Control and assessment of the uterus and cervix during pregnancy and labour

R.E.Garfield1,3, G.Saade1, C.Buhimschi1, I.Buhimschi1, L.Shi1, S-Q.Shi1 and K.Chwalisz2

1Reproductive Sciences, Department of Obstetrics and Gynecology, University of Texas Medical Branch, Galveston, Texas, USA and 2Research Laboratories of Schering AG, Berlin, Germany

Preterm labour and resultant preterm birth are the most important problems in perinatology. Countless efforts have failed to establish a single effective treatment of preterm labour, partly because the mechanisms regulating the uterus and cervix during pregnancy are not well understood. New knowledge is needed to inhibit early progression of labour (uterine contractility and cervical ripening), and adequate quantitative tools to evaluate the uterus and cervix during pregnancy are lacking. In this review, we outline studies showing that the uterus (myometrium) and cervix pass through a conditioning step in preparation for labour. This step is not easily identifiable with present methods to assess the uterus or cervix. In the uterus, this seemingly irreversible step consists of changes in the electrical properties to make muscle more excitable and responsive to produce forceful contractions. In the cervix, the step consists of softening of the connective tissue components. Progesterone appears to have a dominant role in controlling both the uterus and cervix, as antiprogestins induce early, preterm conditioning leading to preterm labour. Apparently, nitric oxide (NO) also controls conditioning of the uterus and cervix. In the uterus, NO, in concert with progesterone, inhibits uterine contractility. At term, NO production by the uterus and placenta are decreased and allow labour to progress. In contrast, NO in the cervix increases at the end of pregnancy and it may be the final pathway for stimulating cervical ripening by activation of metalloenzymes. The progress of labour can be assessed non-invasively using electromyographic (EMG) signals from the uterus (the driving force for contractility) recorded from the abdominal surface. Uterine EMG bursts detected in this manner characterize uterine contractile events during human and animal pregnancy. A low uterine EMG activity, measured transabdominally throughout most of pregnancy, rises dramatically during labour. EMG activity also increases substantially during preterm labour in humans and rats. This method may be used one day to predict impending preterm labour and identify control steps and treatments. A quantitative method also assesses the cervix, using an optical device which measures collagen fluorescence in the cervix. The collascope estimates cervical collagen content from a fluorescent signal generated when collagen cross-links are illuminated with excitation light of about 340 nm. The system has proved useful in rats and humans at various stages of pregnancy, and indicates that cervical softening occurs progressively in the last one-third of pregnancy. In rats, collascope readings correlate with resistance measurements made in the isolated cervix, which may help to assess cervical function during pregnancy, and indicate control and treatments.

Key words: cervical ripening/diagnosis of labour/myometrium/nitric oxide/parturition

Introduction

Since prematurity is the leading cause of infant mortality and morbidity, the major problem in perinatology is...
preterm labour and subsequent preterm birth. Preterm labour has to be seen as a syndrome of multifactorial origin. Factors such as intrauterine infection, maternal and fetal stress and low socioeconomic status are clearly associated with prematurity. Presently, there is no effective treatment for preterm labour if a reduction in perinatal mortality is considered the chief criterion of efficacy. One of the reasons why efficacious and safe methods for the prevention and treatment of preterm labour are still not available is that the actual mechanisms responsible for the initiation of both term and preterm labour are unclear.

In the past, the emphasis in pregnancy research was placed primarily on mechanisms controlling the release of uterotonic agents, mostly prostaglandins, which are believed to play a central role in both term and preterm labour. Various mechanisms of premature prostaglandin release, including intrauterine infection (Romero et al., 1988), and activation of the fetal cortisol–placental corticotrophin-releasing hormone (CRH) axis under circumstances of fetal compromise (McLean et al., 1995) have been proposed as possible causes of preterm labour. In fact, it has meanwhile been well established that intrauterine infection leads to preterm labour, although it does not seem to be the major cause of prematurity, as it can be demonstrated in only 25–30% of all preterm deliveries (Romero et al., 1988). The relevance of CRH in premature deliveries is still unclear. Hence, the major proportion of preterm births is due to other mechanisms, the majority of which, however, are unknown (idiopathic preterm labour).

During the past decade our attention was mainly focused on studying the basic mechanisms controlling the transition steps from pregnancy to labour in both the myometrium and cervix. We were also interested in investigating those relaxation mechanisms which withdraw and may play a greater role in the onset of labour than the increased release of uterotonins or hormones such as oestrogens. In using the progesterone antagonists as non-invasive tools, we demonstrated that parturition is a two-step process consisting of a conditioning (preparatory) phase followed by active labour (Garfield and Yallampalli, 1993; Chwalisz and Garfield, 1994, 1997). During the conditioning phase of parturition there is a progression of uterine contractility from an inactive to a vigorously active state, and in addition a softening and opening of the rigid and closed cervix. In the uterus, the increase in both propagation and excitability are the key events, since the driving force of uterine contractility during labour is electrical activity. These two crucial phases of parturition are thought to be regulated by a coordinated change in various systems, including the interaction of endocrine, nervous and immune control mechanisms on the uterus and cervix. Experimental and clinical studies with progesterone antagonists clearly demonstrate the pivotal role of progesterone in establishing and maintaining pregnancy. We believe that the withdrawal of progesterone inhibition, either due to a drop in production (rats, rabbits, sheep) or to decreased activity in the target organs (primates, guinea pigs), represents the major mechanism in the initiation of parturition. More recently, nitric oxide (NO) has emerged as a possible element in controlling uterine quiescence and cervical function during pregnancy and labour (for review see Chwalisz et al., 1996).

The relatively slow progress in the treatment and prevention of preterm labour is also related to the lack of clear biophysical and biochemical indicators of true labour, i.e. methods which early on discriminate between unproductive uterine contractions (false labour, pre-labour) and those capable of inducing progressive cervical dilatation and expulsion of the fetus. Moreover, the present diagnostic methods which are suitable to monitor the established labour are of limited value to predict the onset of preterm labour. Furthermore, this diagnostic uncertainty of labour represents the major obstacle in clinical trials with new tocolytic agents. Hence, further progress in perinatal medicine will depend not only on new tocolytic agents, but also on the availability of improved diagnostic methods. The most important clinical question that needs to be addressed is how to predict when a patient at term or preterm will proceed to active labour. Perhaps, the efficacy of tocolytic agents could be increased if the actual treatment were to start earlier, i.e. during the conditioning phase of labour.

In this review we will define the myometrial and cervical conditioning that lead to labour, and events which regulate these processes. In addition, we will discuss our experimental studies on employing non-invasive devices to diagnose uterine contractility and cervical ripening which may be useful in helping to recognize when the uterus or the cervix is prepared for labour and thus dictate effective treatment schedules.

Model of parturition: the importance of the conditioning phase of labour

During pregnancy, the uterine contractile function is suppressed while the cervix remains firm and closed. Uterine quiescence during pregnancy is essential for the normal development of the fetus. Progesterone seems to exert an overall control on uterine quiescence by: (i) suppressing a number of genes in the myometrium which are essential for uterine contractility (connexin 43, calcium channels, receptors, etc.); (ii) upregulating the relaxation
mechanisms, including the nitric oxide system (see below); and (iii) suppressing the release of proinflammatory cytokines and reducing the availability of prostaglandins (for review see Chwalisz, 1993). For many years, labour was viewed as the transition from an inactive to an active muscle either by the addition of a uterotonin or withdrawal from tonic progesterone inhibition (Csapo, 1981). Although these models recognized the importance of progesterone in controlling uterine quiescence, they neither defined precisely the uterine stages of labour nor identified the mechanism of action of the hormones involved. In addition, Csapo’s models of parturition did not consider the changes in the cervix as an important component of parturition.

The results of experimental and clinical studies with progesterone and its antagonists indicate, however, that parturition is composed of two major steps: a relatively long conditioning (preparatory) phase, followed by a short secondary phase (active labour) (Garfield and Yallampalli 1993; Chwalisz and Garfield, 1994, 1997) (Figure 1). The conditioning step, leading to the softening of the cervix, takes place in a different time frame from that of the uterus and myometrium, indicating that the myometrium and cervix are regulated in part by independent mechanisms. In the myometrium this preparatory process involves changes in transduction mechanisms and the synthesis of several new proteins including connexins, ion channels and receptors for uterotonins. At the same time, there is a downregulation of the nitric oxide system which leads to withdrawal of uterine relaxation. In the cervix, the transition involves a change in the composition of the connective tissue and the invasion by inflammatory cells. It is likely that preparatory changes similar to those occurring in the cervix also take place in fetal membranes (Figure 1).

We view the initiation of the conditioning step as the start of parturition. The conditioning phase can be induced with antiprogestins in all species studied thus far, including humans and other primates (Chwalisz and Garfield, 1994). In humans, antiprogestin treatment during pregnancy increases the sensitivity to prostaglandins and oxytocin, supporting the concept of a conditioning phase (Swahn and Bygdeman, 1988). However, our recent studies show that cervical ripening starts prior to the fall in progesterone concentrations in the peripheral blood, which points to the existence of an additional, progesterone-independent mechanism controlling cervical ripening (see also Figure 18). At some point during the conditioning step, the process becomes irreversible and will lead to active labour and delivery. Once active labour has started, delivery cannot be delayed for more than five days in humans because the changes which occur in this preparatory phase have by this time become well established and cannot be reversed, especially not with currently available tocolytics. Active labour, leading eventually to the delivery of the fetus and placenta, starts with the onset of coordinated uterine contractions. In our opinion, the key to understanding parturition and to developing suitable treatments and diagnostic methods is the means by which the myometrium and the cervix undergo conversion to their final stages.

**Fundamental mechanisms of myometrial conditioning**

The myometrium is composed of billions of small, smooth muscle cells. The basic process that controls uterine contractions is the underlying electrical activity in the form of action potentials. Action potentials propagate between muscle cells and open ion channels and allow the entry of calcium ions to produce a contraction (Figure 2). Action potentials usually occur together in a group, forming a burst (Figure 3). The number of bursts in a given time determines the frequency of uterine contractions, whereas the duration of a burst determines the duration of uterine contraction. The force generated by the whole uterus, or the amplitude of a contraction, is dependent upon the propagation of action potentials from cell to cell and the amount of muscle mass involved (Garfield, 1994).

**Cell-to-cell coupling in the myometrium**

Many studies indicate that myometrial cells are coupled together electrically by gap junctions composed of connexin proteins (Garfield et al., 1988; Garfield, 1994). The grouping of connexins provide channels of low electrical resistance between cells and thereby furnish
pathways for the efficient conduction of action potentials. Throughout most of pregnancy, and in all species studied, the cell-to-cell channels or contacts are low, indicating poor coupling and decreased electrical conductance. This condition favours quiescence of the muscle and the maintenance of pregnancy. However, at term, the cell junctions increase and form an electrical syncytium required for effective contractions. The presence of the contacts seems to be controlled by changing oestrogen and progesterone concentrations in the uterus. Progesterone downregulates the myometrial gap junction density, while progesterone antagonists dramatically upregulate their expression.

There are only a few functional studies of electrical coupling between myometrial cells (Sims et al., 1982; Miller et al., 1989). Recently, we examined the currents between pairs of freshly isolated rat myometrial cells using double-whole-cell patch–clamp techniques (Miyoshi et al., 1996). These studies showed that during term and preterm labour, junctional conductance is significantly elevated (from 32 to 26 nS) compared with the antepartum or postpartum period (4 to 7 nS) in both longitudinal and circular muscle layers (Figure 4). The conductance values measured during labour correspond to those reported for connexin 43 (Cx43) in several tissues. These findings demonstrate that heightened electrical coupling between myometrial cells of the uterine wall emerges at the end of pregnancy in preparation for the massive, coordinated contractions of labour.

In order to determine what regulates the expression of the Cx43 protein in the human uterus, we have sequenced and characterized the promoter region of the Cx43 protein (Geimonen et al., 1996). The evidence thus far indicates that transcription of the Cx43 gene is induced by activating the protein kinase C in human myometrial cells, and that this induction involves the upregulation and stimulation of c-Jun and c-Fos through binding to an AP-1 site located in the proximal region of the gene. The function of the AP-1 site suggests that steroid hormones may regulate Cx43 expression by producing c-Fos or c-Jun. These studies may lead to a better understanding of how steroid hormones regulate this crucial step in the initiation of labour in humans.

**Calcium channels**

Calcium enters muscle cells via specific channels, and extracellular calcium is required for myometrial cells to contract forcefully. As action potentials propagate throughout the uterus (via gap junctions, see above), they depolarize muscle cells and open voltage-dependent calcium channels (VDCC). When this occurs, Ca\(^{2+}\) enters the muscle cell down its chemical gradient to activate the myofilaments and provoke a contraction. Since the driving mechanism underlying uterine contractions is electrical activity, an upregulation of both electrical coupling (increase in propagation) and VDCC (increase in excitability) will greatly enhance the action of any agents that stimulate contractility. We have recently demonstrated by reverse transcription–polymerase chain reaction (RT–PCR) that the expression of VDCC subunits in the rat myometrium increases during term and preterm labour (Tezuka et al., 1995). The increased expression, which
appears to be controlled by progesterone withdrawal, may facilitate uterine contractility during labour by increasing portals for Ca\(^{2+}\) entry.

**Nitric oxide**

One of the most exciting recent advances in biology and medicine is the discovery that the diffusible molecule nitric oxide (NO) is produced by endothelial cells, and that it is involved in the regulation of vascular tone, platelet aggregation, peripheral nitrergic transmission in smooth muscle, intracellular communication in the central nervous system, and macrophage defence mechanisms following exposure to bacterial products (Furchgott and Zawadzki, 1980; Moncada and Higgs, 1993). NO is synthesized by a family of nitric oxide synthases (NOS) which are enzymes that convert the amino acid L-arginine to L-citrulline and NO. Three highly related NOS enzymes have been isolated and identified. These include endothelial NOS (ec-NOS, type III), neuronal NOS (nc-NOS, b-NOS, type I) and inducible NOS (i-NOS, type II) (Nathan and Xie, 1994). NO rapidly penetrates smooth muscles to activate guanylate cyclase by binding to the iron-haem moiety, thus resulting in an increase in cellular levels of cyclic guanosine monophosphate (cGMP), which subsequently produces relaxation. However, NO may also act on various systems directly and independent of cGMP.

**Functional studies in vitro and in vivo**

The NO system, which is present in the uterus (Izumi et al., 1993; Jennings et al., 1993; Natuzzi et al., 1993; Sladek et al., 1993), may represent a major mechanism responsible for relaxation, pregnancy maintenance and uteroplacental-fetal perfusion during pregnancy. In-vitro studies with rat and human uterine strips show that NO, NO substrates and donors inhibit spontaneous uterine contractions, whereas the NO synthase inhibitor NG-nitro-L-arginine methyl ester (L-NAME) has a stimulatory effect (Figure 5) (Izumi et al., 1993). Although in-vivo treatment with L-NAME did not induce preterm parturition in rats (Yallampalli and Garfield, 1993), it did induce preterm parturition in late pregnant guinea pigs (Chwalisz et al., 1993). This observation indicates that there are species-related differences in uterine sensitivity to NO and that the NO system plays a pivotal role in the maintenance of pregnancy in species displaying a high NO sensitivity, such as the guinea pig. However, in both guinea pigs and rats, the ability of antiprogestins to induce labour was increased substantially after NOS inhibition with L-NAME (Chwalisz et al., 1993; Yallampalli et al., 1996). Observations in pregnant rats show that the ability of
L-arginine (NO substrate) and 8-bromo-cGMP (a permeable analogue of cGMP) to relax the uterus in vitro diminishes after progesterone withdrawal, both spontaneously at term and after onapristone treatment. This strongly suggests that the NO-dependent relaxation (NO synthesis and/or the effector system of cGMP) in the uterus is controlled by progesterone (Izumi et al., 1993). The results from the in-vitro studies suggest that the NO donors might be less effective at term in suppressing uterine contractility. However, our recent studies in rats, using intrauterine pressure recording, indicate that the response to diethylenetriamine/NO spontaneously-releasing NO donor (DETA/NO) in vivo does not decrease at term or preterm compared with mid-pregnancy (Buhimschi et al., 1997b). In conscious animals, DETA/NO showed a pronounced, long-lasting tocolytic effect during term (day 22 post-conception (p.c.)) and onapristone-induced preterm labour (day 19 p.c.). In this study, the effects of DETA/NO on uterine contractility in vitro were also investigated. Although in vitro, DETA/NO was less effective in tissues collected during spontaneous term labour and antiprogestin-induced preterm labour compared with tissues collected during late pregnancy, studies in vivo demonstrate that tocolytic activity of DETA/NO is even more pronounced during labour. These contrasting effects suggest that NO donors may either act indirectly in vivo to suppress uterine contractility, or higher NO concentrations are released from DETA/NO in the uterus in the presence of the placenta.

Regulation of NO production and NOS expression during pregnancy and labour: role of progesterone

The uterine NO production and NOS expression are gestationally regulated and progesterone-dependent. NO production, reflected in total nitrites produced by uterine tissues, was low in non-pregnant rats, substantially elevated during the mid stage of gestation, and markedly lower at the time of spontaneous delivery and first day postpartum (Figure 7) (Buhimschi et al., 1996). Likewise, there was a decrease in NO synthesis in the uterus and an increase in the cervix during both term (Figure 7) and onapristone-induced preterm birth. Uterine cGMP concentrations in the same tissues followed a similar pattern (Buhimschi et al., 1996). In human tissues, NO donors significantly elevated cGMP levels (Buhimschi et al., 1995).

Furthermore, our studies in rats provide ample evidence that i-NOS is the dominant isoform of NOS in the myometrium. In rats, myometrial i-NOS expression seems to be regulated by progesterone, since i-NOS declines prior to normal parturition when serum progesterone concentrations are low. i-NOS expression was also decreased during onapristone-induced preterm labour, an effect which can be reversed by a progesterone agonist (Buhimschi et al., 1996). NOS activity is not limited to the uterus, but is also localized in the rat placenta (Purcell et al., 1997). Therefore, NO of placental origin may play an important paracrine role in controlling myometrial relaxation.

In the rat uterus, only two NOS isoforms (i-NOS and e-NOS) were detected with immunoblotting. The inducible isoform (i-NOS) appears to play a dominant role in the pregnant rat uterus. The i-NOS enzyme decreased in the uterus during term (Figure 8) and preterm labour in animals treated to deliver prematurely (Buhimschi et al.,...
Contrary changes were observed in the cervix (Figure 8). Similar patterns of uterine and cervical NOS expression were found at the mRNA level using RT–PCR (Ali et al., 1997). Three distinct PCR products (i-NOS, ec-NOS, nc-NOS) were detected in both the uterus and the cervix, the i-NOS PCR product being the most prominent. The uterine i-NOS mRNA decreased on day 22 before labour and decreased further during labour at term. In contrast, cervical mRNA levels were low until delivery (day 22) when it increased and was dramatically elevated during labour. Similarly, 3 h after onapristone treatment, i-NOS mRNA decreased by 45% in the uterus and increased by 245% in the cervix when compared with controls.

These studies indicate that both NO production and NOS expression are reduced in the uterus during term and preterm labour, but are upregulated in the cervix. Moreover, our studies in rats show that progesterone is responsible for the differential regulation of the NO system (Buhimschi et al., 1996; Chwalisz et al., 1996). Due to progesterone action, uterine NO production is increased by i-NOS during pregnancy. Prior to parturition at term, or after antiprogestin treatment at preterm, there is a decline in uterine NO production and a consequential decrease in relaxation. Hence, the NO system may contribute to the maintenance of uterine quiescence during pregnancy when progesterone concentrations are elevated, but not during delivery. Conversely, during term and preterm labour there is an upregulation of the NO system in the uterine cervix as a result of the inflammation cascade being activated, thereby contributing to the remodelling of the extracellular matrix (see below). However, the mechanism responsible for the differential regulation of the NO system in the uterus and the cervix remains to be established.

Recent studies of NOS enzymes and also superoxide dismutase and xanthine oxidase (enzymes that generate superoxide and attenuate NO levels) indicate little change in these systems in human myometrium, fetal membranes and placenta at term labour versus non-labour (Telfer et al., 1997; Thomson et al., 1997b). However, all the samples were obtained at term, at a time when the levels of the enzymes might already have decreased. In addition, all the samples are from the lower uterine segment obtained during Caesarean section. Therefore, it is difficult to interpret from these studies whether the human uterus is different. Our studies of the human uterus support the concept of a decline in NOS at term (Buhimschi et al., 1995).

Dominant role of progesterone in controlling myometrial conditioning

The above studies indicate that progesterone controls NO synthesis and its effects on the uterus and cervix. In the uterus, progesterone has an overall control on uterine quiescence during pregnancy by upregulating the relaxation mechanisms, and NO seems to mediate, at least in part, its effects (Figure 9). On the one hand, progesterone acts as a gene suppressor, perhaps through repression of the
NF-κB activity (van der Burg and van der Saag, 1996), and downregulates a number of genes in the myometrium which are essential for parturition, including the expression of the gap junction protein connexin 43, calcium channels, and oxytocin receptors (OTR; depending on species) (Chwalisz and Garfield, 1994). A number of in-vitro and in-vivo studies have shown clearly that when antiprogestins are administered during advanced pregnancy, they enhance myometrial responsiveness to uterotonics such as prostaglandins and oxytocin in all species investigated to date, including humans (for review see Chwalisz, 1994). Studies performed in pregnant rats and guinea pigs suggest that an increased myometrial responsiveness after antiprogestin treatment is mainly due to the upregulation of gap junctions (Garfield et al., 1988; Chwalisz et al., 1991; Sakai et al., 1992). The onset and progression of labour contractile activity is invariably associated with the presence of sizeable numbers of gap junctions (Garfield et al., 1995). Hence, the increase in gap junctions could be the underlying mechanism of enhanced myometrial responsiveness to uterotonics following antiprogestin treatment. The myometrium of various species, including humans, is most reactive to oxytocin either near or at the time of parturition. The enhanced myometrial responsiveness to oxytocin has been attributed to an increase in myometrial OTR concentrations (Soloff, 1989). It is believed that this increase in OTR concentrations in the myometrium and the decidua at term is one of the principal factors leading to the initiation of parturition. However, our studies performed in late pregnant guinea pigs indicate that, in this species, onapristone dramatically heightened myometrial responsiveness to oxytocin without increasing myometrial OTR (Chwalisz et al., 1991). Similarly, RU 486 increases uterine reactivity to prostaglandins, but not to oxytocin (Sakai et al., 1992) during early human pregnancy when OTR concentrations are low. These data suggest that progesterone does not control OTR expression in guinea pigs or in humans and provide further evidence that a rise in OTR concentrations is not of primary importance in the initiation of parturition.

In summary, our studies demonstrate that progesterone can increase uterine quiescence by stimulating the relaxation mechanisms, i.e. mainly the uterine NO system.
(see above). On the other hand, progesterone suppresses myometrial responsiveness to uterotonic agents, primarily by inhibiting gap junctions. Progesterone can also act by functionally opposing oestrogen action, which is believed to promote uterine contractility. However, progesterone seems to exert a dominant action on uterine contractility, since in the presence of functionally effective progesterone the oestrogen effects on the myometrium are suppressed or completely blocked. In some species, including humans, the ratio of the magnitude of the progesterone to oestrogen effect is more important than the effects of individual hormones.

**Conditioning of the cervix**

Cervical ripening is an active biochemical process, which occurs independent of uterine contractions, and is similar to an inflammatory reaction. During this process the inflammatory cascade is activated, including the release of proinflammatory cytokines, the infiltration of white blood cells, the release and activation of degradative enzymes (matrix metalloproteinases; MMPs), a changing synthesis of extracellular matrix proteins and glycoproteins, an increase in collagen turnover, a disruption of tightly aligned collagen fibrils, changes in the decorin/collagen ratio, and increased extracellular fluid due to hyaluronan (Leppert, 1992, 1995; Rechberger et al., 1996). Various humoral agents are involved in cervical ripening, including progesterone, relaxin, prostaglandins, and local mediators such as proinflammatory cytokines and NO. However, the exact biochemical mechanisms responsible for the rearrangement of extracellular matrix during cervical ripening are still poorly understood. Nevertheless, the dissolution of collagen fibres via enzymatic degradation and/or ‘dilution’ by increasing proteoglycan (decorin) concentrations is the pivotal event during cervical ripening, resulting in the subsequent decrease in cervical resistance.

**Steroid hormones**

Progesterone seems to exert an overall control on cervical ripening. Antiprogestins are effective agents in inducing cervical ripening in all species investigated to date, including humans (for review see Chwalisz, 1994). In guinea pigs, the progesterone agonist R5020 (promegestone) completely blocked onapristone-induced cervical ripening, indicating that this effect was mediated by the progesterone receptor (Chwalisz, 1994). However, our recent studies in rats show that cervical ripening starts long before the spontaneous decrease in progesterone blood concentrations, which suggests that an additional, progesterone-independent mechanism controls cervical ripening (Shi et al., 1996a; see also Figure 19). Major gaps still exist in our knowledge of the role of oestrogens in cervical ripening. In guinea pigs, we were unable to demonstrate the ripening effects of oestradiol and the oestrogen precursor androstenedione during pregnancy, i.e. in the presence of high progesterone concentrations. Paradoxically, oestradiol treatment attenuated the onapristone-induced cervical ripening in pregnant guinea pigs due to an unknown mechanism (Chwalisz et al., 1995). In addition, there is currently no convincing evidence as to the beneficial effects of oestradiol on cervical ripening in women.

**Prostaglandins**

Prostaglandins, particularly PGE2, have for a long time been thought of as the key mediators of cervical ripening (Kelly, 1994). Although locally administered prostaglandins are effective in inducing cervical changes (Formam et al., 1982) and the results of many experimental and clinical studies suggest that endogenous prostaglandins are involved in cervical ripening, there are also studies which question their role in this process. Physiological cervical ripening occurs independent of uterine contractions, i.e. prior to labour and prostaglandin increase. Our extensibility studies in guinea pigs (Chwalisz et al., 1991) and rats (Shi et al., 1996b) show that in normal pregnancy the cervix starts to soften during late mid-pregnancy, i.e. long before the onset of labour. In addition, neither non-selective cyclo-oxygenase (COX) inhibitors nor specific COX-II (cytokine-inducible COX isoform) inhibitors block antiprogestin-induced cervical ripening in humans, guinea pigs and rats, although they do inhibit antiprogestin-induced uterine contractions which are probably mediated by prostaglandins (for review see Chwalisz 1994). Apparently, other local mediators may be more important than prostaglandins in cervical ripening. Our studies indicate that an alternative NO-dependent pathway exists in the cervix (see below). This pathway would explain why COX inhibitors do not block antiprogestin-induced cervical ripening. However, recent studies demonstrate that there is cross-talk between NO and COX in numerous inflammatory responses. NO can directly stimulate COX-II, thus increasing prostaglandin production during inflammation (Sirois and Richards, 1992; Salvemini et al., 1993). Furthermore, NOS inhibitors markedly suppress PGE2 production in acute and chronic models of inflammation (Salvemini et al., 1997). Therefore, the NO regulation of COX-II represents a newly discovered mechanism which may be responsible for the amplification of the inflammatory response. It appears
likely that this mechanism operates in the cervix during the ripening process.

**Cytokines**

In primates and guinea pigs, inflammatory cytokines such as interleukin (IL) -1 and IL-8 play an important role during cervical ripening (Ito et al., 1987; Osmers et al., 1992; Kelly et al., 1993; Chwalisz et al., 1994a). Cytokine production in the cervix and uterus seems to be regulated by progesterone. Progesterone inhibits and RU 486 stimulates IL-8 release in human choriodecidual cells in vitro (Kelly et al., 1993). Progesterone may, therefore, act as an immunosuppressor in both the cervix and the decidua, and antiprogestin treatment may activate the cytokine and leukotriene cascades as well as the neutrophil migration into the tissue. In fact, the neutrophils and macrophages which infiltrate the uterine cervix in response to the activation of the cytokine cascade are well-known sources of metalloproteinases capable of digesting extracellular matrix proteins. Thus, it is likely that these enzymes play a pivotal role in tissue remodelling during cervical ripening. Moreover, macrophages and other migratory cells contain the cytokine-inducible enzymes COX-II and i-NOS and are capable of producing substantial amounts of PGE$_2$ and NO, respectively (Laskin and Pendino, 1995).

Using morphological and biomechanical studies, the efficacy of IL-8, IL-1$\beta$ and tumour necrosis factor-$\alpha$ (TNF-$\alpha$) has been demonstrated in inducing cervical ripening in pregnant guinea pigs after topical-gel administration (Chwalisz et al., 1994). Both IL-8 and IL-1 may play a physiological role in cervical ripening, since their cervical concentrations increase during normal parturition. TNF-$\alpha$, which is mainly inducible via bacterial products (lipopolysaccharides; LPS), may operate to stimulate NO and ripen the cervix, however, during preterm labour associated with ascending infection.

**Nitric oxide as the final common pathway for cervical ripening**

There are several lines of evidence suggesting that NO plays a pivotal physiological role in cervical function. Treatment of pregnant guinea pigs with the NOS inhibitor L-NAME induced preterm parturition but delayed physiological cervical ripening, resulting in prolonged deliveries (Chwalisz et al., 1994). Furthermore, L-NAME treatment of pregnant rats significantly prolonged the duration of delivery, indicating indirectly a cervical dystocia (Buhimschi et al., 1996). Finally, a decrease in cervical extensibility was observed after in-vitro incubation with L-NAME (Buhimschi et al., 1996) and in-vivo treatment (S.Q.Shi et al., unpublished observations). As in the uterus, i-NOS is the most abundant isoform in the cervix during term and preterm labour in rats. In rats, the cervical NO production via i-NOS is low during pregnancy but highly elevated during labour (see Figure 7). The changes in NO production clearly correspond to changes in both i-NOS protein and i-NOS mRNA expressions (Ali et al., 1997).

These studies suggest that NO may not only be involved in the initiation of labour, subsequent to a decline in uterine production, but may also be an essential physiological mediator of cervical ripening during term and preterm birth. Furthermore, in order to investigate this hypothesis we applied the NO donor sodium nitroprusside (SNP) to the cervical canal of pregnant guinea pigs. The ripening effects of SNP were assessed by means of biomechanical evaluation and ultrastructural criteria. In order to compare the efficacy of SNP to reduce cervical resistance with that of the established ripening agents, we treated the pregnant guinea pigs systemically with an antiprogestin (onapristone) and with the prostaglandins PGE$_2$ and sulprostone (Chwalisz et al., 1997). This study showed that the local administration of an NO donor induces cervical ripening. The effects of the local administration of SNP on cervical resistance were comparable with those of systemic treatment with onapristone (10 mg/animal). SNP treatment significantly increased cervical extensibility, whereas electron microscope evaluation revealed a pronounced cervical ripening accompanied by the rearrangement of the extracellular matrix components (Figure 10), by stromal oedema, arterial dilatation, as well as by the infiltration of macrophages, lymphocytes and granulocytes. Morphologically, the NO-induced cervical ripening was indistinguishable from the normal ripening process at term or onapristone-induced ripening at preterm.

In summary, we propose that NO represents the final metabolic pathway of cervical ripening acting in concert with prostaglandins (mainly PGE$_2$) by inducing local vasodilatation, increasing vascular permeability and leukocyte infiltration, and perhaps by activating MMPs and other mechanisms responsible for the extracellular matrix remodelling such as modulation of proteoglycan synthesis. Furthermore, our studies indicate that the NO system represents a new target for novel therapeutic agents capable of both stimulating (NO donors) and inhibiting (NOS inhibitors) cervical ripening. Local application of agents which do not stimulate uterine contractions may have certain advantages over the local administration of prostaglandins. Moreover, the relaxation effects of NO on the myometrium and placental blood vessels may be of
Additional benefit. It may also be advantageous to ripen the cervix prior to the commencement of uterine contractility, given that this is the physiological sequence.

Recent preliminary studies in humans confirm that NO donors ripen the cervix (Thomson et al., 1997a). Given that previous treatments have not reduced the incidence of Caesarean section rates, further studies are warranted.

Non-invasive devices for the diagnosis of labour

At present, there are no methods which objectively evaluate the physiological states of the uterus or cervix (i.e. the conditioning steps; see above). Consequently, there are no reliable methods to predict labour, nor effective strategies for the prevention of preterm labour. It is essential that clinically applicable methods be established for direct assessment of the uterus and cervix in order to measure the progress and evolution of labour. At present, almost all of the methods used clinically, such as manual cervical examination and tocodynamometric monitoring of uterine activity, are limited to the evaluation of the final stage of parturition or active labour.

The diagnosis of labour either at term or preterm is a challenging issue. Currently, only indirect or correlative methods are applied to assess the state of labour. The frequency of the uterine contractions and cervical changes are employed as general indicators of labour progression. External monitors of contractions such as the tocodynamometer are used in about 90% of patients admitted to most labour and delivery units, although the information provided by tocodynamometry is limited. The tocodynamometer may not detect all uterine contractions and is not very useful in distinguishing between contractions that ultimately will lead to delivery or to true labour, and those that will subside spontaneously, i.e. false labour. Faced with these uncertainties, most obstetricians either treat all patients having preterm contractions, or wait for cervical change. The first alternative will in some cases lead to overtreatment, whereas if treatment is delayed, it may prove to be less effective once the cervical dilatation or effacement has occurred. In addition, the tocodynamometric monitoring of uterine contractility is very difficult, if not impossible, in obese women. The abnormality of the uterine cervix during pregnancy is also of major concern to obstetricians. Extensive ripening before term may result in abortion or preterm delivery, whereas the absence of spontaneous ripening may indicate a prolonged labour or post-term pregnancy. At present, palpation is the most common method in clinical examinations of the cervix.

None of the currently available techniques is able to assess the preparatory phase. More novel methods are therefore required to establish effective treatments in order to stimulate or inhibit labour. As with any medical condition, the diagnosis is crucial for appropriate treatment. Recently, we described techniques which can quantitatively and non-invasively determine uterine and cervical changes during the antepartum and peripartum periods.

Abdominal surface recording of electromyographic (EMG) activity

There is no effective, non-invasive manner to assess the contractility of the uterus. This is true in both non-pregnant patients where hypercontractility is associated with dysmenorrhea, and in pregnant patients where the uterus
is sometimes active prior to term. Normally, the uterus is quiescent in non-pregnant women and during most of pregnancy. However, at the end of pregnancy the myometrium undergoes a series of changes (the conversion or conditioning steps; see above) that lead to increased reactivity and synchronous, rhythmic uterine contractions (labour) (Wolfs and van Leeuwen, 1979; Csapo, 1981; Garfield et al., 1988). Since there is some minor spontaneous uterine contractility at all times during pregnancy, it is often not possible to distinguish between this activity and term or preterm labour (i.e. to differentiate between the preconditioned and the conditioned states; see above).

Differentiation between true labour, which ultimately leads to delivery, and false labour, which does not, is the most significant problem faced by obstetricians. In addition, preterm labour, which occurs in about 10% of pregnant patients, is difficult to predict, assess and treat. Frequently term or preterm labour require adjuvant therapy to either stimulate or inhibit contractility. However, there is no currently available technique to assess objectively and accurately the response of the uterus to these uterotonic agents, and no method to detect when the organ has entered a state of increased activity and responsiveness.

The state of the cervix is commonly used as a predictor of spontaneous labour, or as a response to labour induction. However, the relationship between the softening, effacement and dilatation of the cervix and changes in uterine contractility varies. In addition, labour and cervical changes can occur independently. Conversely, the frequency and amplitude of contractions are used as an index of labour, sometimes recorded with a tocdynamometer or intrauterine pressure (IUP) catheter. All of these methods provide subjective estimates of uterine contractility, and IUP can only be accomplished after rupture or puncture of the membranes. Thus, it would be useful to have more direct measures of the progress or evolution of labour.

As noted above, contractions of the uterus are dependent upon the generation and propagation of action potentials in the millions of muscle bundles which comprise the myometrium (Csapo, 1981; Marshall, 1982; Garfield et al., 1988). Unlike single action potentials which initiate contractions of striated muscle, action potentials in the myometrium occur in groups or bursts. Many studies have recorded bursts of uterine myometrial electrical activity using electromyography where electrodes were placed directly on the uterus (Wolfs, and van Leeuwen, 1979; Csapo, 1981; Harding et al., 1982; Demianczuk et al., 1984; Verhoeff et al., 1985; Nathanielsz et al., 1988; Devedeux et al., 1993). These studies show that the myometrium generates little electrical activity prior to labour, while burst activity increases tremendously during labour, reflecting the mechanical events. Previous studies have also attempted to record uterine electrical activity from electrodes placed on the abdominal surface (Steer, 1954; Hon and Davis, 1958; Freundlich and Wingate, 1973). However, a review of studies prior to 1979 (Wolfs and van Leeuwen, 1979) concluded that it has not been shown conclusively that electrical signals recorded from the abdominal surface represent electrical activity of the uterus. Similarly, Devedeux et al. (1993) stated more recently that abdominal monitoring of uterine electrical activity requires further investigation. Studies necessary to establish whether uterine EMG activity can be appraised from abdominal surface measurements had not been successfully carried out. No study had systematically examined EMG activity recorded directly from the uterus and simultaneously from the abdominal surface during term labour, preterm labour, or following methods which inhibit or stimulate activity.

Below, we outline our recent studies which methodically evaluated the relationship between EMG activity recorded non-invasively from the abdominal surface of rats and humans at various times during pregnancy and following various treatments (Buhimschi and Garfield, 1996; Buhimschi et al., 1997a, 1998). The aim of these studies was to develop a procedure to predict the state of preparedness for labour.

**Abdominal and surface EMG recordings from rats**

**Assessment of electrical activity (EMG) recorded from the uterus and abdominal surface during pregnancy**

Figure 11A shows the EMG activity recorded directly from the uterus (Ut; channel 1) and from the abdominal surface (AS; channel 2) along with intrauterine pressure (IUP) measurements (channel 3) in a rat instrumented on day 18 of gestation. At this stage of gestation the EMG bursts are of low amplitude, irregular, and there is little or no correspondence between the activity recorded from the uterus and that of the abdominal surface. The IUP is frequent, but also irregular and generally of low amplitude. Later in gestation (day 21 to delivery), the EMG activity is more regular and uterine activity (Figure 11B, channel 1) coincides well with that recorded from the abdominal surface (Figure 11B, channel 2). There is also a tendency for low-amplitude IUP to coincide with the EMG activity recorded from both sites (Figure 11B, channel 3).

In labouring animals, EMG activity recorded from both the uterus and abdominal surface occurs simultaneously with IUP (Figure 11C). The EMG signals and IUP are
Control of the uterus and cervix

Figure 11. Electromyographic recordings of uterus (Ut, upper channel), abdominal surface (AS, middle channel) and intrauterine pressure (IUP, bottom channel) from pregnant rats in different days of gestation, labour at term, and preterm labour induced with onapristone. (A) Recording from rat on day 18 of gestation. Note bursts of electrical activity (action potentials) recorded from uterus corresponding with low-amplitude pressure change in uterus, but lack of accordance to activity from surface. (B) Typical recording from rat on day 21 of gestation. At this stage of gestation electrical activity of uterus becomes more coordinated with electrical activity recorded from surface. Pressure is characterized by increase in regular low-amplitude changes occurring at same time as electrical bursts. (C) Record from rat on day 22 of gestation, during delivery. Note tremendous increase in duration, amplitude, and frequency of electrical abdominal surface and in regular large-amplitude pressure changes. (D) Recording of rat on day 18 of gestation after premature labour was induced by administration of onapristone (ZK 98299). Note intensity of electrical activity generated by uterus, with almost same aspects as noted during delivery [compare (C) with (D)].

frequent (about one contraction per minute) and of high amplitude. In EMG recorded activity, each burst is composed of many action potentials (about 1/s) and their appearance at the uterine wall corresponds in time with those from the abdominal surface. However, the burst configurations are not identical and the amplitude of the action potentials recorded from the abdominal surface are usually lower that those recorded from the uterus.

Recording during preterm labour
Preterm labour was induced by a subcutaneous injection of 10 mg of the antiprogestin, onapristone. Figure 11D shows the electrical activity generated by the uterus (channel 1) during premature labour as well as its surface (channel 2) and pressure (channel 3) 24 h after onapristone injection. The records are similar to the one obtained during delivery at term (Figure 11C). Records obtained from control animals at the same stage of gestation show a low amplitude, and infrequent and asynchronous activity similar to Figure 11A.

Effects of stimulation and inhibition
The stimulatory effect of oxytocin treatment on day 22 of gestation resulted in an intensification of myometrial electrical activity by the abdominal surface recording. The stimulatory effect was exactly reproduced and accompanied by a rise in IUP. Conversely, the administration of the tocolytic agent isoproterenol on the same day of gestation showed a sustained inhibitory effect which was reflected by the absence of bursts recorded from both the myometrium and the abdominal surface, and by a low IUP (Buhiemshi et al., 1996).

Assessment of uterine electrical activity by recording from the vaginal surface
Studies were performed during delivery on day 22 of gestation. Electrical activity was recorded from electrodes placed directly on the myometrium and compared with the electrical activity recorded from electrodes placed on the surface of the vagina. The electrical events recorded with respect to both the uterine and vaginal wall were nearly identical. Similarly, the entire set of all these uterine activity measures proved to be in good agreement during term labour. To demonstrate that the electrical activity recorded from the vagina is not the result of a vaginal pacemaker, the uterine horns were separated surgically from the vagina. Following this procedure, the electrical activity recorded from the uterus continued to be characterized by regular bursts in the absence of any vaginal activity.
Comments on rat studies

The studies described above demonstrate that abdominal surface recordings of uterine electrical events are indicative of the electrical activity in the uterus, at least during late pregnancy. Electrical activity and therefore uterine contractility can be detected and measured from the body surface. This conclusion is based on the following observations: (i) action potential bursts obtained from the abdominal surface almost exactly mirror bursts occurring in the uterus (Figure 11); (ii) increases and decreases in electrical bursts at both sites are associated with changes in intrauterine pressure; (iii) when uterine activity increases during term and preterm labour the EMG signals are detected simultaneously at the uterine and abdominal surfaces (Figure 11); (iv) either pharmacological stimulation or inhibition leads to similar changes in recordings from both sites; (v) cardiac action potentials are also conducted to the abdominal surface; and (vi) action potentials from the uterus are also propagated to the vaginal surface.

It is well recognized that the frequency, duration and magnitude of bursts of action potentials are directly proportional to the frequency, duration and intensity of the contractions, respectively (Marshall, 1982). Our results support these findings. The timing and the intensity of the observed contractions appeared to be commensurate with electrical activity. Thus, by measuring electrical activity of the uterus at the abdominal surface one can indirectly assess uterine contractility. Although the burst frequency and duration varied somewhat between animals, the amplitude of action potentials was consistently elevated during delivery, even when recorded with surface electrodes (Figure 11). The rise in amplitude is one indication that conduction velocity is enhanced during labour. Action potential amplitude determines the size of the depolarizing current and establishes the probable distance that an action potential will propagate to excite remote tissues and produce a more forceful contraction (Katz, 1992). The basis for improved conduction and excitability during labour is probably due to enhanced electrical coupling afforded by the existence of gap junctions among myometrial cells during labour (see above). Gap junctions develop between myometrial cell prior to labour and their presence may be one of the primary events responsible for preparing the uterus for labour. Therefore, the configuration of the action potentials and the frequency and duration of bursts recorded from the abdominal surface can be used to determine whether the uterus has entered the state necessary for labour (i.e. conditioned phase; see above).

The rat studies also demonstrate that the uterine electrical activity can be accurately recorded from the vaginal surface, which is not surprising since the female reproductive tract is contiguous and muscle cells extend down its length, although muscle is less abundant in the vagina than in the uterus. When the uterus and vagina are separated surgically, electrical activity is present in the uterus but not in the vagina. This demonstrates that electrical activity in the vagina originates in the uterus. It may be possible to use vaginal recordings to measure uterine activity in conditions of obesity where the uterus is insulated from the abdominal surface. It may also be useful to measure activity in non-pregnant states or during early pregnancy, when the uterus is small.

Abdominal EMG recordings from pregnant patients

The results from experimental studies in rats suggest that the EMG technology might be used to determine when the uterus enters the necessary state required for labour in humans. This conclusion is based on experiments showing that electrical activity is greatly elevated during labour and that stimulation of the vagina during labour results in signals that can be detected non-invasively from the abdominal surface. Surface recordings of uterine electrical activity may be of eminent value in assessing the uterine contractility and the ability to diagnose labour in obstetrical practice. Our preliminary studies in humans support these conclusions.

Recordings (see Figure 12) of EMG activity from the abdominal surface of non-labouring and labouring patients are shown in Figure 13. There was little electrical activity in early pregnancy but, when present, it was also detected by the patient (Figure 13A). The bursts of electromyographic activity in labour coincided with the perception of subjective symptoms experienced by patients, such as pressure or pain (Figure 13B). In patients who had a catheter inserted into the uterine cavity, the rise in the level of intrauterine pressure corresponded with the electromyographic bursts at the abdominal surface (Figure 13C). In the patient with no correlation, a Caesarean delivery was performed to arrest the active phase of labour. When comparing EMG bursts with the external tocograph recordings during active labour, high congruity was seen between the bursts of EMG activity and uterine contraction in 14 pregnant patients, all of whom had normal vaginal deliveries. In three severely obese patients, however, EMG activity was difficult to detect.

Five patients were followed longitudinally during their pregnancies. Figure 14A–D shows representative records from a single patient with a normal pregnancy at various
times from 20 weeks of gestation to term. This patient delivered a healthy infant at 37 weeks of gestation. At 20 weeks of gestation, little uterine electrical activity was detected from the abdominal surface (Figure 14A). Between 27 and 36 weeks of gestation (Figure 14B), bursts of electrical activity became evident, but the EMG activity was irregular and of low amplitude. In the instance shown here (27 weeks), the patient reported that she felt contractions during the periods of the most intense electrical activity. At term (37 weeks; Figure 14C), the frequency of the electrical bursts recorded in the non-labouring patient was higher compared with records made earlier in gestation, and the bursts were almost always accompanied by patient recognition of the contraction. During labour (Figure 14D), the bursts were almost always accompanied by patient recognition of the contraction and were much more frequent and of higher amplitude. The labour contractions, as recognized by the patients or detected by the tocograph, and in some patients recorded via an IUP catheter, coincided with the bursts of EMG activity.

EMG activity was also recorded from the abdominal surface in five patients who were in preterm labour. The frequency and amplitude of the EMG bursts in patients in preterm labour were similar to those noted in term labour. At 24 h after delivery, minimal EMG activity was recorded by electrodes on the abdominal surface. Three of the patients at term underwent Caesarean delivery for failure to progress. In these patients, despite a standard oxytocin stimulation protocol (Cunningham et al., 1993), an arrest

Figure 12. Configuration of equipment used for recording electromyographic activity from the abdominal surface of pregnant patients. The device consists of transdermal electrodes, an amplifier, an analog/digital converter (ADC) and a computer.

Figure 13. Electromyographic (EMG) recordings from the human abdominal surface. (A) A record registered from two sites (AS1 and AS2) on the abdominal surface in a patient at 27 weeks. The small bursts of action potentials, when present, appeared concurrently with perception of contractions by the patient (marked with arrows by the attending physician). (B) Record of the electrical activity as recorded from the abdominal surface of the patient during term active labour (40 weeks). (C) Record of the EMG activity measured simultaneously with the intruterine pressure (IUP) in a patient at term (38 weeks) in active labour. (D) Expanded view of bursts from a non-labour (top) and a labouring patient (bottom).
of dilatation and incoordination of uterine contractility were registered.

Figure 15 (top) shows a representative burst of EMG activity in magnified view along with its power density spectrum [power in microvolt-seconds (µVs) versus frequency of electrical events] during labour. The lower part of Figure 15 is an expanded view of a representative EMG burst recorded from the abdominal surface at 42 weeks of gestation and during term labour. The duration of the bursts were similar before and during active labour. However, the bursts recorded during labour were of higher amplitude. There were also differences in the frequency content of EMG events. Power density spectral analysis revealed that at term, but in non-labouring patients (37–38 weeks), power density peaked at 0.48 ± 0.03 Hz, though was significantly higher during active term labour (0.71 ± 0.05 Hz at 38–43 weeks) and preterm labour (0.78 ± 0.06 Hz at 33–36 weeks). The power density peak values were relatively low in non-labouring patients during the entire period of gestation (37–38 weeks; 14.11 ± 2.31 µVs), reaching high levels during active labour (38–43 weeks; 60.2 ± 13.87 µVs) and then declining dramatically postpartum (4.4 ± 0.6 µVs). During preterm labour, the power density peak values also reached high levels (62.27 ± 22.93 µVs). The high standard errors obtained in the labouring groups (i.e. term and preterm labour) were due to two outlier values that generated a non-normal distribution of the data. It is interesting that the outlier values were obtained from patients who received neither epidural anaesthesia nor oxytocin infusion because of the advanced stage of labour at admission. Further data are needed to understand fully the effects of these treatments. It also appears likely that intra-patient changes in uterine electrical activity are more important than inter-patient differences.

A three-dimensional plot of the mean power density levels of the bursts (i.e. energy levels) versus frequency and weeks of gestation is shown in Figure 16. This figure illustrates a substantial increase in power density during term labour. Similarly, the values obtained from patients in preterm labour were increased compared with those at similar gestational age who were not in labour.

Comments on EMG recordings from humans

These studies demonstrate that abdominal surface recordings of uterine electrical activity can be used reliably to characterize contractile events in humans during pregnancy. The EMG bursts correlated with: (i) subjective symptoms such as pressure or pain (Figure 13A); (ii) an increase in the IUP recorded with an intrauterine catheter or a surface tocodynamometer (Figure 13C); and (iii) increased energy content of EMG activity during labour (Figures 15 and 16). In the future, this technology may be seen as a helpful adjunct in the diagnosis of labour and in assessing of the contractile state of the uterus.

The above studies in pregnant rats, involving simultaneous measurement of EMG activity from the uterus and abdominal surface, demonstrate that signals recorded from the abdominal surface correspond to those generated in the uterus (Buhimschi and Garfield, 1996),
either spontaneously or after pharmacological stimulation. As expected, the abdominal surface recording is attenuated compared with the uterine EMG activity recorded with electrodes placed directly on the myometrial surface (unpublished results). Otherwise, the recordings from the uterine and abdominal surfaces in the rat gave similar information. This study confirms that similar techniques can be used in human patients to detect uterine electrical events from the abdominal surface. We placed electrodes at points where external palpation of the uterus suggested proximity to the abdominal wall (Buhimschi and Garfield, 1996). In human subjects, we have not been able to compare directly myometrial recordings with those of the abdomen, but these studies show high congruency between EMG bursts and pressure changes, especially during active labour. Thus far, in our studies we have limited our analysis to the frequency and magnitude of uterine electrical events.

Figure 15. Power density spectral analyses of bursts of electrical activity recorded from the abdominal surface of a woman during pregnancy, term labour. Typical analysis of one burst. Power spectrum analysis (top) and burst of electromyographic activity analysed (bottom). The first peak in the spectrum (top) is a low-frequency artefact. The second peak represents the electrical frequencies (about 0.75 Hz) within the burst (bottom).

However, with further refinements, these methods have the potential to yield considerably more detailed information about the excitability of the myometrium and further analysis of the configuration, directionality and spatial distribution of the electrical signals.

Uterine EMG activity increases during either term or preterm labour, corresponding to an increase in contractility that occurs at this time (Wolfs and van Leeuwen, 1979; Csapo, 1981). The increases in EMG activity and subsequent contractility are reflected in the power density spectrum of the electromyograph, which changes dramatically from the non-labouring condition to that present during term labour (see Figures 14 and 15). Previous studies in monkeys demonstrated the feasibility of obtaining power density spectral analyses of EMG (recorded from the uterine surface) and contractile activity during pregnancy, but did not compare the labour and non-labour states (Nathanielsz et al., 1988). The increase in power density of the action potentials observed in this way represents an approximately 5-fold increase in energy during labour, reflecting the changes that occur in the electrical properties of the myometrium at term (Figures 14 and 15). Moreover, the frequency of the bursts also increases. Hence, the total energy developed by the uterus (determined by the total number of bursts per unit time) and the energy of each individual burst, increases substantially during labour compared with the non-labour state. On the other hand, in some of our patients followed longitudinally,
we were not able to record regular high-amplitude EMG activity at term (37–38 weeks), prior to the onset of labour. In addition, three patients underwent Caesarean delivery for failure to progress. Although they were considered clinically to be in active labour, based on cervical dilatation of >4 cm, examination and frequency of uterine contractions, we failed to detect regular EMG activity. These findings suggest that, even in late pregnancy, examination of EMG activity can be indicative of a lack of specific developmental changes in electrical activity at this time and the myometrium not being prepared to contract effectively.

Interference from other physiological electrical activity, such as heart rhythm, was not a major problem in our study (Figure 16). It should be mentioned that we found it difficult to record electromyographic activity from three morbidly obese patients. This probably represents the inability of electrical signals to be conducted via adipose tissue. Using an animal model (Buhimschi and Garfield, 1996), we demonstrated previously that it is possible to record the electrical events of the uterus from the vagina and suggested that this might be a better recording site in such situations. It seems that epidural anaesthesia or oxytocin infusion changes the power density spectra of the burst, but we do not have sufficient data at this point to draw a clear conclusion. Because these are routine treatments in many institutions, it will be essential to clarify these issues.

Current methods used in the analysis of labour are generally subjective. We suggest that the recording of uterine EMG activity from the abdominal or vaginal surfaces may provide healthcare workers with a very powerful tool to pursue the evolution of uterine contractility and for the proper diagnosis of labour. In addition, these techniques should provide us with further information about uterine contractility disorders such as preterm labour or dysmenorrhoea. The clinical relevance of the insights provided by the electrocardiogram into the behaviour of the ion currents that govern cardiac function cannot yet be matched by any other technology. In a similar fashion, the electromyogram has the potential to provide the same type of information about the uterus.

The information obtained from surface EMG recordings may guide the physician in the choice of treatment either to stimulate or to inhibit uterine contractions. The current treatment of preterm labour is clearly unsatisfactory (Higby et al., 1993), yet accurate diagnosis of medical conditions has always been essential for the development of effective treatment. If the state of preparedness for labour can be determined more precisely, then measures to stop preterm labour (or to augment term labour) may be applied more effectively. Agents applied to intervene in labour may be of immense value during certain periods in the progression to delivery. It would be helpful to have better objective criteria to evaluate tocolytics and uterine stimulants and to classify patients more properly in clinical trials.

In summary, our studies indicate that the use of abdominal surface recordings of EMG activity could offer tremendous benefits in diagnosing the evolution in electrical activity that precedes labour, in addition to differentiating between the non-prepared state before labour and the prepared state during labour. However, it will be important to conduct several types of longitudinal studies of pregnant women to determine the patterns that evolve spontaneously, leading to the labour process. The patterns in preterm labour and delivery, and the events that presage them, should receive special attention with the objective of identifying candidates for tocolytic and glucocorticoid therapy. Characterization of patterns during the induction of labour and during failed induction should furthermore prove instructive from both a clinical and a basic scientific standpoint. Whether this methodology will be more effective than present methods for diagnosing failure to progress or for predicting a delay in the onset of labour should also be the subject of future clinical trials.

Cervical collagen content measured by light-induced autofluorescence

Fluorescence spectroscopy has been widely applied in bioscience research for almost half a century. Recently, several investigations have introduced the unique possibility of employing the fluorescence spectra (from intrinsic fluorophors) in biological tissue to analyse the tissue’s physiological state. The great promise that fluorescence spectroscopy holds is the prospect of using it as a non-invasive medical test. The aim of studies reviewed below was to assess the concentration of collagen in cervical tissue in an optical, non-invasive manner. It is known that collagen degradation and rearrangement plays a crucial role in the softening and ripening process of the uterine cervix during pregnancy and labour (see above). Collagen comprises approximately 70% of cervical tissue (Leppert and Yu, 1991), nearly 62–80% of which is type I collagen (the rest is type III collagen) (Kleissl et al., 1978; Ito et al., 1979). Collagen degradation reduces cervical rigidity and increases its extensibility (Harkness and Nightingale, 1962). Although the mechanisms of spontaneous cervical ripening are still unknown, there is ample evidence to suggest that cervical collagen changes during pregnancy and labour. Changes such as: (i) an
increase in the interior space between collagen fibres; (ii) a shortening of the length of collagen fibres; and (iii) an increase in the collagen acidic solubility, occur as the pregnancy approaches term (Fosang and Handley, 1988; Osmers et al., 1990; Yu et al., 1995).

**Optical instrument to measure collagen content in the cervix (collascope)**

Our studies on collagen content in the cervix represent further evidence utilizing the fluorescence from pyridinoline, one of the major cross-links of type I and III collagen, to evaluate cervical connective tissue changes during various female reproductive periods. The stiffness and tensile strength of collagen are highly dependent on the cross-link bonds between collagen fibres and other matrix proteins (Marvin, 1973). Pyridinoline, a mature collagen cross-link, is known to exist in the uterus (Gunja-Smith and Boucek, 1981) and may very well exist in the cervix (Kaidi et al., 1995). A decrease or deformation of collagen cross-links could contribute to rearrangement as well as a loosening and shortening of the collagen fibres. The decrease in pyridinoline concentrations directly affects the measured fluorescence intensity. Collagen gives a characteristic fluorescence spectrum, the maximum being around 390 nm (see Figure 18). The intrinsic fluorophor is believed to be a pyridinoline, which is considered to be one of the major cross-links within the primary structure of collagen fibrils (Fujimoto, 1977).

Based on our previous studies, a prototype instrument has been constructed (Figure 17), designed specifically for the purpose of vaginal examinations of cervical connective tissue by measuring light-induced fluorescence directly from the surface of the external os of the cervix. This first prototype unit (collascope) was assembled by Instrument S.A., Inc. (Edison, New Jersey, USA) under the specifications given by the investigators and based upon previous studies. The instrument includes four main portions. The first part comprises a non-ozone xenon lamp (250 W) and a selective filter system which filters out the UV light with wavelength centred at 339 ± 3 nm. The second portion includes a monochromator and CCD spectroscopic analysis system. They both are placed in an enclosed unit which has links to the third portion for data acquisition and a notebook computer as a monitor and controller. The fourth portion includes a collascope probe with an optical fibre cable connected to the main unit (which includes the first and second portions). The selected excitation light (339 nm) is delivered from the first portion to the probe, which also carries the fluorescence signal from the measurement site back to the second portion for data recording and analysis. An additional optical fibre also links the light source, probe and CCD detector in order to obtain a reference signal which represents the intensity of the excitation light. The collascope probe, which is in contact with the measuring site, is made up of two parts: the fibre-optical probe and a sheath. The fibre-optical probe is a stainless steel rod with an optical fibre bundle in the centre. The sheath, used for isolating the fibre-optical probe from the measuring site, is hollow stainless steel with a fixed sapphire window on one end. During application, the fibre-optical probe is inserted into the sheath and fixed with a thumbscrew. The probe is then inserted into the vagina with the sapphire window contacting the surface of the external cervical os for measurement. Once the probe is in position, the data collection process is operated using the notebook computer terminal with a programmed process. The time needed for accumulation measurements is 2 s for rats and 8 s for humans. In the presented spectra, all the data are normalized to the peak of the reference light. The ratio between fluorescence signal from cervix to reference signal is calculated.

**Measurements in rats during normal pregnancy, term labour and preterm labour**

We were the first to introduce the method of using light-induced autofluorescence to measure cervical tissue changes during gestation and labour (Glassman et al., 1995). Our previous investigations were carried out by measuring the serosal surface of the medium band of the cervix in vitro (Figure 18A–D), resulting in decreased fluorescence intensity during advanced gestation and at parturition. Our findings also showed a drop in collagen fluorescence intensity in those rats treated with the antiprogestin RU 486 (Figure 18C). The ratio, $R$, ...
between the peak value of the fluorescence (in the range of 370–430 nm) and the peak value of the reference peak (in range 330–345 nm) were calculated after each measurement. In the spectra, the sharp peak with the centre around 339 nm is the reference peak of the excitation light. The peak centred around 393 nm is known as the fluorescence from collagen (from pyridinoline). The small elevation occurring at around 460 nm most probably reflects intracellular NADH. The increase in the spectra at the tail around 495 nm is likely due to the CCD detector.

With an improved instrument setting and a scope style probe containing optical fibres, we were able to measure the signal from the surface of the external os by approaching it via the vagina. Figure 18E shows the average fluorescence spectra profiles from the external os of rats which were: (i) non-pregnant and day 17 gestation; (ii) in gestation and in delivery; and also (iii) in the postpartum period. A decrease in the collagen (pyridinoline) fluorescence intensity was noted when rats on day 13 of gestation were compared with non-pregnant rats. The $R$ value (counts in spectrum/reference peak) changed from $3.7 \pm 0.34$ to $0.72 \pm 0.08$. During pregnancy, there was a gradual decrease in the collagen fluorescence intensity as the gestation progressed. During the mid-term pregnancy, the $R$ value decreased relatively quickly from $2.8 \pm 0.12$ on day 13, to $0.72 \pm 0.08$ on day 17 of gestation. The $R$ value then decreased relatively slowly from day 17 to day 22 during the non-delivery period. At the time of delivery, the $R$ value dropped to $0.26 \pm 0.04$, compared with $0.57 \pm 0.07$ on day 22 in non-delivering rats. During postpartum, the collagen fluorescence intensity gradually increased in the first few days to $2.1 \pm 0.3$ on postpartum day 5. The mean $R$ value then remained at $2.2\text{--}2.7$ from postpartum day 5 to postpartum day 17. By the end of the experiment on postpartum day 17, the $R$ value had not yet returned to non-pregnant levels.

**Human studies**

We have also initiated human studies with the collascope. Non-pregnant, pregnant and postpartum human volunteers were recruited for the study. The first step in the human study was to establish a longitudinal distribution profile according to the weeks of gestation and postpartum. The cervical external os was gently cleaned of residuals with...
rayon-tipped proctoscopic swabs prior to measurements being taken. The measuring sites were selected at the 12 o’clock position and away from any observable lesion site. Human subject data are not presented here, since the studies are ongoing. However, \( R \) values calculated as the tissue fluorescence counts at 393 nm divided by counts at 339 nm (the reference peak for the excitation light) decreased progressively during the final 15 weeks of pregnancy. So far, a total of 24 patients (including eight non-pregnant patients) have been recruited. Several of the subjects have been measured two to three times during their pregnancy and postpartum period, for a total of 32 measurements. The results show a gradual decrease of fluorescence as pregnancy approaches term, followed by recovery during the postpartum period.

**Conclusions about light-induced autofluorescence**

It is feasible, as shown in these studies, to measure non-invasively collagen fluorescence from the surface of the external os of the rat and human cervix. The results in rats suggested that there was a small amount of change during early pregnancy (non-pregnancy to day 13), and then a quick restructuring of collagen from days 13 to 17, followed by a slowing of the process during the later stages of pregnancy. There was then a quick drop from day 22 non-delivery to day 22 delivery time, indicating a further collagen structural change. This corresponded to the study performed using light and electron microscopy (Marvin, 1973), which demonstrated that collagen fibres were well-organized and densely packed in the cervix of non-pregnant rats and early pregnant rats (<10 days’ gestation). Between days 18 and 21, the structure of the collagen fibres changed to more fragmented fibrous networks with increased spaces (loosely packed). At term, the ripened cervix was filled with collagen microfibres, with far fewer long fibres. Furthermore, the fluorescence measurements displayed a gradually increasing signal level postpartum, which represented the recovery of the cervical collagen structure. It was interesting to note that the signal level does not return to that seen in non-pregnant animals. This may indicate that there is some kind of permanent change after first-time parturition, though our observation period was limited to 17 days.

The results of fluorescence measurements confirm our previous findings employing an invasive method to analyse cervical extensibility (Figure 19), and other studies in the literature of cervical collagen content and cervical collagenase enzymes regulation. In view of the important role of collagen fibres and their turnover in the process of cervical function during pregnancy), light-induced autofluorescence of cervical collagen could be a useful tool for evaluating cervical treatment strategies. Moreover, this instrument could serve as a device for the non-invasive estimation of cervical status in the clinic and the diagnosis of the changes in the cervix during the preparation for labour. We do not yet have a clear understanding of the changes in collagen in the human cervix during pregnancy and postpartum. This is because cervical biopsies, the only method available to date, are invasive and unacceptable to many patients. Some human studies have offered results suggesting that prolonged labour is related to the higher collagen content in the cervix, and that a breakdown of cervical collagen fibres occurs following treatment with the antiprogestin RU 486. The non-invasive measurements obtained with the collascope provide a good tool for the study of gestational changes in the human cervix. Our initial human data show that the in-vivo data correlate with the in-vitro studies performed on cervical biopsies. More human research is needed, however, to reach a better understanding of how cervical collagen change progresses during human pregnancy.

**Concluding remarks**

In this brief review, we describe the conditioning phase of labour for both myometrial and cervical functions. We have also indicated some of the important events that are thought to regulate this process. We believe that further progress in this field will depend largely on the availability of new non-invasive diagnostic techniques such as recording EMG activities or collagen analysis to assess the conditioning changes in the myometrium and cervix prior to the onset of an active labour. In this review, we have
outlined several studies involving two devices which appear promising for measuring of uterine and cervical functions in future.

References


