses, i.e. soluble progesterone equivalent to doses up to 5 mg of crystalline progesterone (Fig. 6). We also infused micronized progesterone dissolved in small amounts of ethanol and other solubilizing agents. These experiments also failed to show any inhibition but it was difficult to demonstrate because ethanol alone, and other agents used to solubilize progesterone, inhibited contractions by themselves. These studies, although somewhat incomplete and hard to interpret, suggest that progesterone does not exert an immediate, nongenomic action of the

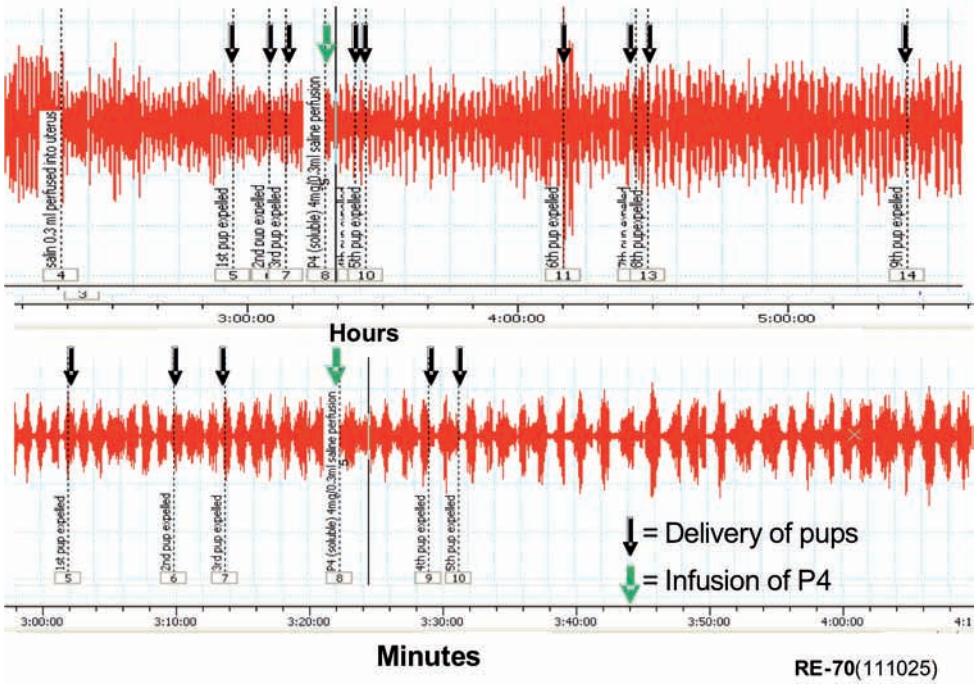


Fig. 6. — Examples of EMG records from delivering (pup delivery indicated with black arrows) rat perfused with soluble progesterone (equivalent to 4 mg of authentic progesterone, P4, 4 mg at green arrow) as shown during 4 hours recording (top) and the same record shown enlarged with recording time equal to about 70 minutes. Note the perfusion with progesterone had no effect on the EMG activity.

uterine muscle during delivery to suppress uterine EMG and contractile activity. Thus the question of whether progesterone treatment may immediately inhibit acute preterm labor by direct action on the uterus is not completely resolved.

Discussion

Preterm birth and term delivery present enormous challenges to physicians, their patients and premature infants. We suggest that preterm labor and the resultant early birth are the consequence of premature uterine contractions and therefore can be considered as a uterine contractility disorder. Recently treatment of preterm birth has been attempted using weekly 17 alpha hydroxyprogesterone caproate injections and daily vaginal progesterone. Our studies indicate that neither of these progesterone preparations is useful to suppress term or preterm birth. We suggest that transdermal (topical) treatment of progesterone or progesterone injections may be useful. The transdermal route may be particularly helpful because progesterone, with a half-life of only about 30 hours, must be given daily and daily injections are not ideal. Progesterone inhibits uterine contractions through genomic mechanisms probably involving the classical progesterone receptors. Progesterone may not act through nongenomic mechanisms, although our studies with human myometrial tissues seems to indicate otherwise (Ruddock et al., 2008) and membrane progesterone receptors have been identified on human myometrial cells (Wu et al., 2011). The studies above involving infusion of progesterone into the uterine cavity indicate little effect but soluble progesterone and authentic progesterone in solvent may not be the best way to demonstrate this action.

Transabdominal measurement of EMG activity is a powerful tool to evaluate function of the uterus during pregnancy and identify true preterm or term labor. This method also could be useful to identify the best treatment strategies for preterm labor treatment. Lucovnik et al. (2011) estimate that less patients would be needed to examine the effectiveness of any treatment on preterm labor using EMG methods compared to other clinical determinates used to make clinical decisions on preterm birth treatment. Our studies above with progesterone treatment clearly show that progesterone suppresses uterine EMG activity after 1 to 2 days treatment and inhibits birth in rats even at 8 hours before normal delivery. This short time frame indicates that the site of progesterone action is not on the cervix. Ultimately analogs of progesterone might prove more effective for inhibition of preterm birth because 17 alpha hydroxyprogesterone caproate and authentic progesterone are both weak agonists for the progesterone receptor. Further studies are urgently needed to demonstrate better preparations of progesterone on human EMG activity and on preterm birth. This paper represents some Facts, our Views and Visions in ObGyn.

References

Buhimschi C, Garfield RE. Uterine contractility as assessed by abdominal surface recording of electromyographic activity in rats during pregnancy. Am J Obstet Gynecol. 1996;174: 744-53.

Buhimschi C, Boyle MB, Garfield RE. Electrical activity of the human uterus during pregnancy as recorded from the abdominal surface. Obstet Gynecol. 1997;90:102-11.

Buhimschi C, Boyle MB, Saade GR et al. Uterine activity during pregnancy and labor assessed by simultaneous recordings from the myometrium and abdominal surface in the rat. Am J Obstet Gynecol. 1998;178:811-22.

Chwalisz K, Garfield RE. Antiprogestins in the Induction of Labor. Ann N Y Acad Sci. 1994;734:387-413.

Chwalisz K, Stockemann K, Fuhrmann U et al. Mechanism of Action of Antiprogestins in the Pregnant Uterus. Ann N Y Acad Sci. 1995;761:202-24.

Chwalisz K, Garfield RE. Regulation of the uterus and cervix during pregnancy and labor: Role of progeseterone and nitric oxide. Ann N Y Acad Sci. 1997;828:238-53.

Cole WC, Garfield RE, Kirkaldy JS. Gap junctions and direct intercellular communication between rat uterine smooth muscle cells. Am J Physiol. 1985;249(18):C20-C31.

Csapo AI. Force of labor, in Principles and Practice of Obstetrics and Perinatology, Iffy I and Kamientsky HA, Eds, Wiley, New York, 1981, 761-99.

da Fonseca EB, Bittar RE, Carvalho MHB et al. Prophylactic administration of progesterone by vaginal suppository to reduce the incidence of spontaneous preterm birth in women at increased risk: A randomized placebo-controlled double-blind study. Am J Obstet Gynecol. 2003;188:419-24.

Demianczuk N, Towell ME, Garfield RE. Myometrial electrophysiological activity and gap junctions in the pregnant rabbit. Am J Obstet Gynecol. 1984;149(5):485-91.

Facchinetti F, Paganelli S, Comitini G et al. Cervical length changes during preterm cervical ripening: effects of 17-alpha-hydroxyprogesterone caproate. Am J Obstet Gynecol. 2007;196:453.e1-4.

Garfield RE, Saade G, Buhimschi C et al. Control and assessment of the uterus and cervix during pregnancy and labour. Hum Reprod Update. 1998;4:673-95.

Garfield RE, Maner WL, MacKay LB et al. Comparing uterine electromyography activity of antepartum patients versus term labor patients. Am J Obstet Gynecol. 2005;193:23-9.

Garfield RE, Chwalisz K, Shi L, et al. Instrumentation for the diagnosis of term and preterm labour. J Perinat Med. 1998; 26:413-36.

Garfield RE, Sims S, Daniel EE. Gap junctions: Their presence and necessity in myometrium during parturition. Science. 1977;198:958-60.

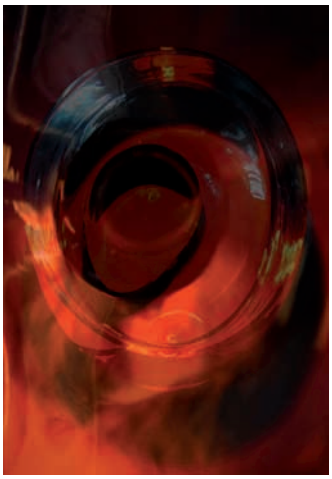
Garfield RE, Hayashi RH. Appearance of gap junctions in the myometrium of women during labor. Am J Obstet Gynecol. 1981;140(3):254-60.

Garfield RE, Puri CP, Csapo AI. Endocrine structural and functional changes in the uterus during premature labor. Am J Obstet Gynecol. 1982;142(1):21-7.

Garfield RE, Gasc JM, Baulieu EE. Effects of the antiprogesterone RU 486 on preterm birth in the rat. Am J Obstet Gynecol. 1987;157(5):1281-5.

Garfield RE, Blennerhassett MG, Miller SM. Control of myometrial contractility: Role and regulation of gap junctions. Oxf Rev Reprod Biol. 1988;10:436-90.

Hassan SS, Romero R, Vidyadhari D et al. Vaginal progesterone reduces the rate of preterm birth in woman with a sonographic short cervix: a multicenter, randomized, double-blind, placebo-controlled trial. Ultrasound Obstet Gynecol. 2011:38:18-31.
Kao CY. Electrical properties of uterine smooth muscle. In: Biology of the Uterus. Wynn R.M., Ed., Plenum Press, New York, 1977, 423-96.
Kuon RJ, Shi S-Q, Maul H et al. Pharmacologic actions of progestins to inhibit cervical ripening and prevent delivery depend on their properties, the route of administration, and the vehicle. Am J Obstet Gynecol. 2010;202:455.e1-9.
Kuon RJ, Shi, S-Q, Maul, H, et al. A Novel Optical Method to Assess Cervical Changes During Pregnancy and Use to Evaluate the Effects of Progestins on Term and Preterm Labor. Am J Obstet Gynecol. 2011;205:82.e15-20.
Kuriyama H. Recent studies on the electrophysiology of the uterus. In: Progesterone and the Defense Mechanism of Pregnancy. Ciba Foundation Study Group 9, Little Brown, Boston, 1961, 51-70.
Lucovnik M, Maner WL, Chambliss LR et al. Noninvasive Uterine Electromyography For Prediction of Preterm Delivery. Am J Obstet Gynecol. 2011;204:228.e1-10.330.
Lucovnik M, Kuon RJ, et al. Use of Uterine Electromyography to Diagnose Term and Preterm Labor. Acta Obstet Gynecol Scand. 2011;90:150-7.
Lucovnik M, Kuon, RJ, Chambliss LR et al. Use of Uterine Electromyography to Diagnose Term and Preterm Labor. Acta Obstet Gynecol Scand. 2011;90:150-7.
Maner WL, Garfield RE, Maul H et al. Predicting term and preterm delivery with transabdominal uterine electromyography. Obstet Gynecol. 2003;101:1254-60.
Maner WL, Garfield RE. Identification of human term and preterm labor using artificial neural networks on uterine electromyography data. Ann Biomed Eng. 2007;35:465-73.
Marshall JM. Regulation of activity in uterine smooth muscle. Physiol Rev. 1982;42: 213-27.
Maul H, Maner WL, Olson, G et al. Non-invasive transabdominal uterine electromyography correlates with the strength of intrauterine pressure and is predictive of labor and delivery. J Matern Fetal Neonatal Med. 2004;15:297-301.
Meis PJ, Klebanoff M, Thom E et al. Prevention of recurrent preterm delivery by 17 alpha-hydroxyprogesterone caproate. N Engl J Med. 2003;348:2379-85.
Miller SM, Garfield RE, Daniel EE. Improved propagation in myometrium associated with gap junctions during parturition. Am J Physiol. 1989;256(25):C130-141.
Miyoshi H, Boyle MB, MacKay L et al. Voltage-Clamp Studies of Gap Junctions Between Uterine Muscle Cells During Term and Preterm Labor. Biophys J. 1996;71(3):1324-34.
Miyoshi H, Boyle MB, MacKay LB et al. Gap junction currents in cultured muscle cells from human myometrium. Am J Obstet Gynecol. 1998;178(3):588-93.
Ruddock N, Shi S-Q, Jain S et al. Progesterone (P4), but not 17 alpha hydroxyprogesterone caproate (17P) inhibits uterine contractility during pregnancy. Am J Obstet Gynecol. 2008; 199(4):391.e1-7.
Shi SQ, Maner WL, MacKay LB et al. Identification of term & pre-term labor in rats by using artificial neural networks on uterine electromyography signals. Am J Obstet Gynecol. 2008;198:235.e1-235.e1-4.
Shi L, Shi SQ, Saade GR et al. Studies of cervical ripening in pregnant rats:effects of various treatments. Mol Hum Reprod. 2000;6(4):382-9.
Sims, SM, Daniel, EE, Garfield RE. Improved electrical coupling in uterine smooth muscle is associated with increased numbers of gap junctions at parturition. J Gen Physiol. 1982; 80(3):353-75.
Wu W, Shi, S-Q, Huang H-J, Balducci J et al. Changes in PGRMC1, a Potential Progesterone Receptor, in Human Myometrium during Pregnancy and Labour at Term and Preterm. Mol Hum Reprod. 2011;17(4):233-42.
World Health Organization. Born Too Soon: The Global Action Report on Preterm Birth. 2012;1-111.



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Postterm pregnancy

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Abstract

Postterm pregnancy is a pregnancy that extends to 42 weeks of gestation or beyond. Fetal, neonatal and maternal complications associated with this condition have always been underestimated. It is not well understood why some women become postterm although in obesity, hormonal and genetic factors have been implicated. The management of postterm pregnancy constitutes a challenge to clinicians; knowing who to induce, who will respond to induction and who will require a caesarean section (CS). The current definition and management of postterm pregnancy have been challenged in several studies as the emerging evidence demonstrates that the incidence of complications associated with postterm pregnancy also increase prior to 42 weeks of gestation. For example the incidence of still-birth increases from 39 weeks onwards with a sharp rise after 40 weeks of gestation. Induction of labour before 42 weeks of gestation has the potential to prevent these complications; however, both patients and clinicians alike are concerned about risks associated with induction of labour such as failure of induction and increases in CS rates. There is a strong body of evidence however that demonstrates that induction of labour at term and prior to 42 weeks of gestation (particularly between 40 & 42 weeks) is associated with a reduction in perinatal complications without an associated increase in CS rates. It seems therefore that a policy of induction of labour at 41 weeks in postterm women could be beneficial with potential improvement in perinatal outcome and a reduction in maternal complications.

Key words: **Body mass index, induction of labour, perinatal complications, postterm pregnancy, ultrasound.**

Introduction

Post term pregnancy is associated with an increased risk of fetal and neonatal mortality and morbidity (Olesen et al., 2003a;2003b) as well as an increased maternal morbidity (Caughey et al., 2007). Antepartum stillbirth at and beyond term (37-43 weeks gestation) is a major public health problem accounting for a greater contribution to perinatal mortality than either deaths from complications of prematurity or the sudden infant death syndrome (Cotzias et al., 1999). Increased fetal mortality from postterm pregnancy could therefore be prevented by induction of labour (IOL) at term, however, both clinicians and patients alike are concerned about the risks of induction of labour including uterine hyper-stimulation, failed induction and increased Caesarean section rates. Postterm pregnancy is also associated with increased costs related to antenatal fetal monitoring and induction of labour (Allen et al., 2005; Fonseca et al., 2003) and can be a source of significant anxiety for the pregnant woman (ACOG, 1997). Optimisation of these conflicting pressures is a clinical challenge.

Definitions

Postterm pregnancy is defined as pregnancy that has extended to or beyond 42 weeks of gestation (294 days), or estimated date of delivery (EDD) +

14 days (ACOG, 2004). The terms prolonged pregnancy, postdates and postdatism are synonymously used to describe the same condition. The terms postdate and prolonged pregnancy are ill-defined and best avoided (ACOG, 2004).

Postmaturity, postmaturity syndrome and dysmaturity are not synonymous terms to postterm pregnancy. They are often used to describe the features of a neonate who appears to have been in utero longer than 42 weeks of gestation. They describe the effects of intrauterine growth restriction (IUGR) secondary to utero-placental insufficiency encountered in a postterm pregnancy (Shime et al., 1986).

Epidemiology

The incidence of postterm pregnancy is about 7% of all pregnancies (Martin et al., 2007). The prevalence varies depending on population characteristics and local management practices. Population characteristics that affect the prevalence include: the percentage of primigravidas in the studied population, the prevalence of obesity, a prior postterm pregnancy as well as genetic predisposition. The proportion of women with pregnancy complications and the frequency of spontaneous preterm labour also influence the rate of postterm pregnancy. The link between ethnicity and overall duration of pregnancy is not well established (Collins et al., 2001; Caughey et al., 2009).

Local management practices such as scheduled IOL, differences in the use of early ultrasound (US) for pregnancy dating, , and elective Caesarean section (CS) rates will affect the overall prevalence of postterm pregnancy. In the United States for example, the increase in the incidence of IOL in the last decade was associated with a drop in the number of pregnancies continued beyond 41 and 42 weeks from 18%&10% respectively in 1998 (Ventura et al., 1998) to 14%& 4% respectively in 2005 (Martin et al., 2005). Similarly, the use of early US for pregnancy dating has been associated with a significant reduction in the incidence of postterm pregnancy from 12% to 3% (Savitz et al., 2002).

Aetiology and risk factors

The most common cause of prolonged pregnancies is inaccurate dating (Neilson, 2000; Crowley, 2004). The use of standard clinical criteria to determine the estimated delivery date (EDD) tends to overestimate gestational age and consequently increases the incidence of postterm pregnancy (Gardosi et al., 1997; Taipale and Hiilermaa, 2001). Clinical criteria which are commonly used to confirm gestational age include last menstrual period (LMP), the size of the uterus as estimated by bimanual examination in the first trimester, the perception of fetal movements, auscultation of fetal heart tones, and fundal height in a singleton pregnancy.

When postterm pregnancy truly exists the cause is usually unknown. Common risk factors include primiparity, previous postterm pregnancy (Alfirevic and Walkinshaw, 1994; Mogren et al., 1999; Olesen et al., 1999), male fetus (Divon et al., 2002), obesity (Usha Kiran et al., 2005; Stotland et al., 2007), hormonal factors and genetic predisposition (Laursen et al., 2004).

It is not known how body mass index (BMI) affects the duration of pregnancy and timing of delivery, but interestingly obese women have a higher incidence of postterm pregnancy (Usha Kiran et al., 2005), while women with low BMI have a higher incidence of preterm labour (delivery before 37 weeks of gestation) (Hickey et al., 1997). Because adipose tissue is hormonally active (Baranova et al., 2006), and because obese women may have an altered metabolic status, it is possible that endocrine factors involved in the initiation of labour are altered in obese women.

Perhaps amongst all the factors which could influence the incidence of postterm pregnancy obesity is the one modifiable risk factor which could theoretically improve by dietary and exercise behavioural modifications before or during pregnancy. Such modifications would have an impact on other health outcomes as well, but because postterm pregnancy is associated with a number of perinatal complications, its prevention would be clearly beneficial (Ingemarsson and Kallen, 1997).

Using a number needed to treat calculation, it was found that for approximately every 20 women who successfully decreased BMI below the obesity range, one fewer woman would go past 41 weeks of gestation (adjusted odds ratio of 1.26; based on baseline risk of reaching 41 weeks gestation of approximately 20%) (Caughey et al., 2009).

Altered levels of circulating hormones that are thought to play a role in spontaneous labour may also play a role in the causation of postterm pregnancy. Placental sulphatase deficiency for example, is a rare X-linked recessive disorder that can prevent spontaneous labour due to a defect in placental sulphatase activity and the resulting decreased oestriol levels (E3). Fetal adrenal insufficiency and fetal adrenal hypoplasia as well as fetal anencephaly (in the absence of polyhydramnios), despite being rare, are all associated with postterm pregnancies (Doherty and Norwitz, 2008).

Genetic factors may be involved with prolongation of pregnancy. Women who were themselves products of a prolonged pregnancy are at higher risk of postterm pregnancy (relative risk is 1.3) (Mogren et al., 1999). Women with prior prolonged pregnancy

have an increased risk of subsequent postterm pregnancy (27% with one prior prolonged pregnancy & 39% with 2 prior prolonged pregnancies) (Kistka et al., 2007). Twin studies also support a genetic predisposition. Rates of prolonged pregnancy are increased in women whose twin sister has had a previous postterm birth. This association is greater in monozygotic than in dizygotic twins (Laursen et al., 2004). There also appears to be a paternal role in the recurrence risk of prolonged pregnancy. The risk of recurrence of postterm pregnancy was reduced from 19.9% to 15.4% when the father of the baby changed between the first and second pregnancy (Olesen et al., 2003).

Pathogenesis

The pathogenesis of postterm pregnancy is not clearly understood. As demonstrated above some risk factors associated with postterm pregnancy were identified with some possible explanations, however, the pathogenesis of the condition is not yet clear. Despite improved understanding of parturition in recent years, we still lack clarity about the exact mechanisms which initiate labour and allow its progression. To have a better understanding of the pathogenesis of postterm pregnancy it is essential to shed some lights on the pathophysiology of parturition and try to understand why these mechanisms fail to be triggered in postterm pregnancies or conversely are triggered earlier in preterm labour. It seems logical that a common ground or a link does exist between these three conditions. The mechanisms of parturition include interactions between hormonal, mechanical and inflammatory processes, in which placenta, mother and fetus each play a vital role.

Placental production of the peptide corticotrophin releasing hormone (CRH) has been related to the length of gestation (McLean et al., 1995). Synthesis of CRH by the placenta increases exponentially as pregnancy advances and peaks at the time of labour. In women who deliver prematurely the exponential rise is more rapid than those delivering at term, while in women who deliver postterm the rate of rise is slower (Ellis et al., 2002; Torricelli et al., 2006). This data suggests that postterm delivery is due to a change in the biological mechanisms regulating the length of gestation. This may be due to an inherited predisposition due to polymorphisms in the genes on the physiological pathway linking CRH to birth. It is also possible that the maternal phenotype may change the response of maternal tissues to the usual hormonal signals to birth as may occur in the obese woman.

CRH can directly stimulate fetal adrenal production of DHEAs, the precursor for placental oestriol synthesis (Smith et al., 1998). Maternal plasma CRH concentrations correlate with oestriol concentrations (Smith et al., 2009). The rising oestriol driven by CRH increases at the end of gestation more rapidly than oestradiol levels leading to an increase in the oestriol to oestradiol ratio which has been postulated to produce an estrogenic environment in the last weeks of pregnancy. Concurrently the rise in maternal plasma progesterone concentrations that occurs across gestation slows at the end of pregnancy or even falls. This may be due to CRH inhibition of placental progesterone synthesis (Yang et al., 2006). Thus the pro-pregnancy effect of progesterone (promoting relaxation) is declining as the pro-labour actions of oestriol (promoting contraction) are increasing. These changes in ratios have been observed in preterm births, singletons delivering at term and in twin gestations (Smith et al., 2009). The situation in postterm pregnancies is unknown. It is likely to be similar in postterm women who go into spontaneous labour or those who respond to IOL, based on one study of postterm women (Torricelli et al., 2011).

Complications of postterm pregnancies

Postterm pregnancies are associated with increased fetal and neonatal motality and morbidity as well as maternal morbidity. These risks are greater than it was originally thought. Risks have been underestimated in the past for two reasons. First, earlier studies on postterm pregnancy were published before the routine use of ultrasound for pregnancy dating. As a result many pregnancies included in the studies were not actually postterm. The second reason rests within the definition of stillbirth itself. Stillbirth rates were traditionally calculated using pregnancies delivered at a given gestational age rather than ongoing (undelivered) pregnancies. This would lower the stillbirth rates in postterm pregnancies as once the fetus is delivered it is no longer at risk of intra-uterine fetal death (IUFD). The appropriate denominator is therefore not all deliveries at a given gestational age but ongoing (undelivered) pregnancies (Rand et al., 2000; Smith, 2001; Caughey et al., 2003).

One retrospective study of over 170,000 singleton births, using the appropriate denominator demonstrated a 6-fold increase in stillbirth rates in postterm pregnancies from 0.35 to 2.12 per 1000 ongoing pregnancies (Hilder et al., 1998).

Fetal and neonatal complications

The perinatal mortality rate, defined as stillbirths plus early neonatal deaths, at 42 weeks of gestation is twice as high as that at term (4-7 versus 2-3 per 1000 deliveries, respectively). It increases 4-fold at 43 weeks and 5-7-fold at 44 weeks (Bakketeig and Bergsjo, 1989; Feldman, 1992; Hilder et al., 1998;

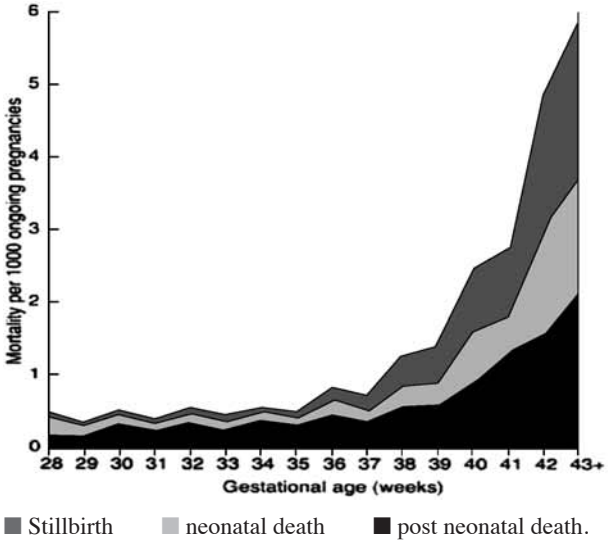

Fig. 1. — Perinatal mortality per 1000 ongoing pregnancies. Reproduced from BJOG Volume 105 (Hilder L, et al., 1998).

Cotzias et al., 1999). These data also demonstrate that when calculated per 1000 ongoing pregnancies, fetal and neonatal mortality rates increase sharply after 40 weeks (Hilder et al., 1998) (Fig. 1). It is believed that utero-placental insufficiency, meconium aspiration and intrauterine infection are the underlying causes of the increased perinatal mortality rates in these cases (Hannah, 1993).

Fetal morbidity is also increased in postterm pregnancies and pregnancies that progress beyond 41 weeks gestation. This includes passage of meconium, meconium aspiration syndrome, macrosomia and dysmaturity. Post term pregnancy also is an independent risk factor for low umbilical cord pH levels (neonatal acidaemia), low 5-minute Apgar scores (Kitlinski et al., 2003), neonatal encephalopathy (Badawi et al., 1998), and infant death in the first year of life (Hilder et al., 1998; Cotzias et al., 1999; Rand et al., 2000). Although some of these infant deaths clearly result from peripartum complications such as meconium aspiration syndrome, most have no known cause.

Meconium aspiration syndrome refers to respiratory compromise with tachypnea, cyanosis, and reduced pulmonary compliance in newborn infants exposed to meconium *in utero*. It is seen in higher rates in postterm neonates (Kabbur et al., 2005). In the United States the incidence of meconium aspiration syndrome has shown a 4-fold reduction between 1990 and 1998 (from 5.8% to 1.5% in infants more than 37 weeks; $P < 0.003$). This has been attributed primarily to a reduction in postterm pregnancy rates (Yoder et al., 2002). Conventional interventions such as amnio-infusion (Hofmeyr, 2002; Fraser et al., 2005)) or routine nasopharyngeal and oro-pharyngeal suction of meconium at the perineum at the time of delivery, have made very little contribution to this improvement (Vain et al., 2004).

Postterm infants are larger than term infants and have a higher incidence of fetal macrosomia (2.5-10% in postterm versus 0.8-1% at term) (Spellacy et al., 1985; Rosen and Dickinson, 1992). Fetal macrosomia, defined as an estimated fetal weight ≥ 4.5 kg (ACOG, 2000), is associated with prolonged labour, cephalo-pelvic disproportion and shoulder dystocia. Shoulder dystocia is associated with risk of orthopaedic injury (e.g. fractured humerus and clavicle) as well as neurological injury such as brachial plexus injury and cerebral palsy (Spellacy et al., 1985; Rosen and Dickinson, 1992). However, there is no evidence that IOL as a preventative measure in these cases is associated with a reduction in complication rates or improvement in perinatal outcome (ACOG, 2004).

About 20% of postterm fetuses have dysmaturity syndrome, which refers to infants with characteristics resembling chronic intrauterine growth restriction from utero-placental insufficiency (Vorherr, 1975; Mannino, 1988). This includes thin wrinkled peeling skin (excessive desquamation), thin body (malnourishment), long hair and nails, oligohydramnios and frequently passage of meconium. These pregnancies are at increased risk of umbilical cord compression from oligohydramnios, meconium aspiration, and short-term neonatal complications such as hypoglycemia, seizures, and respiratory insufficiency. They also have an increased incidence of non-reassuring antepartum and intrapartum fetal testing (Knox et al., 1979). Whether such infants are at increased risk of long-term neurologic sequelae is not clear. In a large, prospective, follow-up study of children at ages 1 and 2 years, the general intelligence quotient, physical milestones, and frequency of intercurrent illnesses were not significantly different between normal infants born at term and those born postterm (Shime et al., 1986).

Although much of the work above has been conducted in postterm pregnancies, some risks such as stillbirth, passage of meconium, and neonatal acidaemia have been described as being greater at 41 and even 40 weeks of gestation as compared to 39 weeks gestation (Caughey et al., 2005; Caughey and Musci, 2004). A study from Scotland published in 2010 demonstrated increased risk of stillbirth (both overall and unexplained stillbirth) as pregnancy advances especially after 39 weeks of gestation (Sutan et al., 2010) (Fig. 2). Yudkin et al. (1987) also demonstrated that the risk of unexplained stillbirth rises fourfold after 39 weeks to a maximum at 41 weeks (Fig. 3). The rates of meconium aspiration and neonatal acidaemia both increase as term pregnancies progress beyond 38 weeks (Bruckner et al., 2008). Neonatal morbidity including birth inju-

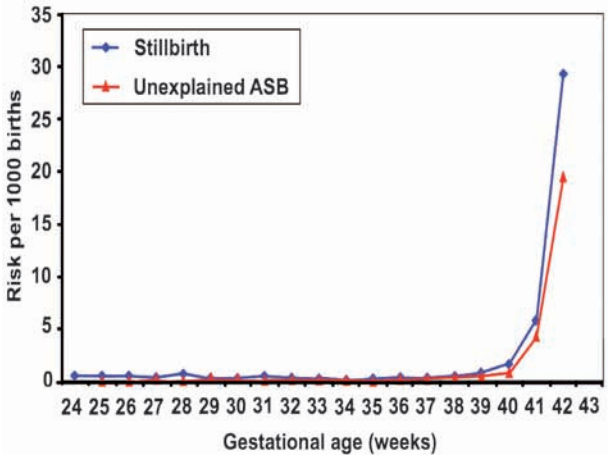

Fig. 2. — Risk of Stillbirth by week of gestation (rate calculated per 1000 ongoing pregnancies) (Sutan et al. 2010).

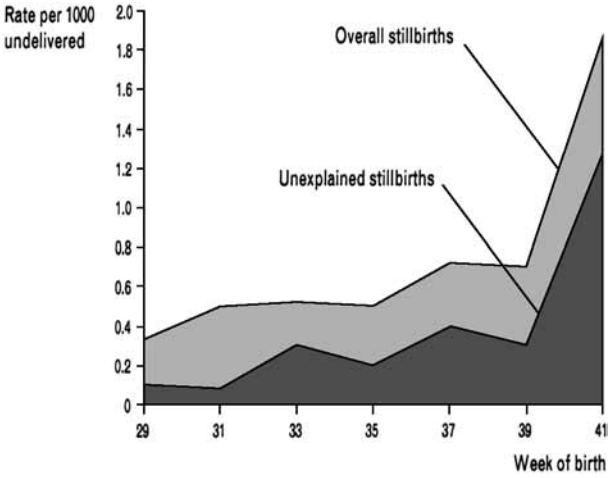

Fig. 3. — Relationship between stillbirth and gestational age (Yudkin et al., 1987).

ries seems to nadir at around 38 weeks and increase in a continuous fashion thereafter (Nicholson et al., 2006). Therefore, 42 weeks of gestation does not represent a a thershold below which risks are uniformly distributed. Hence, the definition and management of postterm pregnancy have been questioned and challenged in several studies in recent years (Caughey et al., 2007; Doherty et al., 2008).

Maternal risks

Postterm pregnancy is associated with significant risks to the mother. There is an increased risk of: 1) labour dystocia (9-12% versus 2-7% at term); 2) severe perineal lacerations (3rd & 4th degree tears), related to macrosomia (3.3% versus 2.6% at term); 3) operative vaginal delivery; and 4) doubling in caesarean section (CS) rates (14% versus 7% at term) (Rand et al., 2000; Campbell et al., 1997; Alexander et al., 2000; Treger et al., 2002). Caesarean delivery is associated with higher incidence of endometritis, haemorrhage, and thromboembolic disease (Alexander et al., 2001; Eden et al., 1987).

The emotional impact of prolonged pregnancy should not be underestimated either. In one randomised controlled trial of women at 41 weeks of gestation, women who were induced desired the same management 74% of the time, whereas women with serial antenatal monitoring desired the same management only in 38% of the time ($P < 0.001$) (Heimstad et al., 2007).

Similar to neonatal outcomes, maternal morbidity also increases in term pregnancies before 42 weeks of gestation. Complications such as chorioamnionitis, severe perineal lacerations, Caesarean delivery, postpartum haemorrhage, and endomyometritis all increase progressively after 39 weeks of gestation (Yoder et al., 2002; Caughey and Bishop, 2006; Heimstad et al., 2006; Caughey et al., 2007; Bruckner et al., 2008;).

A large retrospective study (Caughey et al., 2007), which included 119,254 singleton-low risk pregnancies, demonstrated a statistically significant increase in the rate of maternal complications beyond 40 weeks of gestation and even beyond 39 weeks' gestation for some morbidities.

The study also showed that the increase in maternal complications persisted at statistically and clinically significant levels even allowing for the increase in operative deliveries. This is true for all of the trends except for the rate of endomyometritis among women undergoing vaginal delivery. For this complication alone, the increase in and among women experiencing caesarean deliveries accounted for the bulk of the increase by gestational age (Caughey et al., 2007).

Management of postterm pregnancy

A) *Pregnancy Dating*

Accurate pregnancy dating is crucial to the diagnosis and management of postterm pregnancy (Mandruzzato et al., 2010). Last menstrual period has traditionally been used to calculate the expected date of delivery (EDD). But many inaccuracies could exist because of cycle irregularity, recent use of hormonal contraception or because of bleeding early in pregnancy.

Routine ultrasound examination for pregnancy dating demonstrated a reduction in the rate of false positive diagnosis and thereby the overall rate of postterm pregnancy from 10-15% to approximately 2-5%, and thereby minimized unnecessary intervention (Bennett et al., 2004; Caughey et al., 2008a; 2009). A Cochrane systematic review in 2000 found

a similar reduction in the overall rates of induction of labour for postterm pregnancy (OR, 0.68; 95% CI, 0.57-0.82) among women who underwent sonographic gestational age assessment before 24 weeks of gestation (Neilson, 2000).

When using ultrasound for dating it is necessary to understand the margin of error reported at various times during gestation. The variation by ultrasonography generally is ± 7 days up to 20 weeks of gestation, ± 14 days between 20 and 30 weeks of gestation, and ± 21 days beyond 30 weeks of gestation (ACOG, 2004).

A calculated gestational age by ultrasound must be therefore considered as an estimate and must take into account the range of possibilities. If the estimated gestational age by a patient's last menstrual period differs from the ultrasound estimate by more than these accepted variations, the ultrasound estimate of gestational age should be used instead of the patient's menstrual cycle estimate (ACOG, 2004).

Due to the lower margin of error first trimester ultrasonography seems to be superior to mid-trimester ultrasound for pregnancy dating (Mandruzzato et al., 2010). In a small prospective randomised controlled trial, routine first trimester ultrasound for pregnancy dating reduced the incidence of postterm pregnancy from 13% to 5% when compared with second trimester ultrasound (Bennett et al., 2004). Another study of this issue, showed that prolonged pregnancy is less common in women dated by ultrasound before 12 weeks compared with women scanned between 12 and 24 weeks (2.7 versus 3.7% respectively; P=0.02). Another interesting finding of this study was that better dating revealed a greater difference in the rate of perinatal complications between term and postterm pregnancies (Caughey et al., 2008a).

B) *Prevention of postterm pregnancy*

Prevention of postterm pregnancies seems to be the best management. Induction of labour at term is the most decisive way of prevention. However, clinicians and patients alike are concerned about risks associated with induction of labour including an increased caesarean section rate. To avoid formal induction and encourage spontaneous onset of labour at term, several minimally invasive interventions have been recommended. This includes membrane sweeping, unprotected sexual intercourse, nipple stimulation and acupuncture.

Membrane sweeping or stripping is a relatively simple technique usually performed without admission to hospital. it has the potential to initiate labour by increasing local production of prostaglandins and, thus, reduce pregnancy duration or pre-empt formal induction of labour with either oxytocin, prostaglandins or amniotomy.

Some studies show that membrane sweeping may reduce the interval to spontaneous onset of labour and in turn the proportion of women with postterm pregnancy. However, there is no consistent evidence that it reduces the incidence of operative vaginal delivery, Caesarean section rates, maternal or neonatal complications (Kashanian et al., 2006; de Miranda et al., 2006).

A Cochrane review (Boulvain et al., 2005) on membrane sweeping for induction of labour in 2010 concluded that sweeping of the membranes, performed as a general policy in women at term, was associated with reduced duration of pregnancy and reduced frequency of pregnancy continuing beyond 41 & 42 weeks. To avoid one formal induction of labour, sweeping of membranes must be performed in eight women (NNT = 8). There was no evidence of a difference in the risk of maternal or neonatal infection. Discomfort during vaginal examination and other adverse effects (bleeding, irregular contractions) were more frequently reported by women allocated to sweeping. Studies comparing sweeping with prostaglandin administration are of limited sample size and do not provide evidence of benefit.

Sexual intercourse is widely believed to facilitate the onset of labour (Schaffir, 2002). The action of sexual intercourse in stimulating labour is unclear, it may in part be due to the physical stimulation of the lower uterine segment, endogenous release of oxytocin as a result of orgasm, uterine activity which is thought to be provoked by orgasm (Chayen et al., 1986), or from the direct action of prostaglandins in semen (Taylor and Kelly, 1974) as human semen is the biological source that is presumed to contain the highest prostaglandin concentration. Some studies show that unprotected sexual intercourse results in earlier onset of labour, reduction in postterm pregnancy rates and fewer interventions with labour induction (Tan et al., 2006). However, a Cochrane review concluded that the role of sexual intercourse as a method of induction of labour is uncertain and that further studies of sufficient power are needed to assess its value (Kavanagh et al., 2001). Another study in 2009 reported that women who had coitus were less likely to go into spontaneous labour prior to their scheduled induction date (Tan et al., 2009).

Acupuncture has long been used in China and other Asian countries for pregnancy-related conditions, including breech presentation (Tiran, 2004), labour pains (Qu and Zhou, 2007), and hyperemesis gravidarum (Helmreich et al., 2006). The Shanghai College of Traditional Medicine recommends acupuncture for labour induction (John Ed O'Conner, 1981), and it is used routinely for labour induction in some societies (West, 1997). Additionally, there

do not appear to be significant maternal or fetal risks associated with acupuncture (Neri et al., 2002; Scharf et al., 2003). A Cochrane review on this issue concluded that fewer women receiving acupuncture required induction compared to standard care (RR 1.45, 95% CI 1.08, 1.95; three trials) (Smith and Crowther, 2004). In conclusion, acupuncture cannot be definitely assessed because of the paucity of trial data and the need for further evaluation (Rabl et al., 2001; Smith and Crowther, 2004).

Breast stimulation is thought to promote labour onset and has been suggested as a means of inducing labour. It is a non-medical intervention allowing women greater control over the induction process. A Cochrane review on breast stimulation for cervical ripening and induction of labour (Kavanagh et al., 2005) concluded that breast stimulation appears beneficial in reducing the number of women not in labour after 72 hours, and reducing postpartum haemorrhage rates. However, until safety issues have been fully evaluated it should not be used in high-risk women. Further studies are required before recommending its adoption in practice.

C) *Antepartum fetal surveillance*

Women who reached 42 weeks gestation and opt to continue their pregnancy with conservative management should undergo antenatal fetal surveillance. Despite the fact that there is no evidence to suggest that antepartum fetal surveillance in postterm pregnancies decreases perinatal mortality, antenatal fetal surveillance has become a common practice in these cases on the basis of universal acceptance. The reasons are: 1) data suggest a gradual increase in perinatal morbidity and mortality during this period (Fig. 3) (Hilder et al., 1998); 2) there is no evidence that antenatal fetal monitoring adversely affects postterm women; 3) the published studies are of insufficient power to demonstrate a benefit of monitoring in these cases; 4) because of ethical and medico-legal considerations, no studies have included postterm patients who were not monitored, and it is unlikely that any future studies will do so.

Women who have passed their EDD but who have not yet reached 42 weeks of gestation constitute another group for whom antenatal fetal surveillance has been proposed. Despite the lack of evidence demonstrating a beneficial effect, antenatal fetal surveillance is often performed during this period. Some studies report a greater complication rate among women giving birth during the latter half of this 2-week period (Bochner et al., 1988; Guidetti et al., 1989; Alexander et al., 2000; Alexander et al., 2001; Treger et al., 2002). Although the data are inconsistent, there is a suggestion that antenatal testing at 40 to 42 weeks of gestation may be associated with improvements in perinatal outcome. In one retrospective study, women with routine antenatal testing from 41 weeks had lower rates of caesarean delivery for non-reassuring fetal testing than women in whom testing was started at 42 weeks (2.3% vs. 5.6%, respectively; $P < 0.01$) (Bochner et al., 1988). Furthermore, the group with delayed antenatal testing experienced 3 stillbirths and 7 other neonatal morbidity events as compared with none in the 41-week antenatal testing group ($P < 0.05$). However, no randomized controlled trial has demonstrated an improvement in perinatal outcome attributable to fetal surveillance between 40 and 42 weeks of gestation (Usher et al., 1988).

The literature is inconsistent regarding both the type and frequency of antenatal surveillance among postterm patients (Cardozo et al., 1986; Martin et al., 1989; Hannah et al., 1992; Almstrom et al., 1995; Crowley, 2004). Options for evaluating fetal well-being include nonstress testing (CTG), biophysical profile (BPP) or modified BPP (CTG plus amniotic fluid volume estimation), contraction stress testing, and a combination of these modalities. Practices vary widely and no single method has been shown to be superior (Crowley, 2004).

Ultrasound assessment of amniotic fluid volume appears to be important. Delivery should be considered if there is evidence of fetal compromise or oligohydramnios (Crowley et al., 1984; Phelan et al., 1985). Adverse pregnancy outcome (e.g. non-reassuring fetal heart rate tracing, neonatal intensive care unit admission, low Apgar score) is more common when oligohydramnios is present (Bochner et al., 1987; Tongsong and Srisomboon, 1993). Oligohydramnios may result from feto-placental insufficiency or increased renal artery resistance (Oz et al., 2002) and may predispose to umbilical cord compression, thus leading to intermittent fetal hypoxemia, meconium passage, or meconium aspiration. Frequent (twice weekly) screening in postterm pregnancies is suggested because amniotic fluid can become drastically reduced within 24 to 48 hours (Clement et al., 1987). However, there is no consistent definition of oligohydramnios in the postterm pregnancy. Options include 1) largest vertical fluid pocket < 2 cm in depth or 2) amniotic fluid index (AFI) <5cm (Crowley et al., 1984; Chamberlin et al., 1984). A prospective, double blind, cohort study of 1584 women after 40 weeks of gestation found that AFI < 5cm, but not largest vertical fluid pocket < 2cm, was associated with birth asphyxia and meconium aspiration, although the sensitivity for adverse outcome was low (Morris et al., 2003).

Umbilical artery Doppler velocimetry has no proven benefit in monitoring the postterm fetus and is not recommended for this indication (Guidetti et al., 1987; Stokes et al., 1991). Although no firm

recommendation can be made on the basis of published research regarding the frequency of antenatal surveillance among postterm women, it seems that twice-weekly testing is widely acceptable by many clinicians (ACOG, 2004). It also seems that testing, using CTG and AF volume assessment, constitutes an acceptable standard by many clinicians.

D) *Induction of labour*

Induction of labour is indicated when the benefits of delivery outweighs the risks associated with induction. The main concern around induction of labour in postterm low risk pregnancies is related to uterine overstimulation, fetal distress, failure of induction and increase in caesarean section rates. There are also risks associated with induction in particular groups of patients with specific risk factors such as risk of uterine rupture in women with previous caesarean section. Induction of labour is more likely to succeed when the cervix is favourable. Several techniques have been evaluated to assess cervical favourability and to predict the likelihood of success in women undergoing labour induction. These include digital cervical examination (Bishop score), ultrasound assessment of cervical length and more recently biochemical markers (oestriol/oestradiol ratio).

A favourable cervix is defined as a cervix with Bishop score of ≥ 6. Digital cervical assessment has been shown to be superior to trans-vaginal ultrasound assessment of cervical length at term to predict the time interval from IOL to delivery (Rozenberg et al., 2005). However, digital cervical assessment remains subjective and could lack reproducibility.

Oestrogens have been demonstrated to be important hormones involved in the regulation of several functions during pregnancy (Goodwin, 1999). Oestriol (E3), oestradiol (E2), and the oestriol/oestradiol ratio in particular play an important role in the control of parturition by creating a specific oestrogenic environment at the onset of labour (Smith et al., 2009). Oestrogens were therefore, studied on the basis that they may contribute to a better assessment of women with postterm pregnancy that are at risk of unsuccessful induction, such as women with an unfavourable cervix. In one study, E3/E2 ratio was presented as a biochemical marker to predict the responsiveness to IOL (Torricelli et al., 2011)

It was found that maternal serum E3/E2 ratio is significantly higher in women responding to IOL (Torricelli et al., 2011). These data was in accord with other studies (Walsh et al., 1984; Al-Shawarby et al., 2006). The study suggested that when pregnancy is approaching labour the levels of oestriol and oestradiol change in maternal circulation causing an increase in their ratio (E3/E2 ratio). These data also suggested that oestrogen activation in human parturition is mediated at the functional level by an increase in myometrial oestrogen responsiveness. The study concluded that a combination of ultrasound assessment of cervical length and the E3/E2 ratio shows a good performance in the prediction of successful IOL in postterm pregnancy (Torricelli et al., 2011).

Induction of labour in women with a favourable cervix

Clinicians are less concerned about IOL in women with a favourable cervix; these women are more likely to go into spontaneous labour on their own, and if induced, induction is more likely to succeed. It seems therefore that IOL in this group could be less cost effective as the intervention might not be required in the first place. Most studies of postterm pregnancy comparing outcomes of labour induction with those of expectant management excluded women with a favourable cervix (Dyson et al., 1987; Heden et al., 1991; Hannah et al., 1992; Shaw et al., 1992; NICHHD, 1994). Moreover, when women in the expectant management group experienced a spontaneous change in their cervical status, expectant management ceased and labour was induced (Augensen et al., 1987; Witter et al., 1987; NICHHD, 1994).

In postterm pregnancy studies in which women with a favourable cervix were managed expectantly, there was no indication that expectant management had a deleterious effect on the outcome of the pregnancy, but results were not stratified according to the condition of the cervix (Cardozo et al., 1986; Bergsjo et al., 1989; James et al., 2001; Chanrachakul and Herabutya, 2003). When the ongoing risk of stillbirth is weighted against the very low risk of failed induction in this group, it is suggested that elective IOL may be a reasonable option for such women at 39-41 weeks of gestation. However, such a conclusion requires a large well designed trial to determine whether this approach would reduce complications and improve fetal, neonatal and/or maternal outcomes. At 41-42 weeks of gestation it seems that the risks of IOL are outweighed by the benefits and it is a common practice to offer IOL to such patients (Caughey et al., 2008b).

Induction of labour in women with a unfavourable cervix

As many as 80% of women who reach 42 weeks gestation have an unfavourable cervix (Bishop Score < 6). Using cervical ripening prior to induction in these cases appears to have some advantage in terms

of outcome regardless of parity or method of induction. Pre-induction cervical ripening has resulted in fewer failed inductions, reduced fetal and maternal morbidity, reduced medical cost, and possibly a reduced rate of caesarean delivery in the general obstetric population (Xenakis et al., 1997; Poma, 1999; Sanchez-Ramos et al., 2002).

Cochrane systematic reviews demonstrated that prostaglandins (PGs) improve cervical ripeness and could initiate uterine contractions (Boulvain et al., 2007; Kelly et al., 2009). However, their value in reducing induction-delivery interval and CS rate in postterm women is debatable (Rayburn et al., 1988; Papageorgiou et al., 1992; Sawai et al., 1994).

Although multiple studies have used PG to induce labour in postterm pregnancies, no standardized dose or dosing interval has been established. Overall, the medications were well tolerated with few reported side effects. Higher doses of PG (especially PGE1) have been associated with an increased risk of uterine tachysystole and hyper-stimulation leading to non-reassuring fetal testing results (How et al., 2001). As such lower doses (e.g. 25 microgram intravaginal misoprostol) are preferable to 50 microgram (Sanchez-Ramos et al., 2002). When PG is used, fetal heart rate monitoring should be performed routinely to assess fetal well-being because of the risk of uterine hyper-stimulation.

Although postterm pregnancy is defined as a pregnancy of 42 weeks or more of gestation, several large multi-centre randomized studies of management of pregnancy beyond 40 weeks of gestation reported favourable outcomes with routine IOL as early as the beginning of 41 weeks of gestation (Hannah et al., 1992; NICHHD, 1994; Crowley, 2004). The largest study to date randomly assigned 3,407 low-risk women with uncomplicated singleton pregnancies at 41 weeks of gestation to labour induction (with or without cervical ripening agents) within 4 days of randomization or expectant management until 44 weeks of gestation (Hannah et al., 1992). Elective induction resulted in a lower caesarean delivery rate (21.2% versus 24.5%), primarily related to fewer surgeries performed for non-reassuring fetal heart rate tracings. Patient satisfaction was significantly higher in women randomly assigned to labour induction.

A meta-analysis of 19 trials of routine versus selective labour induction in postterm patients found that routine induction after 41 weeks of gestation was associated with a lower rate of perinatal mortality (OR, 0.2; 95% CI, 0.06-0.7) and no increase in the caesarean delivery rate (OR, 1.02; 95% CI, 0.75-1.38) (2). Routine labour induction also had no effect on the instrumental delivery rate, use of analgesia, or incidence of fetal heart rate abnormality. The risk of meconium-stained amniotic fluid was reduced, but the risks of meconium aspiration syndrome and neonatal seizures were unaffected (Crowley, 2004). The actual risk of stillbirth during the 41st week of gestation is estimated at 1.04-1.27 per 1,000 undelivered women, compared with 1.55-3.1 per 1,000 women at or beyond 42 weeks of gestation (Caughey et al., 2008b). Taken together, these data suggest that routine induction at 41 weeks of gestation has fetal benefit without incurring the additional maternal risks of a higher rate of caesarean delivery (Rand et al., 2000; Crowley, 2004). This conclusion has not been universally accepted (Cardozo et al., 1986; Witter et al., 1987; Heden et al., 1991; NICHHD, 1994).

Induction of labour in postterm women with previous caesarean section

Vaginal birth after caesarean delivery (VBAC) has been promoted as a reasonable alternative to elective repeat caesarean delivery for some women. The risk of uterine rupture does not appear to increase substantially after 40 weeks of gestation (Callahan et al., 1999; Zelop et al., 2001), but the risk appears to be increased with IOL with prostaglandins or syntocinon regardless of gestational age (Zelop et al., 2001; Lydon-Rochelle, 2001). In a population-based, retrospective cohort analysis, the risk of uterine rupture with VBAC was 1.6 per 1000 women with previous one caesarean delivery without labour, 5.2 per 1000 women with spontaneous onset of labour, 7.7 per 1000 women with IOL without PG, and 24.5 per 1000 women with PG induction of labour (Lydon-Rochelle, 2001). There is limited evidence on the efficacy or safety of VBAC after 42 weeks of gestation. As such, no firm recommendation can be made for this particular group (ACOG, 2004).

Conclusion

Postterm pregnancy is associated with fetal, neonatal and maternal complications including morbidity and perinatal mortality. These risks were originally underestimated because of inaccurate pregnancy dating and the denominator used to define stillbirth. The use of routine ultrasound for dating in the first trimester has decreased the overall rate of postterm pregnancy and demonstrated higher complication rates in postterm pregnancies due to better distinction between term and postterm gestation. Also the use of ongoing pregnancies as a denominator for stillbirth rather than pregnancies delivered has shown a six-fold increase in perinatal complications in postterm women.

Forty two weeks of gestation does not represent a threshold under which risks are uniformly distributed, and there is emerging evidence that fetal, neonatal and maternal complications do increase

before 42 weeks (from 38-39 weeks onwards with an obvious rise after 40&41 weeks gestation). Therefore the definition and management of post-term pregnancy have been challenged in several studies in recent years**.** In the light of the current evidence earlier intervention with IOL at 41 weeks appears appropriate management.

We conclude that in the light of the current evidence IOL at 41 weeks is justified to minimise both fetal and maternal complications.

References

Alexander JM, McIntire DD, Leveno KJ. Forty weeks and beyond: pregnancy outcomes by week of gestation. Obstet Gynecol. 2000; 96:291-4.

Alexander JM, McIntire DD, Leveno KJ. Prolonged pregnancy: induction of labor and cesarean births. Obstet Gynecol. 2001; 97:911.

Alfirevic Z, Walkinshaw SA. Management of post-term pregnancy: to induce or not? Br J Hosp Med. 1994;52:218-21.

Allen VM, O'Connell CM, Farrell SA et al. Economic implications of method of delivery. Am J Obstst Gynecol. 2005; 193:192-7.

Almstrom H, Granstrom L, Ekman G. Serial antenatal monitoring compared with labor induction in post-term pregnancies. Acta Obstet Gynecol Scand. 1995;74:599-603.

Al-Shawarby SA, Connell RJ. Induction of labour at term with vaginal prostaglandins preparations: a randomized controlled trial of Prostin vs Propess. J Obstet Gynaecol. 2006;26:627-30.

ACOG (American College of Obstetricians and Gynecologists). Management of Postterm Pregnancy. ACOG Practice Bulletin No. 6 (1997). Int J Gynaecol Obstet. 1998;60:86-91.

ACOG (American College of Obstetricians and Gynecologists). Fetal Macrosomia. Washington, DC; ACOG; 2000. ACOG Practice Bulletin #22.

ACOG (American College of Obstetricians and Gynecologists). Management of Postterm Pregnancy. ACOG Practice Bulletin No. 55. Obstet Gynecol. 2004;104:639-46.

Augensen K, Bergsjo P, Eikeland T et al. Randomised comparison of early versus late induction of labour in post-term pregnancy. Br Med J. (Clin Res Ed) 1987;294:1192-5.

Badawi N, Kurinczuk JJ, Keogh JM et al. Antepartum risk factors for newborn encephalopathy: the Western Australian case-control study. Br Med J. 1998;317:1549-53.

Bakketeig LS, Bergsjo P. Post-term pregnancy: magnitude of the problem. In: Enkin M, Keirse MJ, Chalmers I, eds. Effective Care in Pregnancy and Childbirth. Oxford: Oxford University Press, 1989:765-75.

Baranova A, Gowder SJ, Schlauch K et al. Gene expression of leptin, resistin, and adiponectin in the white adipose tissue of obese patients with non-alcoholic fatty liver disease and insulin resistance. Obes Surg. 2006;16:1118-25.

Bennett KA, Crane JM, O'Shea P et al. First trimester ultrasound screening is effective in reducing postterm labor induction rates: a randomized controlled trial. Am J Obstet Gynecol. 2004;190:1077-81.

Bergsjo P, Huang GD, Yu SQ et al. Comparison of induced versus non-induced labor in postterm pregnancy. A randomized prospective study. Acta Obstet Gynecol Scand. 1989;68:683-7.

Bochner CJ, Medearis AL, Davis J et al. Antepartum predictors of fetal distress in postterm pregnancy. Am J Obstet Gynecol. 1987;157:353-8.

Bochner CJ, Williams J 3rd, Castro L et al. The efficacy of starting postterm antenatal testing at 41 weeks as compared with 42 weeks of gestational age. Am J Obstet Gynecol. 1988; 159:550-4.

Boulvain M, Stan C, Irion O. Membrane sweeping for induction of labour. Cochrane Database Syst Rev. 2005;1:CD000451.

Boulvain M, Kelly AJ, Irion O. Intracervical prostaglandins for induction of labour. Cochran Database Syst Rev. 2007; CD006971.

Bruckner TA, Cheng YW, Caughey AB. Increased neonatal mortality among post-term births in California. Am J Obstet Gynecol. 2008;199:421.e1-7.

Callahan C, Chescheir N, Steiner BD. Safety and efficacy of attempted vaginal birth after cesarean beyond the estimated date of delivery. J Reprod Med. 1999;44:606-10.

Campbell MK, Ostbye T, Irgens LM. Post-term birth: risk factors and outcomes in a 10-year cohort of Norwegian births. Obstet Gynecol. 1997;89:543-8.

Cardozo L, Fysh J, Pearce JM. Prolonged pregnancy: the management debate. Br Med J. (Clin Res Ed) 1986;293: 1059-63.

Caughey AB, Bishop J. Maternal complications of pregnancy increase beyond 40 weeks of gestation in low risk women. J Perinatol. 2006;26:540-5.

Caughey AB, Musci TJ. Complications of term pregnancies beyond 37 weeks of gestation. Obstet Gynecol. 2004;103:57-62.

Caughey AB, Nicholson JM, Washington AE. First versus second trimester ultrasound: the effect on pregnancy dating and perinatal outcomes. Am J Obstet Gynecol. 2008a; 198: 703.e1-e5.

Caughey AB, Sengovskikh VV, Norwitz ER. Postterm pregnancy: How Can We Improve Outcomes? Obstet Gynecol Survey. 2008b;63:715-24.

Caughey AB, Stotland NE, Escobar GJ. What is the best measure of maternal complications of term pregnancy: ongoing pregnancies or pregnancies delivered? Am J Obstet Gynecol. 2003;189:1047-52.

Caughey AB, Stotland NE, Washington AE et al. Who is at risk for prolonged and postterm pregnancy. Am J Obstet Gynecol. 2009;200(6):683e1-683e5.

Caughey AB, Stotland NE, Washington AE et al. Maternal obstetric complications of pregnancy are associated with increasing gestational age at term. Am J Obstet Gynecol. 2007;196:155.e1-e6.

Caughey AB, Washington AE, Laros RK. Neonatal complications of term pregnancies: rates increase in a continuous, not threshold fashion. Am J Obstet Gynecol. 2005; 192(1):185-9.

Chamberlain PF, Manning FA, Morrison I et al. Ultrasound evaluation of amniotic fluid volume. I. The relationship of marginal and decreased amniotic fluid volumes to perinatal outcome. Am J Obstet Gynecol. 1984;150:245-9.

Chanrachakul B, Herabutya Y. Postterm with favorable cervix: is induction necessary? Eur J Obstet Gynecol Reprod Biol. 2003;106:154-7.

Chayen B, Tejani N, Verma UL et al. Fetal heart rate changes and uterine activity during coitus. Acta Obstet Gynecol Scand. 1986; 65:853-5.

Clement D, Schifrin BS, Kates RB. Acute oligohydramnios in postdate pregnancy. Am J Obstet Gynecol. 1987;157:884-6.

Collins JW, Schulte NF, George L et al. Postterm delivery among African Americans, Mexican Americans and Whites in Chicago. Ethn Dis. 2001;11:181-7.

Cotzias CS, Paterson-Brown S, Fisk NM. Prospective risk of unexplained stillbirth in singleton pregnancies at term: population based analysis. Br Med J. 1999;319:287-8.

Crowley P, O'Herlihy C, Boylan P. The value of ultrasound measurement of amniotic fluid volume in the management of prolonged pregnancies. Br J Obstet Gynaecol. 1984;91: 444-8.

Crowley P. Interventions for preventing or improving the outcome of delivery at or beyond term (Cochrane review). In: The Cochrane Library, Issue 2, 2004. Chicester, UK: John Wiley & Sons, Ltd.

de Miranda E, can der Bom JG, Bonsel GJ et al. Membrane sweeping and prevention of post-term pregnancy in low-risk pregnancies: a randomised controlled trial. Br J Obstet Gynaecol. 2006;113:402-8.

Divon MY, Ferber A, Nisell H et al. Male gender predisposes to prolongation of pregnancy. Am J Obstet Gynecol. 2002; 187:1081-3.

Doherty L, Norwitz R. Prolonged Pregnancy: When should we intervene? Curr Opin Obstet Gynecol. 2008;20:519-27.
Dyson DC, Miller PD, Armstrong MA. Management of prolonged pregnancy: induction of labor versus antepartum fetal testing. Am J Obstet Gynecol. 1987;156:928-34.
Eden RD, Seifert LS, Winegar A et al. Perinatal characteristics of uncomplicated postdate pregnancies. Obstet Gynecol. 1987;69:296-9.
Ellis MJ, Livesey JH, Inder WJ et al. Plasma corticotrophin-releasing hormone and unconjugated estriol in human pregnancy: gestational patterns and ability to predict preterm delivery. Am J Obstet Gynecol. 2002;186:94-9.
Feldman GB. Prospective risk of stillbirth. Obstet Gynecol. 1992;79:547-53.
Fonseca L, Monga M, Silva J. Postdates pregnancy in an indigent population: the financial burden. Am J Obstet Gynecol. 2003;188:1214-6.
Fraser WD, Hofmeyr J, Lede R et al. Amnioinfusion for the prevention of the meconium aspiration syndrome. N Engl J Med. 2005;353:909.
Gardosi J, Vanner T, Francis A. Gestational age and induction of labor for prolonged pregnancy. Br J Obstet Gynaecol. 1997;104:792-7.
Goodwin TM. A role for estriol in human labor, term and preterm. Am J Obstet Gynecol. 1999; 180:S208-13.
Guidetti DA, Divon MY, Cavalieri RL et al. Fetal umbilical artery flow velocimetry in postdate pregnancies. Am J Obstet Gynecol. 1987;157:1521-3.
Guidetti DA, Divon MY, Langer O. Postdate fetal surveillance: is 41 weeks too early? Am J Obstet Gynecol. 1989; 161:91-3.
Hannah ME, Hannah WJ, Hellmann J et al. Induction of labor as compared with serial antenatal monitoring in post-term pregnancy. A randomized controlled trial. The Canadian Multicenter Post-term Pregnancy Trial Group. N Engl J Med. 1992;326:1587-92.
Hannah ME. Postterm pregnancy: should all women have labour induced? A review of the literature. Fetal Matern Med Rev. 1993;5:3.
Heden L, Ingemarsson I, Ahlstrom H et al. Induction of labor versus conservative management in prolonged pregnancy: controlled study. Int J Feto-Maternal Med. 1991;4:231-6.
Heimstad R, Romundstad PR, Eik-Nes SH et al. Outcomes of pregnancy beyond 37 weeks of gestation. Obstet Gynecol. 2006;108:500-8.
Heimstad R, Romundstad PR, Hyett J et al. Women's experiences and attitudes towards expectant management and induction of labor for post-term pregnancy. Acta Obstet Gynecol Scand. 2007;86:950-6.
Helmreich RJ, Shiao SY, Dune LS. Meta-analysis of acustimulation effects on nausea and vomiting in pregnant women. Explore (NY). 2006;2:412-21
Herabutya Y, Prasertsawat PO, Tongyai T et al. Prolonged pregnancy: the management dilemma. Int J Gynaecol Obstet. 1992;37:253-8.
Hickey CA, Cliver SP, McNeal SF et al. Low pregravid body mass index as a risk factor for preterm birth: variation by ethnic group. Obstet Gynecol. 1997;89:206-12.
Hilder L, Costeloe K, Thilaganathan B. Prolonged pregnancy: evaluating gestation-specific risks of fetal and infant mortality. Br J Obstet Gynaecol. 1998;105:169-73.
Hofmeyr GJ. Amnioinfusion for meconium-stained liquor in labour. Cochrane Database Syst Rev. 2002; 1:CD000014.
How HY, Leaseburge L, Khoury JC et al. A comparison of various routes and dosages of misoprostol for cervical ripening and the induction of labor. Am J Obstet Gynecol. 2001; 185:911-5.
Ingemarsson I, Kallen K. Stillbirths and rate of neonatal deaths in 76,761 postterm pregnancies insweden, 1982-1991: a register study. Acta Obstet Gynecol Scand. 1997; 76:658-62.
James C, George SS, Gaunekar N, Seshadri L. Management of prolonged pregnancy: a randomised trial of induction of labour and antepartum foetal monitoring. Natl Med J. India. 2001;14:270-3.
John Ed O'Conner DB. Acupuncture: A Comprehensive Text. Seattle: Eastland Press; 1981.
Kabbur PM, Herson VC, Zaremba S et al. Have the year 2000 neonatal resuscitation program guidelines changed the delivery room management or outcome of meconium-stained infants? J Perinatol. 2005;25:694-7.
Kashanian M, Akbarian A, Baradaran H et al. Effect of membrane sweeping at term pregnancy on duration of pregnancy and labor induction: a randomized trial. Gynecol Obstet Invest. 2006;62:41-4.
Kavanagh J, Kelly AJ, Thomas J. Breast stimulation for cervical ripening and induction of labour. Cochrane Database Syst Rev. 2005;(3):CD003392.
Kavanagh J, Kelly AJ, Thomas J. Sexual intercourse for cervical ripening and induction of labour. Cochrane Database Syst Rev. 2001;(2):CD003093.
Kelly AJ, Malik S, Smith L et al. Vaginal prostaglandin (PGE2 and PGF2a) for induction of labour at term. Cochrane Database Syst Rev. 2009; (4):CD003101.
Kistka ZA, Palomar L, Boslaugh SE et al. Risk for postterm delivery after previous postterm delivery. Am J Obstet Gynecol. 2007;196:241e1-241e6.
Kitlinski ML, Kallen K, Marsal K et al. Gestational age-dependent reference values for pH in umbilical cord arterial blood at term. Obstet Gynecol. 2003;102:338-45.
Knox GE, Huddleston JF, Flowers CE Jr. Management of prolonged pregnancy: results of a prospective randomized trial. Am J Obstet Gynecol. 1979;134:376-84.
Laursen M, Bille C, Olesen AW et al. Genetic influence on prolonged gestation: a population-based Danish twin study. Am J Obstet Gynecol. 2004;190:489-94.
Lydon-Rochelle M, Holt VL, Easterling TR et al. Risk of uterine rupture during labor among women with a prior cesarean delivery. N Engl J Med. 2001;345:3-8.
Magann EF, Chauhan SP, McNamara MF et al. Membrane sweeping versus dinoprostone vaginal insert in the management of pregnancies beyond 41 weeks with an unfavorable cervix. J Perinatol. 1999;19:88-91.
Magann EF, Chauhan SP, Nevils BG et al. Management of pregnancies beyond forty-one weeks' gestation with an unfavorable cervix. Am J Obstet Gynecol. 1998;178:1279-87.
Mandruzzato G, Alfirevic Z, Chervenak F et al. Guidelines for the management of postterm pregnancy. J Perinat Med. 2010;38(2):111-9.
Mannino F. Neonatal complications of postterm gestation. J Reprod Med. 1988; 33:271-6.
Martin JA, Hamilton BE, Sutton PD et al. Births: final data for 2005. Natl Vital Stat Rep. 2007; 56:1-103.
Martin JN Jr, Sessums JK, Howard P et al. Alternative approaches to the management of gravidas with prolonged-postterm-postdate pregnancies. J Miss State Med Assoc. 1989;30:105-11.
McLean M, Bisits A, Davies J et al. A placental clock controlling the length of human pregnancy. Nat Med. 1995;1:460-3.
Mogren I, Stenlund H, Hogberg U. Recurrence of prolonged pregnancy. Int J Epidemiol. 1999; 28:253-7.
Morris JM, Thompson K, Smithey J et al. The usefulness of ultrasound assessment of amniotic fluid in predicting adverse outcome in prolonged pregnancy: A prospective blinded observational study. BJOG. 2003;110:989-94.
Neilson JP. Ultrasound for fetal assessment in early pregnancy. Cochrane Database Syst Rev. 2000;2:CD000182.
Neri I, Fazzio M, Menghini S et al. Non-stress test changes during acupuncture plus moxibustion on BL67 point in breech presentation. J Soc Gynecol Investig. 2002;9:158-62.
NICHHD (The National Institute of Child Health and Human Development) Network of Maternal-Fetal Medicine Units. A clinical trial of induction of labor versus expectant management in post-term pregnancy. Am J Obstet Gynecol. 1994; 170:716-23.
Nicholson JM, Kellar LC, Kellar GM. The impact of the interaction between increasing gestational age and obstetrical risk on birth outcomes: evidence of a varying optimal time of delivery. J Perinatol. 2006;26:392-402.
Olesen AW, Basso O, Olsen J. An estimate of the tendency to repeat postterm delivery. Epidemiology. 1999;10:468-9.

Olesen AW, Basso O, Olsen J. Risk of recurrence of prolonged pregnancy. Br Med J. 2003a; 326:476.
Olesen AW, Westergaard JG, Olsen J. Perinatal and maternal complications related to postterm delivery: A national register-based study, 1978-1993. Am J Obstet Gynecol. 2003b;189:222-7.
Oz AU, Holub B, Mendilcioglu I et al. Renal artery Doppler investigation of the etiology of oligohydramnios in postterm pregnancy. Obstet Gynecol. 2002;100:715-8.
Papageorgiou I, Tsionou C, Minaretzis D et al. Labor characteristics of uncomplicated prolonged pregnancies after induction with intracervical prostaglandin E2 gel versus intravenous oxytocin. Gynecol Obstet Invest. 1992;34:92-6.
Phelan JP, Platt LD, Yeh SY et al. The role of ultrasound assessment of amniotic fluid volume in the management of the postdate pregnancy. Am J Obstet Gynecol. 1985;151:304-8.
Poma PA. Cervical ripening. A review and recommendations for clinical practice. J Reprod Med. 1999;44:657-68.
Qu F, Zhou J. Electro-acupuncture in relieving labor pain. Evid Based Complement Alternat Med. 2007;4:125-30.
Rabl M, Ahner R, Bitschnau M et al. Acupuncture for cervical ripening and induction of labor at term--a randomized controlled trial. Wien Klin Wochenschr. 2001;113:942-6.
Rand L, Robinson JN, Economy KE et al. Post-term induction of labor revisited. Obstet Gynecol. 2000; 96:779-83.
Rayburn W, Gosen R, Ramadei C et al. Outpatient cervical ripening with prostaglandin E2 gel in uncomplicated postdate pregnancies. Am J Obstet Gynecol. 1988;158:1417-23.
Rosen MG, Dickinson JC. Management of post-term pregnancy. N Engl J Med.1992; 326:1628-9.
Rozenberg P, Chevret S, Ville Y. Comparison of pre-induction ultrasonographic cervical length and Bishop score in predicting risk of cesarean section after labor induction with prostaglandins. Gynecol Obstet Fertil. 2005;33:17-22.
Sanchez-Ramos L, Kaunitz AM et al. Labor induction with 25 micro versus 50 micro intravaginal misoprostol: a systematic review. Obstet Gynecol. 2002;99:145-51.
Savitz DA, Terry JW Jr, Dole N et al. Comparison of pregnancy dating by last menstrual period, ultrasound scanning, and their combination. Am J Obstet Gynecol. 2002; 187:1660-6.
Sawai SK, O'Brien WF, Mastrogiannis DS et al. Patient-administered outpatient intravaginal prostaglandin E2 suppositories in post-date pregnancies: a double-blind, randomized, placebo-controlled study. Obstet Gynecol. 1994;84:807-10.
Schaffir J. Survey of folk beliefs about induction of labor. Birth. 2002;29:47-51.
Scharf A, Staboulidou I, Gunter HH et al. Influence of antenatal acupuncture on cardiotocographic parameters and maternal circulation -a prospective study. Z Geburtshilfe Neonatol. 2003;207:166-72.
Shaw KJ, Medearis AL, Horenstein J et al. Selective labor induction in postterm patients. Observations and outcomes. J Reprod Med. 1992;37:157-61.
Shime J, Librach CL, Gare DJ et al. The influence of prolonged pregnancy on infant development at one and two years of age: a prospective controlled study. Am J Obstet Gynecol. 1986; 154:341-5.
Smith CA, Crowther CA. Acupuncture for induction of labour. Cochrane Database of Systematic Reviews. 2004:CD002962.
Smith GC. Life-table analysis of the risk of perinatal death at term and post term in singleton pregnancies. Am J Obstet Gynecol. 2001;184:489-96.
Smith R, Mesiano S, Chan EC et al. Corticotropin-releasing hormone directly and preferentially stimulates dehydroepiandrosterone sulphate secretion by human fetal adrenal cortical cells. J Clin Endocrinol Metab. 1998;83:2916-20.
Smith R, Smith JI, Shen X et al. Patterns of plasma corticotropin-releasing hormone, progesterone, estradiol, and estriol change and the onset of human labor. J clin Endocrinol Metab. 2009;94:2066-74.
Spellacy WN, Miller S, Winegar A et al. Macrosomia – maternal characteristics and infant complications. Obstet Gynecol 1985;66:158-61.
Stokes HJ, Roberts RV, Newnham JP. Doppler flow velocity waveform analysis in postdate pregnancies. Aust N Z J Obstet Gynaecol. 1991;31:27-30.
Stotland NE, Washington AE, Caughey AB. Pre-pregnancy body mass index and length of gestation at term. Am J Obstet Gynecol. 2007;197:378.e1-e5.
Sutan R, Campbell D, Prescott GJ et al. The risk factors for unexplained antepartum stillbirths in Scotland, 1994 to 2003. J Perinatology. 2010;30:311-8.
Taipale P, Hiilermaa V. Predicting delivery date by ultrasound and last menstrual period on early gestation. Obstet Gynecol. 2001;97:189-94.
Tan P C, Yow C M, Omar S Z. Coitus and orgasm at term: effect on spontaneous labour and pregnancy outcome. Singapore Med J. 2009;50:1062-8.
Tan PC, Andi A, Azmi et al. Effect of coitus at term on length of gestation, induction of labor, and mode of delivery. Obstet Gynecol. 2006;108:134-40.
Taylor PL, Kelly RW. 19-Hydroxylated E prostaglandins as the major prostaglandins of human semen. Nature. 1974;250: 665-7.
Tiran D. Breech presentation: increasing maternal choice. Complement Ther Nurs Midwifery. 2004;10:233-8.
Tongsong T, Srisomboon J. Amniotic fluid volume as a predictor of fetal distress in postterm pregnancy. Int J Gynaecol Obstet. 1993;40:213-7.
Torricelli M, Ignacchiti E, Giovannelli A et al. Maternal plasma corticotrophin-releasing factor and urocortin levels in postterm pregnancies. Eur J Endocrinol. 2006;154:281-5.
Torricelli M, Novembri R, Voltolini C et al. Biochemical and biophysical predictors of the response to the induction of labour in nulliparous postterm pregnancy. Am J Obstet Gynecol. 2011;204:39.e1-6.
Treger M, Hallak M, Silberstein T et al. Post-term pregnancy: should induction of labor be considered before 42 weeks? J Matern Fetal Neonatal Med. 2002;11:50-3.
Usha Kiran TS, Hemmadi S, Bethel J et al. Outcome of pregnancy in a woman with an increased body mass index. BJOG. 2005;112:768-72.
Usher RH, Boyd ME, McLean FH et al. Assessment of fetal risk in postdate pregnancies. Am J Obstet Gynecol. 1988;158: 259-64.
Vain NE, Szyld EG, Prudent LM et al. Oropharyngeal and nasopharyngeal suctioning of meconium-stained neonates before delivery of their shoulders: multicentre, randomised controlled trial. Lancet. 2004;364:597.
Ventura SJ, Martin JA, Curtin SC et al. Births: final data for 1998. Natl Vital Stat Rep. 2000;48:1-100.
Vorherr H. Placental insufficiency in relation to postterm pregnancy and fetal postmaturity. Evaluation of fetoplacental function; management of the postterm gravida. Am J Obstet Gynecol. 1975;123:67-103.
Walsh SW, Stanczyk FZ, Novy MJ. Daily hormonal changes in the maternal, fetal, and amniotic fluid compartments before parturition in a primate species. J Clin Endocrinol Metab. 1984; 58:929-39.
West Z. Acupuncture within the National Health Service: a personal perspective. Complement Ther Nurs Midwifery. 1997;3:83-6.
Witter FR, Weitz CM. A randomized trial of induction at 42 weeks gestation versus expectant management for postdates pregnancies. Am J Perinatol. 1987;4:206-11.
Wong SF, Hui SK, Choi H et al. Does sweeping of membranes beyond 40 weeks reduce the need for formal induction of labour? BJOG. 2002;109:632-6.
Xenakis EM, Piper JM, Conway DL et al. Induction of labor in the nineties: conquering the unfavorable cervix. Obstet Gynecol 1997;90:235-9.
Yang R, You X, Tang X et al. Corticotropin-releasing hormone inhibits progesterone production in cultured human placental trophoblasts. J Mol Endocrinol. 2006. 37(3):533-40.
Yoder BA, Kirsch EA, Barth WH et al. Changing obstetric practices associated with decreasing incidence of meconium aspiration syndrome. Obstet Gynecol. 2002; 99:731.
Yudkin PL, Wood L, Redman CW. Risk of unexplained stillbirth at different gestational ages. Lancet. 1987;1(8543):1192-4.
Zelop CM, Shipp TD, Cohen A et al. Trial of labor after 40 weeks' gestation in women with prior cesarean. Obstet Gynecol. 2001;97:391-3.