DEPARTMENT OF WOMEN AND CHILD HEALTH
DIVISION OF OBSTETRICS AND GYNAECOLOGY
Karolinska Institute, Stockholm, Sweden

Mediators of cervical ripening in preterm birth: Experimental and clinical investigations

Susanne Abelin Törnblom

Stockholm 2005

Published and printed
Repro Print AB,
Box 21085, 100 31 Stockholm, Sweden

ISBN 91-7140-305-1

ABSTRACT

Mediators of cervical ripening in preterm birth:
Experimental and clinical investigations

Susanne Abelin Törnblom, MD

Department of Women and Child Health, Division of Obstetrics and Gynaecology, Karolinska Institute and Karolinska University Hospital Solna, S-171 76 Stockholm, Sweden

Background: Preterm birth (PTB) is by far the leading worldwide cause of infant mortality and morbidity. Despite decades of research, the frequency of PTB has not decreased and the basic mechanisms initiating the onset of labour are still poorly understood. Vaginal preterm birth cannot take place without cervical softening and remodelling. Cervical ripening at term is an inflammatory-like process, in which complex interactions between cytokines, prostaglandins and nitric oxide (NO) are believed to play key roles, with NO acting as the final mediator. An understanding of the mediators regulating this process in connection with spontaneous preterm labour is of equally great importance, e.g., when, due to maternal/foetal complications, induction of premature cervical ripening and onset of labour is desired.

Aim: Compare preterm cervical ripening to the analogous process occurring at term.

Methods: Transvaginal cervical biopsy specimens were obtained and serum samples taken. Women, exhibiting no clinical signs of infection and undergoing preterm labour with a ripe cervix or preterm birth without labour (unripe cervix) were compared to un-infected women delivering at term, with or without labour. Peripheral white blood cell counts (WBC) and serum levels of C-reactive protein (CRP) were determined by routine procedures in the clinical laboratory. The other parameters monitored and procedures employed were as follows: prostaglandin dehydrogenase, (PGDH), and cyclooxygenase, (COX) proteins (immunohistochemistry (IHC) and dual immunofluorescence) and mRNA´s (Northern blot). NO synthase; bNOS, eNOS and iNOS proteins (IHC) and mRNA´s (Real-time RT-PCR) IL-6, IL-8, MCP-1, RANTES and TNF-α proteins (ELISA/ Immulite) and IL-8, MCP-1 and RANTES mRNA (RT-PCR). Induction of cervical ripening and labour was performed using local administration of PgE_2 and/or intravenous infusion of oxytocin.

Results: The only significant differences between women undergoing preterm and term labour were in the levels of expression of bNOS, eNOS, and iNOS mRNA´s, which where all higher in the preterm group. Other significant changes were only seen upon comparisons of women undergoing and not undergoing labour, irrespective of the gestational age of the foetus. Thus, cervical levels of IL-8 and MCP-1 mRNA´s and proteins, and of IL-6 protein and serum WBC counts and levels of CRP were all higher in connection with labour. An indication of increased prostaglandin activity, i.e., reduced cervical expression of PGDH mRNA, was also observed in women in labour. In the clinical investigation induction of cervical ripening and labour with PgE_2 applied locally, preterm, term or at postterm was not associated with any differences, in the mode of delivery or neonatal outcome. The fact that postterm pregnancy is a high risk obstetric situation, was emphasized by the four-fold higher frequency of extensive postpartum bleeding, >1000 ml, in this situation compared to labour at term.

Conclusions:

This study supports the hypothesis that preterm cervical ripening involves an inflammatory process, which may constitute a normal physiological adaptation to the onset of labour. The elevations in WBC count and serum level of CRP are striking indicators of active labour. NO appears to play a crucial role in preterm cervical ripening, consistent with its presumed acting as the final mediator. Local application of PgE_2 to induce preterm cervical ripening and labour is effective and safe, for both mother and child.

Key words; cervix, preterm, cervical ripening, cytokines, chemokines, cyclooxygenase (COX), prostaglandin dehydrogenase (PGDH), induction, labour, nitric oxide, PgE_2-gel, pregnancy, preterm labour

LIST OF PUBLICATIONS

This thesis is based on the following articles and manuscripts, which will be referred to in the text by their Roman numerals:

I. Susanne Abelin Törnblom, Falguni A. Patel, Birgitta Byström, Diana Giannoulias, Anders Malmström, Maria Sennström, Stephen J. Lye, John R.G. Challis, Gunvor Ekman
15-HydroxyProstaglandin Dehydrogenase and Cyclooxygenase 2 Mesenger Ribonucleic Acid Expression and Immunohistochemical Localization in Human Cervical Tissue During Term and Preterm Labor
Clinical Journal of Endocrinology & Metabolism, 2004, 89, 2909-15

II. Susanne Abelin Törnblom, Holger Maul, Robert E. Garfield, Birgitta Byström, Anders Malmström, Gunvor Ekman-Ordeberg
mRNA Expression and Localization of bNOS, eNOS and iNOS in Human Cervix at Preterm and Term Labour
Submitted for publication

III. Susanne Abelin Törnblom, Aurelija Klimaviciute, Birgitta Byström, Milan Chromek, Anders Malmström, Annelie Brauner, Gunvor Ekman-Ordeberg
Cytokines in Human Cervix in Non-Infected Preterm Birth.
Submitted for publication

IV. Susanne Abelin Törnblom, Eva Östlund, Lena Granström , Gunvor Ekman
Preterm Cervical Ripening and Labor Induction
European Journal of Obstetrics & Gynecology and Reproductive Biology. 2002;104:120-3

CONTENTS

LIST OF ABBREVIATIONS

15-OH PGDH	15-Hydroxyprostaglandin dehydrogenase
ANOVA	Analysis of variance
ASA	Acetylcystein salicylic acid
bNOS	Brain/neuronal nitric oxide
BSA	Bovine serum albumin
c/s	Caesarean section
cDNA	Core deoxyribonucleotide
CI	Confidence intervals
COX	Cyclooxygenase
CRP	C-reactive protein
ECM	Extracellular matrix
ELISA	Enzyme-linked immunosorbent assay
eNOS	Endothelial nitric oxide
fFN	Foetal fibronectin
IHC	Immunohistochemistry
IL	Interleukin
iNOS	Inducible nitric oxide
IUFD	Intrauterine foetal death
IUGR	Intrauterine foetal growth restriction
Kb	Kilobases
kDA	Kilo Daltons
M	Molar
MCP-1	Monocyte chemoattractant protein 1
MMP	Matrix metalloproteinase
mRNA	Messenger ribonucleic acid
NICU	Neonatal intensive care unit
NO	Nitric oxide
NSAID	Non-steroidal anti-inflammatory drug
ODFS	Operative delivery for foetal distress
PBS	Phosphate-buffered saline
PCR	Polymerase chain reaction
PG	Proteoglycan
Pg	Prostaglandin
pPROM	Preterm premature rupture of membranes
PROM	Premature rupture of membranes
PTB	Premature/preterm birth
PTD	Preterm delivery
PTL	Preterm labour
PTnotL	Preterm not in labour
RANTES	Regulated upon activation, normal T-cell expression and secreted
rRNA	Ribosomal ribonucleic acid
RT-PCR	Reverse transcriptase polymerase chain reaction
SP	Substance P
TL	Term labour

TNF-α	Tumour necrosis factor alpha
TnotL	Term not in labour
WBC	White blood cell count

1 BACKGROUND

1.1 INTRODUCTION

Preterm birth (PTB) is by far the leading cause of infant mortality and morbidity around the world. It is estimated that 85 % of the mortality among preterm infants without lethal malformations is due to their immaturity itself (Abrahams and Katz 2002; Kurki 1998; Marvin *et al.* 2002). The frequency of PTB varies from approximately 5% in certain countries with a well-developed system of health-care accessible to everyone, to around 30% of all births, depending on the geographical and demographic features of the population studied (Creasy 1991; Slattery and Morrison 2002). In Sweden and Europe, the frequency of PTB is 5-7%, compared to 12% in the United States, although the incidence of PTB at a gestational age of < 32 weeks is similar in all three of these regions, i.e., 1-2 % (Hagberg and Wennerholm 2000; Tucker and McGuire 2004). To be born prematurely can be a devastating event, with greatly enhanced long-term morbidity and important social implications for both the child and his family. In the United States, health-care expenditures for the care of preterm neonates have been estimated to amount to $8 billion annually (Challis *et al.* 2001).

Despite intense research, the frequency of PTB has not decreased during the last three decades and the basic mechanisms underlying the onset of labour remain obscure. Few biological processes as central to the survival of a species as parturition are so poorly understood (Mahendroo *et al.* 1999; Romero *et al.* 2002b).

Normal term parturition requires close coordination of the functions of the uterus and cervix (Figure 1). In humans, cervical ripening precedes normal onset of labour and is indeed, required for uterine contractions to be effective. There will be no PTB without cervical softening. The cervix slowly undergoes this ripening process during the final weeks of pregnancy, achieving extensive and complete remodelling of the extracellular matrix by the time of onset of labor. This physiological ripening, softening and dilatation occurs independently of uterine contractions (Chwalisz and Garfield 1998).

The various molecular and cellular processes involved in the onset and maintenance of human parturition are exceedingly complex. To dates, research in this area has

focused primarily on myometrial activities and potential inhibitors of PTL. However, as early as 1974, Danfort et al. suggested that ”to focus on the uterus as the site for onset of labour overlooks the important observation that dilation of the cervix begins before the day of birth in women”.

In numerous investigations in our laboratory the mechanisms involved in cervical ripening at term have been elucidated. Remodelling of the extracellular matrix (ECM) at term is an inflammatory process involving hormones (i.e. prostaglandins (Pg), oestrogen and progesterone), cytokines, nitric oxide synthases (NOS), neurotransmitters and degradative enzymes (metalloproteinases MMP) resulting in a changed composition of the ECM-molecules and results in softening of the cervix, which is crucial for normal dilation.(Ekman-Ordeberg and Malmstrom 1998; Ekman-Ordeberg *et al.* 2003; Gibb 1998; Hertelendy and Zakar 2004; Liggins 1978; Stjernholm *et al.* 1997; Uldbjerg *et al.* 1983a; Wang *et al.* 2001a). Comparison of the mechanisms underlying preterm cervical ripening to those occurring at term is of high priority in connection with attempts to improve our understanding of the pathophysiology of PTB.

1.2 THE UTERUS (CORPUS UTERI)

In contrast to the cervix, the uterus is a muscular organ in which smooth muscle cells account for approximately 70% of the tissue weight. These cells are oriented in an ordered fashion to promote effective contractions during labour (Rorie and Newton 1967). The uterus can be divided into an upper, region, the fundus, and a more distally area close to the cervix, the isthmus. Since the isthmus uterus is predominantly

muscular, containing smaller amounts of ECM, than the cervix, and in addition undergoes less extensive remodelling in connection with labour, this region is considered to be part of the corpus, rather than belonging to the cervix (Danforth *et al.* 1974).

The extracellular matrix (ECM) surrounding the smooth muscles consists primarily of collagen I and III arranged in a stabilizing network together with various proteoglycans (PG), proteins and cells such as fibroblasts and monocytes (Young and Hession 1999), (Hjelm *et al.* 2002; Hjelm Cluff *et al.* 2005) (Figure 2). The major function of this ECM is to provide tissue strength, hold the cells together and mediate cell communication in a manner, which, at every stage of pregnancy, is functionally/physiologically optimal for the well-being of the foetus. As pregnancy progresses, the uterus adjusts to the growing foetus by increasing in size, expanding from approximately 60 g in the non-pregnant state to approximately 1000 g at term pregnancy. Various hormones, including progesterone, corticotrophin-releasing hormone (CRH) and nitric oxide (NO), inhibit contractility and promote quiescence in the uterus. This physiological adaptation of the uterus during human pregnancy represents one of the most active processes of normal tissue remodeling that occur in adults. During normal labour well-timed interactions between the ECM and the smooth muscle cells in the uterus give rise to the uterine contractions resulting in expulsion of the foetus (Challis *et al.* 2002), (Garfield *et al.* 1998), (Gibb and Challis 2002), (Hjelm Cluff *et al.* 2005).

1.3 THE CERVIX (CERVIX UTERI)

The cervix plays important roles in both pregnancy and parturition. During pregnancy, it is of vital importance that the cervix remains rigid and stiff and that its internal os remain closed, in order to ensure maintenance of an optimal physiological environment for the growing foetus, as well as to inhibit a preterm delivery (PTD). During labour and delivery the demands made on this organ are completely the opposite, i.e., the cervix must now become smooth, soft and resilient to allow as gentle passage of the foetus as possible (Fig.3). Unlike the uterus, the cervix does not increase in size during pregnancy. Extensive angiogenesis occurs in this tissue, resulting in increased exposure to blood-born factors including immunocompetent cells.

The major component of the human cervix is the ECM, which constitutes more than 85% of the weight of the non-pregnant cervix. Muscle cells are scarce in this organ, comprising approximately 6 % of the total number of cells, in the distal part of the cervix (from which the cervical biopsies examined in the present study were taken) (Schwalm and Dubrauszky 1966). This ECM performs important functions in holding the cervical cells together; stabilizing the tissue and providing strength; containing and producing important mediators; and facilitating cell-cell and cell-matrix communication. The cervical ECM consists primarily of collagen fibrilles and the small proteoglycan decorin (Danforth 1954), (Uldbjerg *et al.* 1983b). Proteoglycans influences the organisation of the ECM and also contains binding sites for growth factors, cytokines and other regulatory molecules (Kjellen and Lindahl 1991; Kovacs and DiPietro 1994) (Figure 2).

1.4 CERVICAL RIPENING AT TERM IS AN INFLAMMATORY PROCESS

Cervical ripening identified clinically as softening, dilation and effacement, involves intense remodeling of the ECM and is a prerequisite for effective labour and delivery of the child. Cervical ripening can be divided into two phases; a slow ripening throughout pregnancy, reflected biochemically as a gradual decrease in collagen content, and the highly rapid cervical changes that occur just prior to or during early labour (Granstrom *et al.* 1989; Uldbjerg *et al.* 1983b). As early as 1978, Liggins, a pioneer in the field suggested that this latter phase involves an inflammatory reaction (Liggins 1978). During this phase marked infiltration of neutrophils into the stroma occurs and later investigations in our laboratory and others have revealed an up to100-fold increase in the local concentrations of cytokines, (i.e., IL-6, IL-8 and Granulocyte colony stimulating factor) and up-regulation of the genes (Junqueira *et al.* 1980; Sennstrom 2000; Sennstrom *et al.* 2000). Moreover increased influxes of neutrophils and macrophages, resulting in the production of degradative enzymes such as elastase and MMPs, have been reported (Osmers *et al.* 1995; Sennstrom *et al.* 2003; Stygar *et al.* 2002; Uldbjerg *et al.* 1983c).

This inflammatory reaction causes changes in the ECM which give rise to the altered physicochemical properties of the ripe cervix. Collagen metabolism is increased to yield decreased cross-linking, degradation, a reduction in the level of decorin by 50% and elevation of the content of the large PG versican by 15% (Ekman *et al.* 1986; Uldbjerg *et al.* 1983b; Uldbjerg *et al.* 1985; Uldbjerg *et al.* 1983c; Westergren-Thorsson *et al.* 1998). This decrease in the decorin content destabilises the collagen fibrils, while versican disintegrates their arrangement. Both versican and hyaluronan attract water, leading to swelling and dispersion of the collagen fibrils. These alterations are recognized clinically as softening and dilation.

1.5 PRETERM BIRTH (PTB)

A preterm delivery (PTD) is defined by the World Health Organization as one that occurs at greater than 20 and less than 37 full weeks of gestation. No more than approximately 20% of all PTB are thought to involve obstetric intervention for maternal or foetal health. The rest are believed to be spontaneous and of unknown pathogenesis (Pschirrer and Monga 2000).

The present study focuses on the enigma of the pathophysiological mechanisms involved in inducing premature onset of cervical ripening and labour. In particular, I have investigated the regulation of prostaglandin synthesis and of nitric oxide synthases (NOS), as well as alterations in the levels of proinflammatory cytokines, all known to be key regulatory factors in connection with the highly complex, and mutually supportive pathways leading to term birth (Chwalisz and Garfield 1998), (Hertelendy and Zakar 2004), (Maul *et al.* 2003). In addition, clinical evaluation of the capacity of PgE_2, applied locally, to induce preterm cervical ripening has been performed in attempt to evaluate the involvement of this prostaglandin in PTB.

1.5.1 Risk factors of PTB

The factors most frequently associated with an increased risk for PTB are a history of a previous PTB, belonging to the black race and multiple gestation (Pschirrer and Monga 2000). The earlier the gestational age at which the prior PTB occurred, the greater the risk for a subsequent early and spontaneous PTB (Goldenberg *et al.* 2000).

The occurrence of a first PTB may be influenced by various genetic and environmental factors. An increasing evidence supports the hypothesis that certain women exhibit a genetic predisposition to deliver preterm. Thus, women who were themselves born before 37 weeks of gestation demonstrate a significantly increased risk. This risk increases as the women´s gestational age at birth decreases, being more than doubled for women born prior to 32 weeks of gestation (Dizon-Townson 2001).

Other well-known risk factors include low socioeconomic status, a maternal age of less than 17 or greater than 40 years, increasing parity and uterine malformations (Goldenberg *et al.* 2000; Porter *et al.* 1997). Both poor and excessive weight gain during the pregnancy are associated with an increased risk for PTB, and women with a low body mass index (<19, 8 kg/m^2) are at highest risk. Furthermore smoking increases the risk by approximately 20-30 %. Interestingly, investigations indicate that mental stress is significantly associated with spontaneous PTB prior to 35 weeks of gestation (Copper *et al.* 1996; Ruiz *et al.* 2003).

1.5.2 Predictive factors

At present, onset of labour cannot be predicted reliably nor have any effective strategies for the prevention of PTL been developed (Chwalisz and Garfield 1997).

Numerous clinical parameters and biochemical markers of PTL have been examined in this context, most often with fruitless results. The only efficient predictor of spontaneous PTL now known is the presence of a cervix of soft or medium consistency, where the consistency of the internal os is of particular importance (Goldenberg 2002) (Figure 4).

Thus, clinical examination of the cervical status is of utmost importance in cases of threatening PTL. Additional factors of some significance include prior spontaneous PTB, possession of a short, soft cervix and the presence of foetal fibronectin (fFN) in vaginal secretions. fFN is normally absent in vaginal secretion from the 20th week of gestation until near term, this glycoprotein is a marker of choriodecidual disruption. Thus, the absence of fFN during this period is a strong indicator (96%), that the risk for PTL is very low (Keirse 1995).

1.5.3 Preterm birth and infection

Although it is well established that intrauterine infection can lead to PTL, this does not appear to be the major cause of prematurity, since such infection can be demonstrated in only 25-30% of all cases of PTB (Romero *et al.* 1989), (Slattery and Morrison 2002). Labour, both term and preterm with concomitant uterine infection is associated with significantly elevated levels of interleukin (IL)-1, IL-8, the "Regulated upon Activation Normal T cells Expressed and Secreted" (RANTES) factor and especially Il-6 and Tumour Necrosis Factor alpha, (TNF-α) which has been demonstrated in various gestational tissues (Sennstrom *et al.* 2000), (Keelan *et al.* 2003), (Dudley *et al.* 1996). Since cytokines induce cervical ripening and onset of labour, the significantly higher levels of these regulators detected in the gestational tissues and vaginal secretions of women experiencing PTL suggest that an exogenous

infection, either clinical or subclinical, may be present. Accordingly, in the year 2000 Goldenberg et al. stated that a growing body of evidence indicates that infection of the deciduas, foetal membranes and amniotic fluid is associated with PTB

In Sweden, approximately 16 % of all cases of PTB appear to be the consequence of infections (Jacobsson *et al.* 2003), (Hagberg *et al.* 2005). Infection causes PTB and labour more frequently at lower gestational ages, and when preterm premature rupture of membranes occurs (Romero *et al.* 2002a).

However, since it is estimated that less than 50% of all cases of PTL involving elevated levels of cytokines are due to an infection, the question arises as to whether some other signal is responsible for initiating preterm cervical ripening and labour (Steinborn *et al.* 1996), (Winkler *et al.* 2001), (Farina and Winkelman 2005).
Could there be a genetic difference, a polymorphism among women experiencing PTL with an associated infection? Indeed, genetic studies do indicate that there is an interaction between genetic susceptibility and environmental factors to produce an increased risk for spontaneous PTB (Macones *et al.* 2004).

For example, PTL is much more common among Afroamerican women, who are dramatically less likely than caucasian women to carry a low-production allelic variant of a gene that decreases the production of IL-6, (Simhan *et al.* 2003). Furthermore, polymorphisms in the promoter region of the TNF-α gene have also been shown to be associated with varying levels of risk for spontaneous PTB with maternal carriers of the rarer allele being at significantly higher risk. Among the carriers of this rarer allele,("gene susceptility") the risk was increased even further by the presence of an infection (Macones *et al.* 2004).

Clearly, there is a need to test scientifically the hypothesis that preterm cervical ripening and labour without clinical infection also involves elevated cytokine levels. This hypothesis is supported by the fact that if the foetal membranes are intact, antibiotic administration does not reduce the frequency of PTB, nor can such treatment prevent PTL associated with infection.

1.5.4 Diagnosis and management of preterm labour

Correct diagnosis of an actual case of PTL is a challenge for even the most experienced obstetrician. Numerous studies confirm that 50-75 % of women exhibiting

false PTL will, without treatment, go on to deliver at term. On the other hand, almost 50% of women actually experiencing PTL demonstrate no risk whatsoever (Goldenberg *et al.* 2000). Since there are at present no reliable biochemical or clinical tests for early diagnosis nor is there any way to delay PTL for more than 24-48 hours, management focuses on making the delivery as atraumatic as possible. Care is also taken to prevent neonatal complications, through the use of corticosteroids and neonatal treatment with antibiotics in order to avoid sepsis.

1.5.5 Pharmacological treatment of preterm birth

At present, there is no effective treatment for PTL that reduces both perinatal mortality and inhibits PTB (Higby and Suiter 1999). Such treatment will require both early diagnosis and effective intervention in the underlying pathophysiological processes. Most investigations in this area have focused on the development of new tocolytic agents that inhibit myometrial contractions.

Terbutaline is not an optimal drug in this context, since its effect is so temporary, lasting only 24-48 hours. In addition, Terbutaline exerts severe adverse effects on the mother, including tachycardia and an enhanced risk for pulmonary oedema (Kurki 1998).

Oxytocin antagonists such as Atosiban are equally effective as β-mimetics, with fewer maternal and foetal side effects.

Among the COX-2 inhibitors of prostaglandin synthesis, Indomethacin has been most extensively characterized in connection with PTL. The clinical use of this drug is limited by severe adverse effects on the foetus, of which the most serious are oligohydramniosis, constriction of the ductus arteriosus, persistent anuria, neonatal death and necrotizing enterocolitis. Moreover, treatment of women experiencing preterm labour with antibiotics for the sole purpose of preventing preterm delivery has proven ineffective (Gibbs and Eschenbach 1997).

Earlier intervention, during cervical ripening, offers intriguing possibilities for treatment of PTL. Identification and characterization of the regulators of preterm cervical remodelling and onset of PTL would therefore be invaluable in connection with the development of novel pharmacological treatments designed to prevent premature labour and delivery.

1.6 MEDIATORS OF CERVICAL RIPENING AT TERM

Numerous hormones and mediators are involved in the regulation of cervical remodeling at the time of parturition, of which the most important are believed to be prostaglandins, proinflammatory cytokines, NO, neuropeptides, oestrogen and progesterone. However, it must be emphasized that the biochemical mechanisms responsible for rearrangement of the ECM are still poorly understood.
Recent investigations highlight genetic predisposition for PTB (Dizon-Townson 2001; Macones *et al.* 2004; Romero *et al.* 2004; Simhan *et al.* 2003).

1.6.1 Prostaglandins

Prostaglandins were first discovered by the Swedish researcher Hans von Euler in 1934, as a major constituent of the seminal fluid which exhibited vaso-active properties. These were originally thought to be a single substance secreted by the prostate gland and therefore designated "prostaglandin". Prostaglandins are synthesised locally and immediately secreted from the cell. They arise from release of arachidonic acid from membrane phospholipids and subsequent conversion of this precursor via several well-regulated steps to the final products. The three enzymes that regulate the levels of active prostaglandin in tissues are COX-1, COX-2 and 15-hydroxyprostaglandin dehydrogenase (PGDH) (Figure 5). Production of Pg has been observed in the amnion, deciduas, chorion (foetal membranes), myometrium, placenta and cervix with the human cervix synthesizing primarily PgE_2 (Barclay *et al.* 1993; Ekman *et al.* 1983b; Kayem *et al.* 2003; Korita *et al.* 2004; North *et al.* 1991; Zakar *et al.* 1996)

Figure 5. Prostaglandin synthesis and metabolism

Prostaglandins (Pg) exert a wide range of physiological actions in the humans. In connection with pregnancy these hormones play key roles in the biochemistry of labour and the regulation of parturition, both in terms of promoting cervical ripening and initiating uterine contractions (Ekman *et al.* 1983c), (Gibb 1998)

PgE_2 , the major prostaglandin involved in cervical ripening is thought to act principally as a vasoactive agent. This hormone thus facilitates infiltration by inflammatory cells and also regulates the release of many cytokines. Furthermore it acts synergistically with IL-8 to augment neutrophil chemotaxis and stimulates the synthesis of MMP, which plays an important role in cervical remodelling.

1.6.2 15-OH Prostaglandin dehydrogenase (PGDH)

PGDH is expressed in two different forms, encoded by 3.4kb and 2.0kb species of mRNA. The 3.4-kb form is believed to be the active form. This is the enzyme primarily responsible for the metabolism of PGE_2 and PGF_2-α, catalyzing them into their corresponding inactive 15-keto derivates and thereby regulating the tissue levels of these biologically active prostaglandins(Challis *et al.* 1997).

PGDH is expressed at high levels by chorionic trophoblast cells (Keirse and Turnbull 1975). Furthermore, Van Meir et al. (1997) reported that in association with normal labour at term the level of PGDH in foetal membranes in the lower uterine segment decreases and, moreover, that chorionic PGDH activity in the chorion is significantly lower in connection with PTL than with labour at term. Even further reduction of this activity was seen when chorioamnionitis was present in the placenta (Van Meir *et al.* 1997).

In addition, the level of PGDH mRNA in the chorio-decidua of women undergoing spontaneous term or preterm labour is also significantly lower than in the case of elective Cesarean section (c/s) following full term pregnancy (Sangha *et al.* 1994). Patel et al. has showed that the PGDH activity is increased by progestagens and inhibited by antiprogestins (RU486 and onapristone) and cortisol (Patel *et al.* 1999). Together, these observations form the basis for the hypothesis that a PGDH deficiency may be involved in PTL. Thus, failure to metabolize PgE_2, to its inactive metabolites, is a potential causative factor of the PTL syndrome.

1.6.3 Cyclooxygenase (COX) -1 and -2

The cyclooxygenase enzymes (COX) referred to as prostaglandin H synthase (PGHS)-1 and -2. are primarily responsible for regulating the synthesis of PgE_2. COX-1 appears to be constitutively expressed in gestational tissues; whereas COX-2 is up-regulated in connection with inflammation, by, among other factors, certain cytokines (e.g., IL-1, TNF-α, IL-8), NO, growth factors and glucocorticoids, and repressed by progesterone (Gibb 1998), (Kirschenbaum *et al.* 2000).

Earlier studies revealed a significantly higher level of COX-2 mRNA in the amnion of patients who gave birth prematurely (Teixeira *et al.* 1994). Furthermore, the levels of this mRNA and the corresponding protein were higher in the amnion of women experiencing spontaneous term labor than of those not in labor (Hirst *et al.* 1995; Zakar *et al.* 1996). The data presently available are somewhat contradictory, since the levels of COX enzymes in the myometrium have been reported to be decreased, unchanged or even increased in women undergoing term labour (Hertelendy and Zakar 2004). These conflicting findings motivated us to re-address the question as to whether the changed expression of COX enzymes is altered in connection with preterm cervical ripening.

1.6.4 Nitric oxide (NO)

The "endothelial-derived relaxing factor" discovered in 1980 was shown in 1987 to be NO and acclaimed as "The molecule of the year" in 1992 (Ignarro *et al.* 1987). Since then, numerous investigations have revealed the importance of NO as a major mediator in many different biological processes in humans. Thus, NO controls many of the key events that enable reproduction. For instance, during pregnancy, NO plays an essential role in maintaining uterine quiescence by inducing smooth muscle relaxation (Maul *et al.* 2003).

The inorganic gas, NO is a highly reactive small free radical with a biological half-life of only a few seconds. Three different NO synthases (NOS), neuronal bNOS, endothelial eNOS and inducible iNOS catalyze conversion of the amino acid L-arginine and oxygen to equal amounts of NO and citrulline (Palmer *et al.* 1988). All three of these activities are detectable in the human cervix at the end of a full-term pregnancy.

Although, NO has been proposed to act as the final regulator of cervical ripening at term its role as an inflammatory mediator of this process is complex and not yet fully

understood (Chwalisz and Garfield 1998). NO amplifies the cytokine cascade associated with acute inflammatory responses, enhances prostaglandin synthesis by activating COX-2 and, together with PGE_2 and PGI, causing potent vasodilatation (Ianaro *et al.* 1994). NO also stimulates the MMP activity of cervical tissue and NO-donors administered locally induce cervical ripening in humans (Chatziantoniou *et al.* 1998; Ianaro *et al.* 1994), (Thomson *et al.* 1997; Thomson *et al.* 1998).
To our knowledge, no corresponding investigations have been performed in connection with PTB.

1.6.4.1 Neuronal/brain nitric oxide synthase, bNOS

The constitutively expressed neuronal NOS, bNOS, was the first isozyme of this enzyme to be isolated and characterized. It was later found in brain neuronal tissue, and is consequently also referred to as bNOS or nNOS. bNOS requires calcium/calmodulin for activation and can rapidly and transiently produce small amounts of NO, which is thought to act as a neurotransmitter in these tissues. Earlier reports have documented both an increase in the protein expression of the bNOS protein in the cervix at the time of labour, as well as no change in this expression in association with labour (Bao *et al.* 2001; Ekerhovd *et al.* 2000). Both Ledingham and Bao and their co-workers observed interaction with anti-bNOS antibodies in epithelial and stromal cells.

1.6.4.2 Endothelial nitric oxide synthase, eNOS

The endothelial eNOS is also constitutively expressed and its activity dependent on calcium. This protein was originally purified and cloned from the vascular endothelium, but has since also been detected in the brain, blood platelets, cardiac myocytes and elsewhere. The analysis of the cervical level of expression has produced contradictory results (Tschugguel *et al.* 1999), (Ledingham *et al.* 2000a), (Ledingham *et al.* 2000b). eNOS has also been observed immunohistochemically in the endothelial and glandular cells of the cervix (Ekerhovd *et al.* 2000), (Ledingham *et al.* 2000b), (Tschugguel *et al.* 1999), (Yoshida *et al.* 2001).

1.6.4.3 Inducible nitric oxide synthase, iNOS

The activity of inducible NOS, first identified in immunoactivated macrophage cell lines, is independent of calcium. Subsequently, this protein has been detected in many other types of cells, including neutrophils, mast cells, endothelial cells and vascular

smooth muscle cells. In general, expression of iNOS is elicited by inflammatory mediators, including the cytokines TNF-α and IL-1 (Ledingham *et al.* 2000a).The iNOS enzyme produces NO in large quantities during an extended period of time (Knowles and Moncada 1994). Elevated levels of iNOS mRNA have been observed in cervical tissue during term labour, compared to the non-pregnant state (Tschugguel *et al.* 1999).

1.6.5 Cytokines

Cytokines, small proteins produced by virtually all nucleated cells, regulate a wide variety of physiological functions in humans. Initially, these mediators were named according to their function or the cells found to produce them. Since the first source of cytokine production identified was white blood cells, many cytokines are referred to as interleukins, i.e., molecules that mediate communication between white blood cells. Cytokines play key roles in connection with inflammatory processes; influence the concentrations of many plasma proteins; mediate the immune response to infections, regulate the production of prostaglandins and affect metabolism and homeostasis (Farina and Winkelman 2005), (Garcia-Velasco and Arici 1999), (Kelly 2002), (Yellon *et al.* 2003). An improved understanding of the roles played by cytokines during the process of labour may well suggest strategies for interrupting PTL and promoting full-term gestation.

1.6.5.1 IL-6

The cytokine IL-6, an important mediator in the cascade of host responses to infection, is produced in human gestational tissues and cervix (Keelan *et al.* 1997; Rath *et al.* 1998; Saji *et al.* 2000), (Sennstrom *et al.* 2000). Since this cytokine activates the acute phase response of plasma proteins, stimulates T lymphocytes, induces the terminal differentiation of B lymphocytes and induces production of C-reactive protein, it was initially employed as a marker of intramniotic infection in patients experiencing PTL (Papanicolaou *et al.* 1998), (Mitchell *et al.* 1993). This approach is questioned today since studies of the cervix, by Sennström et al. have revealed that the expression of both IL-6 and IL-8 is up regulated significantly at both the mRNA and protein levels in patients undergoing normal term labour (Sennstrom *et al.* 1997), (Sennstrom *et al.* 2000). Control of IL-6 expression at the genetic level may be a factor in the risk for prematurity (Simhan *et al.* 2003).

1.6.5.2 IL-8

IL-8, produced by human endometrium, choriodecidua, myometrium, pregnant and non-pregnant cervices and cervical fibroblasts in culture, is a potent chemoattractant and activator of neutrophils (Kelly 2002), (Sennstrom *et al.* 1997), (Sennstrom *et al.* 2000). This cytokine induces plasma exudation and a massive local infiltration of neutrophils from the endothelial wall of blood vessels into the cervix, thereby resulting in their activation and the associated release of collagenases (e.g.MMP-8) from specific granules. In vitro studies have shown that PgE_2 and NO stimulate, while progesterone and dexamethasone inhibit IL-8 release from cervical explants (Denison *et al.* 1999). Sennstrom et al demonstrated that cervical level of the IL-8 mRNA is five-fold higher in the postpartum state than in the term-pregnant and that the protein is elevated several-fold in the postpartum compared to the non-pregnant state (Sennstrom *et al.* 1997), (Sennstrom *et al.* 2000).

1.6.5.3 Tumour necrosis factor- alpha (TNF-α)

Under normal circumstances tissue levels of TNF-α are barely detectable. This cytokine is released by decidual cells in response to bacterial products and in turn stimulates decidual and amnion cells, among others, to release prostaglandins by enhancing their expression of COX-2 (Keelan *et al.* 2003), (Casey *et al.* 1989). Numerous investigations have documented elevations in the level of TNF-α in various tissues during PTL associated with preterm premature rupture of membranes (pPROM) and infection (Romero *et al.* 1992), (Dudley *et al.* 1996; Keelan *et al.* 2003). Therefore, like IL-6, TNF-α is an indicator of infection. Interesting genetic studies have assessed the relationship between the TNF-α allele and PTL, with or without pPROM PTL (Macones *et al.* 2004).

1.6.5.4 Monocyte Chemotactic Protein-1 (MCP-1) and Regulated-upon-Activation Normal-T cell-Expressed and Secreted (RANTES) protein

MCP-1 and RANTES, members of the same group of chemokines, are produced in gestational tissue by endometrial, myometrial, stromal and epithelial cells. These proteins are potent chemoattractants and activators of macrophages, monocytes, T cells, natural killer cells, basophils and mast cells (Garcia-Velasco and Arici 1999), (Denison *et al.* 2000), (Kayisli *et al.* 2002), (Esplin *et al.* 2003). Their production is stimulated by local growth factors and cytokines, including TNF-α and IL-1, while production of MCP-1 is inhibited by glucocorticoids. The concentrations of the MCP-1 and RANTES

proteins in amniotic fluid increase during spontaneous labour at term (Esplin *et al.* 2003), (Athayde *et al.* 1999). Furthermore, women without any clinical signs of infection and who gave preterm birth exhibited higher concentrations of RANTES in their amniotic fluid than did those who delivered at term (Athayde *et al.* 1999).

1.6.6 Corticotropin releasing hormone (CRH)

In response to stress, the corticotropin releasing hormone (CRH), also a protein, is released from the hypothalamus and subsequently activates the pituitary-adrenal axis. This hormone is produced by the placenta and the myometrium during pregnancy as well (Berkowitz *et al.* 1996; Challis 2000). Human reproductive tissues express different CRH receptors, of which the CRF1α receptor is the most important (Hillhouse *et al.* 1993), (Stevens *et al.* 1998).

The exact functions and mechanisms of CRH action in connection with pregnancy and labour remain unknown. This mediator has been proposed to exert dual regulatory effects on uterine contractility, probably as a result of interactions with different members of the wide variety of receptor subtypes expressed by the myometrium. Thus, CRH is believed during most of pregnancy, to play a 'protective' role by promoting myometrial quiescence via the generation of cAMP and cGMP, and up-regulation of nitric oxide synthase (NOS). At the same time CRH stimulates intrauterine prostaglandin production and probably acts synergistically with oxytocin in connection with labour.

Inflammatory cytokines, such as IL-1 and IL-6 and glucocorticoids stimulate the production and release of CRH, thereby elevating prostaglandin production and an enhancing cervical ripening and onset of labour. On the other hand, NO and progesterone reduce CRH levels resulting in the decreased prostaglandin synthesis that contributes to the maintenance of uterine quiescence during pregnancy (Grammatopoulos *et al.* 1998). These apparently contradictory effects might simply reflect the fact that CRH plays different roles at different times during gestation.

During pregnancy plasma levels of CRH are elevated in women with high scores on anxiety tests. Moreover, the level of CRH in plasma of women delivering preterm, after 26-32 full weeks of gestation is increased significantly (Korubrits et al et Challis –98). It has been suggested that CRH induced stimulation of PG production in

gestational tissues is responsible for the enhanced risk for preterm delivery associated with maternal or foetal stress (Challis and Smith 2001).

1.6.7 Oestrogen and progesterone

During pregnancy systemic concentrations of oestrogen and progesterone increase as much as 100-fold and remain elevated until parturition (Speroff *et al.* 1989). Progesterone antagonizes oestrogen activity and inhibits cytokine release by suppressing the expression of iNOS and COX-2, thereby resulting in decreased levels of MMP-9, and slowing down the cervical ripening process. In contrast to the situation in other species, no alterations in the serum levels of these steroids herald the onset of human labour, although a gradual rise in the serum oestradiol level and decline in the serum progesterone level are observed after 35 weeks of gestation (Csapo *et al.* 1971), (Turnbull *et al.* 1974).

Both, oestrogen and progesterone receptors (ER and PR) are expressed in the human cervix. In the endometrium and cervical tissue, oestrogen induces whereas progesterone appears to suppress the expression of both these receptors (Aronica and Katzenellenbogen 1993; Sanborn *et al.* 1978). Following term pregnancy a significant down-regulation of the PR and the ERα and up-regulation of the oestrogen receptor β (ERβ) are observed in the pregnant cervix, in comparison to the non-pregnant state (Stjernholm *et al.* 1996; Wang *et al.* 2001a). This enhanced level of ERβ could lead to decreased progesterone activity, similar to that caused by antiprogestin treatment, by decreasing the level of the PR (Frydman *et al.* 1992). However, following parturition a switch appears to occur and the level of ERβ mRNA expression decreases again to the corresponding non-pregnant level (Wang *et al.* 2001a).

In 1996, Robertson et al. reported that oestrogen has a role to play in leukocyte migration (Robertson *et al.* 1996). Several years later, Wang and co-workers documented intense immunostaining for ERβ in leukocytes, macrophages and the endothelial cells of the vessels in the human cervix (Wang *et al.* 2001a). Therefore, it appears that neutrophils possess the ability to respond to oestrogen in the cervix via the ERβ (Stygar *et al.* 2001), (Young *et al.* 2002).

1.7 MARKERS OF INFLAMMATION IN THE PERIPHERAL BLOOD

1.7.1 The White blood cell count (WBC)

The number of white blood cells per 1000 ml in peripheral blood is often used as an indicator of bacterial infection. Although it is well known that this total white blood cell count increases, possible alterations in this parameter in relationship to the process of parturition have not yet been examined in detail (Siegel and Gleicher 1981), (Kuhnert *et al.* 1998; MacLean *et al.* 1992).

1.7.2 C-Reactive protein (CRP)

In connection with inflammation the C-reactive protein (CRP) is the acute phase reactant whose level in the peripheral plasma increases most rapidly and to the greatest extent (Black *et al.* 2004). Receptors for this protein are expressed by T-lymphcytes. Elevated levels of CRP are indications of cell necrosis and this parameter is used clinically to differentiate between bacterial and virological infections. Kaapa and Koistinen (1993) have reported that maternal peripheral levels of CRP were low at birth, but rose significantly during the first day following normal term vaginal delivery. However, earlier analyses of the relationship between the CRP in peripheral plasma and chorioamnionitis, and for preterm delivery failed to find any predictive value (Kornman *et al.* 1988), (Farb *et al.* 1983), (Foulon *et al.* 1995).

1.8 PRETERM CERVICAL RIPENING AND INDUCTION OF LABOUR

Induction of cervical ripening and labour remains one of the therapeutic challenges in the field of obstetrics. When the foetus must be delivered preterm due to maternal or foetal complications, the cervix is usually very rigid and unripe, so that, to date, Caesarean section has been the method of choice for such premature delivery. In such women, with foetuses that are often at risk in some way, powerful contractions against an unripe cervix can cause foetal distress, including hypoxia. It is therefore of vital importance to coordinate onset of labour with adequate cervical ripening (Calder and Embrey 1975), (Wiqvist *et al.* 1986), (Ulmsten *et al.* 1979). Induction of labour by intravenous infusion of oxytocin and or amniotomy in women with unripe cervices is associated with a high risk for perinatal complications (Calder *et al.* 1977), (Ekman *et al.* 1983a; Wiqvist *et al.* 1986).

Local intracervical or vaginal application of PGE_2 in gel form, is today the “golden standard” procedure for achieving cervical ripening and inducing labour at term and

postterm, but has only been employed for preterm cervical ripening in women with missed abortions or in cases of intrauterine foetal death (IUFD), (Ekman *et al.* 1983d), (Keirse 1993). Intracervical application has been found to be more effective than vaginal application in women with highly rigid cervices, since the former induces cervical ripening with a minimum of myometrial contractions, which is important when handling delicate foetuses (Granstrom *et al.* 1990). Such intracervical application has been shown to significantly decrease both the length of labour and the frequency of Caesarean section and it is therefore important to evaluate the safety, effectiveness and perinatal outcome of employing this procedure to induce preterm labour (Keirse 1992), (Calder and Embrey 1975).

The intense research conducted in this area during the past three decades has been focused primarily on the mechanisms regulating uterine contractility, even though preterm birth also requires cervical softening. Improved understanding of the mediators regulating the ripening process is important to improving health-care both in cases of spontaneous premature birth and when premature induction of labour is desired due to maternal/foetal complications.

2 AIMS OF THE PRESENT STUDY

The overall aim of this investigation has been to determine whether the mediators regulating preterm ripening of the non-infected cervix differ from those involved in this same process at term. In this context the following questions have been asked:

- Does the level of expression of COX and/or PGDH change in connection with preterm cervical ripening?

- Is NO produced endogenously in connection with preterm cervical ripening and could this substance mediate preterm labour?

- Do the preterm cytokine levels in the cervix differ from those at term?

- Are the levels of inflammatory markers in the peripheral blood the same in connection with preterm and term birth?

- Is induction of preterm cervical ripening and labour with Prostaglandin E_2 equally safe as the use of this procedure is at term?

3 METHODOLOGICAL CONSIDERATIONS

3.1 SUBJECTS

The experimental studies (I-III)

A total of 50 women undergoing singleton pregnancy without clinical signs of infection, either during parturition or during the postpartal period were included in these studies. Preterm birth was defined as birth before 36+6 full weeks of gestation (fgw), while the term births included births between 37 +0 to 42+0 fgw. There were no significant differences between the four groups with respect to maternal age, parity and previous preterm births.

The two study groups were as follows:

Preterm Labour (PTL, n=17): women undergoing spontaneous, active preterm labour with a cervical dilation of > 4cm, i.e., a ripe cervix, delivered either vaginally or by emergency Cesarean section (c/s) due to malpresentation. In study I all of the preterm women in labour were delivered vaginally and this group is therefore referred to as Preterm spontaneous vaginal delivery (Preterm S.V.D).

Preterm not Labour (PTnotL, n=8): women with an unripe cervix (dilation < 2cm), delivered by c/s prior to onset of labour, referred to in study I as Preterm c/s.

The two control groups were as follows:

Term Labour (TL, n=14 :) women at term, delivered either vaginally or by emergency c/s due to malpresentation or foetal distress. Otherwise, these women had the same features as those in the PTL group. In study I all of these women were also delivered vaginally and are therefore referred to as Term S.V.D.

Term not Labour (TnotL, n=11): women with unripe cervices (dilation < 2cm), delivered by c/s prior to onset of labour, referred to in study I as Term c/s.

The clinical study (IV)

During a period of two years, from January 4, 1994 – January 12, 1996, all preterm (PT) women undergoing induction following < 37 full weeks of gestation, (n=50) were compared with the next two women delivered, by induction at term (n=50) and postterm (n=47), at the delivery ward of the Karolinska University Hospital. None of these women experienced labour, all had intact membranes and they were matched for age and parity. The reasons for deciding on induction were, in the case of the preterm group, pre-eclampsia, intrauterine growth restriction (IUGR), intrauterine foetal death (IUFD), foetal malformation, and, in the case of the term

group, pre-eclampsia, foetal macrosomia and humanitarian. The postterm women were induced simply because they were overdue. Relevant data on these patients are presented in Tables 1, 2 and 3, in paper IV.

3.2 SAMPLING PROCEDURES

Samples of cervical tissue (I-III)

Immediately following parturition, a transvaginal biopsy was taken at the 12 o'clock position on the upper cervical lip. These samples were immediately frozen in liquid nitrogen and stored at – 70^{o} C until further investigation.

Serum samples (I-III) of venous blood were taken.

Due to the limited amount of tissue obtained from each woman, not all of the different analyses could be performed on every sample. All studies were performed with the informed consent of the women participating and were approved by the local ethics committee**.**

3.3 METHODS

3.3.1 Determination of WBC and CRP (I-III)

The levels of C-reactive protein and white blood cell counts were determined in the routine clinical laboratory at Karolinska Hospital, Solna, (Stockholm, Sweden).

3.3.2 Tissue homogenisation (I-III)

Frozen cervical tissue was cut into small slices on a block of dry ice and thereafter transferred to a capsule containing Teflon-coated tungsten ball and maintained in liquid nitrogen for two minutes. These capsules were subsequently, shaken repeatedly at full speed for two minutes in a dismembranation apparatus, with intermediate freezing, until the tissue had been pulverized into a powder which could be used for RNA extraction or quantitation of cytokines.

3.3.3 ELISA / Immulite (III)

The cytokine proteins IL-6, IL-8 and TNF-α were quantitated employing an IMMULITE Automated Analyser, to perform immulite chemiluminescent enzyme immunometric assays. RANTES and MCP-1 were assayed using quantitative sandwich enzyme-linked immunoassays (ELISA). The levels of all of these proteins were

expressed as picograms of cytokine per mg total protein (pg/mg protein). The total protein concentration was determined with Bio-Rad's Protein Asssay.

3.3.4 Extraction of RNA (I-III)

Total RNA was extracted employing Trizol reagent and the total concentration of RNA thus obtained quantitated by determining the absorption at 260/280 nm in a Eppendorf Bio Photometer, or a spectrophotometer. This extracted RNA was used for Northern blotting, RT-PCR and Real-Time RT-PCR analyses (Figure 6&7).

Figure 6. The structure of DNA

3.3.5 Northern blotting (I)

Twenty μg RNA prepared as described above was fractionated, on the basis of size by agarose1% gel electrophoresis. Thereafter, the RNA bands were transferred to a nylon membrane and hybridized with specific α- [32P] deoxy-CTP-labeled cDNA probes for 15-OH PGDH and COX-2 mRNA for a period of 24 h. The blots thus obtained were exposed to Kodak X-AR film and the relative optical density (R.O.D.) of the resulting autoradiographs determined. 18S ribosomal RNA was employed as an internal standard and the results are expressed as the ratio of the relative optical densities of the hybridization signals for 15-OH PGDH mRNA or COX-2 mRNA to the signal for 18S rRNA.

3.3.6 RT-PCR (III)

Reverse transcription with SuperScript™ RNAse H was performed on the total RNA prepared as described above to produce the corresponding cDNA (Fig. 8). Employing primers specific for IL-8, MCP-1, RANTES and ribosomal 28S cDNA (internal standard) the PCR was carried out using the Master Taq kit and the Eppendorf Mastercycler® gradient. Care was taken to ensure that the level of products obtained was within the linear portion of the curve and the number of cycles and annealing temperatures utilized are documented in Table II in article III. Following staining with ethidium bromide and separation by electrophoresis on a 1.5% agarose gel, the band intensities were measured under UV light, with the Gel Doc 2000 system and expressed as the intensity of the provided band/the intensity of the band corresponding to 28S rRNA.

3.3.7 Real-time multiplex RT-PCR (II)

Primers specific for iNOS, eNOS and bNOS cDNA and Taqman® probes were designed using the Primer Express® software. By running a blast, alignment to other known human genes was excluded and 18S ribosomal RNA was used as an internal standard. Reverse transcription (RT) was performed on the total RNA isolated as

described above in order to produce the corresponding cDNA. PCR was carried out by running 50-60 cycles and the threshold cycle (C_T) at which an increase in reporter fluorescence above the baseline signal could first be detected determined. The relative amounts of target RNA were normalized to the amounts of ribosomal RNA present.

3.3.8 Immunohistochemistry (I-II)

Frozen cryostat sections of cervical tissue, 8 μm thick, were mounted and fixed in 2% paraformaldehyde dissolved in phosphate-buffered saline (PBS). Background staining was minimized by pre-treatment with 0.3% hydrogen peroxide in methanol for 30 minutes, followed by washing in PBS/BSA (0.05%). The slides were incubated overnight with primary monoclonal antibodies directed against iNOS, eNOS or bNOS or polyclonal antibodies towards 15-OH PGDH or COX-2. Subsequently, the secondary antibody was added and incubation with an avidin-biotin-horseradish (ABC) –complex performed. Staining was achieved with the DAB(diaminobenzidine) kit and counterstaining with 10% Mayer's Haematoxylin (NOS) or Carazzi´s haematoxylin for (15-OH PGDH and COX-2) respectively. As a control, sections were stained in the same manners, but without primary antibody. Using light-microscopy the resulting slides were classified by three independent observers.

3.3.9 Dual immunofluorescence (I)

After blocking non-specific binding, tissue sections were incubated with the primary antibodies, i.e., a monoclonal mouse antibody against smooth muscle-α actin together with either polyclonal rabbit anti-human Cox-2 or rabbit anti-human 15-OH PGDH antibodies overnight in a 1% BSA solution. Subsequently, the sections were washed in PBS and incubated with the secondary antibodies in PBS 1%. BSA sheep anti-mouse IgG antibodies conjugated with fluorescein isothiocyanate (FITC) in the case of COX-2 and for 15-OH PGDH, sheep anti-rabbit IgG antibodies conjugated with CY3, for co-visualization of α–actin. Tissue sections were analyzed using a fluorescent Optiphot-2 microscope with a green filter for visualization of FITC and a red filter for CY3. Digital photographs were taken, transferred into a computerized image-analyzing program and superimposed for comparison of the localization of COX-2 or 15-OH PGDH with that of α-actin. When identical pattern the enzyme is present in cells expressing α-actin.

3.3.10 Assessment and induction of cervical ripening (IV)

Cervical status was assessed employing a modified Bishop´s score. In women with unfavourable scores (0-5), prostaglandin E_2 in gel form (Cerviprost (0.5mg ic), Organon or Minprostin (2 mg vaginal gel), Pharmacia was applied locally while women with a favourable cervical score received an intravenous infusion of oxytocin, according to a schedule described previously (Ekman *et al.* 1983a), (Ekman *et al.* 1983e). Induction was considered to have failed if after four applications of gel, followed by therapeutic rupture of the membranes, and intravenous infusion of oxytocin, labour involving regular contractions for 4 hours (3 contractions 60 seconds in duration every 10 minutes was not established. The obstetric end-points monitored were the number of PgE_2 applications, instrumental delivery, unsuccessful induction and postpartal bleeding. The perinatal end-points were the number of operative deliveries motivated for foetal distress (ODFD), Apgar scores of <7 five minutes after delivery and requirement for observation in a neonatal intensive care unit (NICU). These data are documented in Table 2 in paper IV.

3.4 STATISTICAL ANALYSIS

In the case of normally distributed data, Student´s *t* test or the Mann-Whitney U-test were utilized for comparison of two different groups. Data from several groups were analyzed by One-Way Analysis of Variance (ANOVA), with the Tukey or Bonferroni post-hoc tests.

With data that were not normally distributed, the Kruskal-Wallis One-Way ANOVA, followed by Dunn's test was applied. The Chi-square test and Odds ratios were used to analyze associations between groups and outcome variables. A p-value of <0.05 was considered to be statistically significant.

4 RESULTS

None of the women included in study I-III showed clinical or laboratory signs of infection before or after labour.

4.1 THE LEVEL OF EXPRESSION OF PGDH IS DECREASED DURING LABOUR (I)

Study I demonstrates that both term and preterm cervical ripening may be associated with a decreased rate of prostaglandin degradation and, thus elevated tissue levels of biologically active prostaglandin. The cervical level of PGDH mRNA in the two groups of women delivered vaginally was significantly reduced in comparison to the two groups delivered by C/S, without labour, irrespective of the length of gestation (Figure 9). In contrast the cervical level of COX-2 mRNA was similar in all four groups. COX-2 and PGDH were shown histochemically to be expressed by activated fibroblasts (Figures 2&3 in paper I).

Figure 9. Northern blotting of 15-OH PGDH mRNA in the human cervix. Samples associated with preterm elective cesarean section (C/S; n=8) and preterm spontaneous vaginal delivery (S.V.D.; n=16). 1B: Term elective cesarean section (C/S; n=11) and term spontaneous vaginal delivery (S.V.D.; n= 11). The histogram illustrates the ratio (mean ± S.E.M.) of the relative optical densities (R.O.D.) of the hybridization signals for 15-OH PGDH mRNA and 18s rRNA. *$p<0.05$.

4.2 NO MRNA INCREASED AT PRETERM COMPARED TO TERM LABOUR (II)

Employing Real-Time multiplex RT-PCR analysis and immunohistochemistry, respectively, the presence of mRNA encoding for all three isomers of NO synthase and of the proteins themselves was detected in both the preterm and term cervix prior to and after onset of labour. The most prominent findings were that the levels of the mRNA´s for all three isomers were elevated in connection with preterm labour in comparison to term labour and that the level of expression of eNOS mRNA was significantly higher in the preterm group experiencing labour than in the other three groups. At the same time, in the women not in labour, irrespective of gestational age, the cervical level of eNOS mRNA was significantly lower than in those undergoing labour, indicating a role for this enzyme in the very final stages of cervical ripening (Figure 10).

In contrast to these findings on mRNA levels, immunohistochemical staining revealed no clear differences between preterm and term patients, bNOS exhibited the most pronounced staining, which was widely distributed in the stroma, the glandular epithelium and the basal membrane of the squamous epithelium. In all groups staining for eNOS was localized to the endothelium. iNOS was distributed primarily and diffusely in the stroma and in the epithelium (figure 11).

These results indicate that NO plays a specific role in preterm cervical ripening and labour a role which this mediator does not play at term.

4.3 IL-6, IL-8 AND MCP-1 PROTEIN AND MRNA INCREASES AT LABOUR (III)

The levels of the IL-6, IL-8, MCP-1, TNF-α and RANTES proteins and of IL-8, MCP-1 and RANTES mRNA in non-infected preterm cervical tissue were determined here for the first time. No significant differences related to gestational age were observed, nor were there any significant differences between the groups with respect to TNF-α or RANTES protein or mRNA. However, the expression of IL-6, IL-8 and MCP-1 was higher in women undergoing labour (the PTL and TL groups) than those not in labour (PTnotL and TnotL).

Figure 10. Expression of NOS mRNA normalized to the 'Preterm labour' group. Common superscripts indicate no significant differences.The number of patients analyzed in each group are marked in each bar in the bar chart.**The groups are**: **I:** Preterm labour, (**PTL**), **II:** Term labour (**TL**), **III**: Preterm non labour, (**PTnotL**), **IV:** Term non labour, (**TnotL**). **10A: Expression of bNOS mRNA :** Women who delivered preterm had generally higher bNOS mRNA levels compared to those who delivered at term, reaching significance in the labour group (p= 0,007). The lowest values were seen in those who were in labour at term. f patients analyzed in each group are marked in each bar in the bar chart. **10B: Expression of eNOS mRNA:** Significantly higher levels of eNOS mRNA were registered in women with preterm labour compared to term labour (p= 0,009). Women not in labour had significantly lower eNOS mRNA levels compared to preterm labour (p<0,001*)* or term labor (p=0,048). **10C:Expression of iNOS mRNA:** Patients who delivered preterm had higher iNOS mRNA levels compared to those who delivered at term. This relationship reached significance for those who were in labour (p<0,002).

Figure 11. Immunohistochemical localization of nitric oxide synthases in human cervix; **(a)** inducible nitric oxide (iNOS) localized to the stroma in preterm labour **(b)** iNOS localized to the squamous epithelium in preterm non labour patient. In each of the biopsies iNOS localized to the stroma and the epithelium. **(c)** Endothelial nitric oxide (eNOS) localized to the vascular endothelium in all biopsies. This is collected from a woman in preterm labour. Neuronal nitric oxide (bNOS) had a distinct staining and was generally localized to the; **(d)** basal membrane of the squamous epithelium, picture from a women in preterm labour, **(e)** the stroma, biopsy from a term labour patient, **(f)** the cervical glands, in this sample from a term labour patient. Original magnification x200, scale bar 50μm.

Employing ELISA and the Immulite procedure the level of IL-6 protein was found to be significantly higher in the PTL and TL groups than in the PTnotL ($p=0.02$ and $p<0.001$, respectively), and TnotL ($p=0.002$ and $p<0.001$, respectively) groups (Figure 1 in paper III). The median level of this protein was elevated 67-fold in the women undergoing labour compared to those not in labour (the median for the PTL and TL groups = 5.9 pg/mg protein and for the PTnotL and TnotL groups = 397 pg/mg protein) **(**Fig 12**)**.

The level of IL-8 protein was significantly higher in the TL group than in the PTnotL and TnotL groups ($p<0.01$). Although a similar trend was noted in the preterm groups, the difference was not statistically significant in this case (Figure 2a in paper III) (Fig 12).

The level of the MCP-1 protein was significantly higher in the PTL than in the TnotL group ($p=0.02$), and non-significantly higher than in the PTnotL group. For the TL group, this level was significantly higher than in both groups not in labour (Fig.12).

The patterns of expression of IL-8 and MCP-1, as determined by RT-PCR, were in accordance with the protein levels. Thus, the levels of these RNA species were significantly higher in both groups undergoing labour than in both groups not in labour.

Figure 12. Box and whisker plots representing the protein concentration of IL-6, IL-8 and MCP-1 (picograms/mg of total protein) and the expression of the IL-8 and MCP-1 mRNA (expressed as the product/28S intensity ratio) in the cervical tissue of the study groups. The groups are: preterm labour (PTL), term labour (TL), preterm not in labour (PTnotL), term not in labour (TnotL). The number of patients analysed in each group is marked in each bar in the bar chart. The box represents median value with 25%-75% of all data falling within the box. The whiskers extend to the non-outlier range. Outliers are marked as circles. Significant differences between the groups are shown above the box plots.

4.4 NUMBERS OF WBC AND LEVELS OF CRP ARE ENHANCED DURING LABOUR (III)

Interestingly, significantly higher serum levels of two indicators of inflammation, i.e., WBC, ($p<0.001$) and CRP, ($p=0.0221$), were present in the groups undergoing labour compared to those not undergoing labour. As in the case of the cytokine levels, there were no significant differences in WBC count (10^9/l) or serum level of CRP (mg/l) between the preterm and term groups.

4.5 PRETERM LABOUR INDUCTION BY PGE_2 EQUALLY SUCCESSFUL AS AT TERM (IV)

Ours is one of few reports on induction of cervical ripening and labour in connection with preterm birth. The mean cervical score was significantly lower in the preterm group (median +/- S.D = 2.0 +/-1.65), than in the term (3.0 +/_1.35) and postterm (3.0 +/-1.52, p<00.1) groups. The number of PgE_2 applications required did not differ significantly between the groups. Although, the duration of active labour (defined as the time elapsing from 3 cm of dilation to partus) in the preterm group (3 hours) was shorter, than in the term (4.5 hours) and postterm (5 hours) groups, neither of these differences was statistically significant.

There were no differences in the mode of delivery and prolonged labour was considered to be an indication for instrumental delivery only among term and postterm women. The success rate for vaginal delivery was 84%. Myometrial hypercontractility was not observed in any of the woman. The risk for heavy postpartum bleeding (>1000 ml) in the postterm group was almost five-fold higher than among the preterm women (P<0.05; OR=4,8; 95% CI=1.2-18.7) and four-fold higher than for the term women (P<0.05; OR=3.8; 95% CI=1.1-13). Excluding IUFD, the incidence of low Apgar scores was similar in the three groups.

5 DISCUSSION

The frequency o PTB, which is still the leading worldwide cause of neonatal morbidity and mortality, remains high and an estimated 75% of the cases are spontaneous (Goldenberg et al. 1998), (Iams 2003). Our incomplete understanding of the biological and pathophysiological mechanisms underlying a preterm cervical ripening and onset of labour is the major obstacle to preventing PTB, which is most likely to be a multi-factorial disease (Wang et al. 2001b).

The cervix and uterus should be considered as two independent parts of the same organ, which, under ideal conditions in connection with human parturition at term function synergistically to allow successful delivery of the infant. As important as cervical competence is in maintaining pregnancy, cervical ripening and remodelling at the onset of labour are equally important in assuring a well-coordinated and atraumatic delivery. The timing of these events is of the outmost significance, since premature cervical ripening may lead to PTB with an increased risk for mortality and morbidity for the child, while, alternatively, failure to ripe leads to a delay in the onset of labour, i.e., to postterm pregnancies, which are in turn associated with an increased frequency of Caesarean section (c/s) and asphyxia at birth (Chauhan et al. 2003; Smith et al. 1984). Indeed, appropriate cervical ripening is an absolute prerequisite for successful vaginal birth. Consequently, an improved understanding of the mechanisms underlying and the mediators regulating this remodelling of the cervical ECM and the onset of labour are absolutely necessary for more successful future identification, treatment and prevention of abnormal labour, preterm as well as at term.

The active biochemical processes involved in the cervical ripening at term consist of a complex cascade of sequential reactions, including degradation of the proteins and glycoproteins of the ECM, disruption of tightly aligned collagen fibrils, and an increase in hydration caused by hyaluronan (Junqueira et al. 1980; Uldbjerg et al. 1983b). Furthermore, these alterations during labour are associated with local invasion by white blood cells and activation of apoptosis, thus exhibiting several biochemical properties normally associated with an apyretic, inflammatory reaction. For these reasons, researchers in the field today generally agree that cervical ripening at term is an inflammatory process (Farina and Winkelman 2005), (Gibb 1998; Keelan et al. 2003). Complex interactions involving mutually supportive pathways, between cytokines,

prostaglandins and NO are believed to play a key role, with NO acting as the final mediator (Chwalisz and Garfield 1998; Facchinetti et al. 2005).

An extensively body of research concerning the pathways regulating preterm cervical ripening and labour has focused on the biochemical/biophysiological changes that occur in the placenta, gestational tissues, cervical secretion and amniotic fluid, but there have been very few investigations on the alterations occurring in human cervical tissue in this context. Although these tissues have different compositions, it is not unlikely that the events occurring in the cervix, are somewhat similar to those in these other tissues, because of their close proximity (Bennett et al. 2001; Granstrom et al. 1989; Hjelm et al. 2002; Keelan et al. 2003; Maul et al. 2003). Few, if any data concerning the changes in the preterm cervix and, in particular, the possible roles played by PGDH, cytokines and nitric oxide synthases in preterm cervical ripening are presently available, probably due to the difficulties involved in obtaining human cervical biopsies.

In the present investigation we examined exclusively women without signs of infection and made comparisons between preterm and term delivery with and without labour. We analyzed the levels of expression of the mRNA species encoding the key enzyme in the prostaglandin synthesis, COX-2; the central enzyme catabolizing/degrading prostaglandins, 15-OH prostaglandin dehydrogenase; the cytokines IL-8, MCP-1 and RANTES; as well as the isomers of NO synthase, bNOS, eNOS and iNOS. We also quantitated the cytokines listed above, and, in addition, IL-6 and TNF-α at the protein level and examined the distributions of the COX-2, PGDH and three NO synthase proteins immunohistochemically. In addition a maternal blood sample was analyzed for white blood cell count and level of CRP.

Of these parameters, only levels of mRNA encoding bNOS, eNOS and iNOS exhibited significant differences between preterm and term women in labour (Figure 10). Accordingly, the other mediators of concern here appear to play similar roles in the ripening process preterm and at term. Moreover, we also detected significantly increased WBC counts and serum levels of CRP, both indicators of inflammation, as well as elevated cervical levels of the pro-inflammatory cytokine proteins IL-6, IL-8 and MCP-1 and the corresponding mRNA´s in the groups in labour compared to those not in labour (Figure 13&14).

Figure 13. Box and whisker plots representing the protein concentration of IL-6, IL-8 and MCP-1 (picograms/mg of total protein) The two groups are: In labour (including preterm labour (PTL) and term labour (TL) and not in labour (including preterm not in labour (PTnotL) and term not in labour (TnotLabour). The number of patients analysed in each group is marked in each bar in the bar chart. The box represents median value with 25%-75% of all data falling within the box. The whiskers extend to the non-outlier range. Outliers are marked as circles. Significant differences between the groups are shown above the box plots.

Figure 14. Box and whisker plots representing (A) white blood cell count (10^9/l) (B) C- reactive protein (mg/l) in the blood of women in the study groups. The two groups are: In labour (including preterm labour (PTL) and term labour (TL) and not in labour (including preterm not in labour (PTnotL) and term not in labour (TnotLabour). The number of patients analysed in each group is marked in each bar in the bar chart. The box represents median value with 25%-75% of all data falling within the box. The whiskers extend to the non-outlier range. Outliers are marked as circles. Significant differences between the groups are shown above the box plots.

As an indicator of increased prostaglandin activity in the preterm cervix during labour, we could also demonstrate decreased cervical expression of the mRNA encoding the major prostaglandin-catabolizing enzyme, PGDH. Our clinically evaluation of induction of preterm cervical ripening and labour with locally applied PgE_2 is in line with these experimental findings. Therefore, on the basis of our present investigations and earlier research, we conclude cervical ripening both preterm and at term is associated with increased prostaglandin activity.

The decreased cervical level of PGDH mRNA observed here in labouring women, irrespective of the gestational age of the foetus, compared to those not in labour is in line with the reports of Giannoulias et al., on the level of the corresponding protein in myometrial samples from women in labour and not in labour, preterm as well as at term (Giannoulias *et al.* 2002), (Figure 9). We did not detect any changes in the expression of the COX-2 mRNA, which also is consistent with their results concerning COX-2 protein levels in these same groups of women.

For many years it has been almost generally agreed that a subclinical infection constitutes the underlying cause of the inflammatory response observed in tissues associated with gestation during PTB (Goldenberg et al. 2000; Romero et al. 2001). The closeness of the association between clinical infection and histological ammonitis increases as the gestational age of the foetus at the time of delivery decreases, especially prior to 30-32 weeks of gestation. Most PTB related to infection occurs before 30 weeks gestation and most of the bacteria detected in the genitourinary tract in association with spontaneous PTL involving intact membranes are vaginal organisms of low virulence, such as *Ureaplasma urealyticum*, *Mycoplasma hominis* and *Gardnerella vaginalis*. If ascending infection were the predominant cause, an increased frequency of infection-related PTB would be expected in the third trimester, when the cervix is normally less stiff and somewhat open compared to earlier stages in pregnancy (Iams 2003). Goldenberg et al. have even suggested that microorganisms associated with PTB may colonize the endometrial cavity prior to conception (Goldenberg et al. 2000).

The proposed explanation for the increased production of proinflammatory cytokines, prostaglandin and matrix-degrading enzymes and the influx of leukocytes

observed in gestational tissues in connection with PTB has thus been an undiagnosed subclinical infection in the lower genital tract. Here, our comparisons concerning preterm and term labour revealed no differences in the cytokine expression of either the cytokines IL-6, IL-8 and MCP-1, or prostaglandin dehydrogenase (PGDH). The cervical level of TNF-α, which is normally released in response to bacterial products, was the same with and without labour both preterm and term, as was expression of the mRNA encoding the key enzyme in the prostaglandin synthesis, COX-2. This result is in line with the report by Winkler et al. that no change in the level of TNF-α in biopsies from the lower uterine segment, could be detected during labour (Winkler 2003).

Il-6, a cytokine involved in the host response to infection is an important marker of inflammation and acute phase protein and was initially employed as an indicator of intrauterine infection (El-Bastawissi et al. 2000; Foulon et al. 1995; Saji et al. 2000; Splichal and Trebichavsky 2001). Earlier studies by Sennström et al., showed that normal labour at term is associated with significantly elevated levels of both IL-8 and IL-6 mRNA´s and proteins, compared to term delivery without labour (Sennstrom et al. 2000). This observation is in line with findings on the lower uterine segment at the time of parturition (Keelan et al. 2003.; Winkler 2003; Winkler et al. 1999; Winkler and Rath 1999). In addition, another potent chemoattractant, the cytokine MCP-1, is present at elevated levels in the amniotic fluid during normal term labour (Esplin et al. 2003).

Together with these other findings, the lack of any differences between preterm and term labour, documented here support our belief that spontaneous PTB involves an inflammatory process without bacterial infection. If subclinical infection were the cause of PTB, it would be difficult to explain why the same type of reactions can also be observed in connection with normal term labour. Furthermore, it is difficult to believe that the underlying cause of the inflammatory response seen at term, during normal labour, is a subclinical bacterial infection.

Instead of the inflammatory reaction seen in the cervix during preterm labour being a pathological process, this reaction may well be a normal physiological reaction of tissues to the need to adapt to the onset of labour (Thomson *et al.* 1999). Indeed, other physiological processes in the human body, such as menstruation, ovulation and implantation, exhibit similarities. Both the onset of menstrual bleeding and

implantation can be regarded as consequences of inflammatory-like processes involving cytokines, prostaglandins and activation of immunocompetent cells, with influx and activation of leukocytes and release of matrix-degrading proteins (MMP´s) (Finn 1986; Kelly 1994; Kelly et al. 2001; Salamonsen and Woolley 1999). The inflammatory mediators IL-8, MCP-1, and COX-2 are all expressed in the perivascular cells of the blood vessels of the endometrium and demonstrate enhanced immunohistochemical staining premenstrually (Jones et al. 1997). Cyclic variation in the level of the proinflammatory cytokine TNF-α and enhanced prostaglandin levels are also present in the endometrium (Cork et al. 2002).

Our present investigation did not reveal any differences in the cervical levels of cytokines analyzed between preterm and term labour, only differences between women in labour and not in labour (Figure 13). Recent studies have indicated that other cytokines may also be of interest in this context. For example, the anti-inflammatory cytokine IL-10 has been reported to be present in increased concentrations in amniotic fluid (AF) in women undergoing PTL with amnionitis (Dizon-Townson 2001). IL-10 is a potent inhibitor of the synthesis and the secretion of IL-6, IL-8, TNF-α and IL-12 from monocytes/macrophages and is thereby also believed to reduce prostaglandin synthesis.

IL-12, another pro-inflammatory cytokine, has been detected in elevated concentrations in amniotic fluid in connection with preterm as compared to term delivery (Lemancewicz et al. 2001). In addition, Pacora et al. reported increased levels of pro-inflammatory cytokine IL-18 in the AF of women exhibiting both PTL and microbial invasion (Pacora et al. 2000). Clearly, further research on the expression of these and other cytokines at term and preterm is of interest in connection with the mechanisms influencing the onset of labour.

With respect to our serum analyzes, the present findings suggest that elevated WBC counts and levels of CRP can be indicators of active labour as well as of infection (Figure 14). Although few studies have been concerned with these serum markers during pregnancy and labour, it is known that normal pregnancy is associated with a significantly higher WBC count than is observed in non-pregnant women (Kuhnert et al. 1998). Earlier investigations have provided conflicting results, showing elevated serum CRP levels during PTL in comparison to term labour, both in the absence and

presence of infection (Farb et al. 1983; Foulon et al. 1995; Torbe and Czajka 2004; Yoon et al. 2001).

The overall indication from our experimental investigations on cytokines, NO and PGDH that cervical prostaglandin activity is elevated during PTL is strengthened by the results of our clinical study. Unfortunately, our comparison of preterm and at term induction of cervical ripening and labour employing local application of PgE_2 or oxytocin i.v. was not performed in the most optimal manner. This was a retrospective study based on medical records, where the results can be expected to be somewhat less reliable, and with additional confounding factors/biases, than in the case of a prospective study. However, it would be extremely difficult, if at all possible, to perform such a prospective study, due to the difficulties involved in selection and assignment of women to the different groups. Moreover, it would be unacceptable to decide whether a preterm woman should be delivered by c/s or induced into labour on the basis of other than strictly medical considerations.

Another bias involved in the present study was the inclusion of eight women with intrauterine foetal deaths (IUFD) and four women carrying a child with lethal malformations. The physician is likely to be more aggressive in treating such to achieve vaginal delivery. However, exclusion of these women does not alter the results substantially, and the incidence of low Apgar scores (excluding the cases of IUFD), was similar in the three groups (Table 3 in paper IV).

The number of children admitted to the neonatal intensive care unit (NICU) was, for obvious reasons, larger for the PT group. The proportion of vaginal deliveries, approximately 84%, did not differ between the groups. The four-fold higher frequency of heavy postpartum bleeding in the postterm group compared to the term group emphasizes the high level of obstetrical risk associated with postterm pregnancy (Table 4 in paper IV).

An unripe cervix will inhibit normal labour and increase the risk for protracted labour, instrumental delivery and foetal distress. In the case of preterm births, where the health of the foetuses is often compromises, it is of outmost importance that induction of labour is performed in a manner such that the foetal stress is minimized. It is not evident that c/s is less traumatic for the preterm child than well-controlled induction of

cervical ripening and vaginal labour, at least in those cases, when immediate delivery is not required on the basis of foetal and/or maternal indications. Identification of a safe alternative to preterm c/s is therefore of high priority. In summary, the available data support the conclusion that induction of preterm cervical ripening and labour by local application of PgE_2 as a safe and effective alternative to c/s.

Only a few data on the possible role of NO in preterm ripening of the cervix are presently available. To the best of our knowledge, ours is the first study to examine mRNA levels and immunoreactivity in the preterm cervix. In attempt to overcome the problems involved with quantitating NO in the cervix, several analyses of cervical secretions have been performed instead. Thus, the levels of metabolites of NO in cervical fluid have been found to be elevated following cervical ripening and labour at term compared to the corresponding values for non-pregnant subjects; to be reduced in postterm compared to term pregnancy and to be enhanced in connection with PTL compared to term labour (Nakatsuka et al. 2000),(Facchinetti et al. 2005; Vaisanen-Tommiska et al. 2003; Vaisanen-Tommiska et al. 2004).

NO levels are increased by proinflammatory cytokines, oestrogen and additional factors. NO directly stimulates the activity of COX-2, thereby enhancing the rate of prostaglandin synthesis (Ledingham et al. 1999), (Salvemini et al. 1993). These interacting pathways activate the cascade of events involved in the inflammatory process leading to cervical ripening and induction of labour (Facchinetti et al. 2005; Maul et al. 2003). The underlying mechanisms are probably much more complex than we presently realize.

NO exerts quite different effects on the uterus and on the cervix. NO administered transdermally or i.v., as glyceryl trinitrate (GTN), reduces uterine contractility (and can therefore be used to inhibit threatening preterm labour) but does not affect the cervix. On the other hand, NO donors (i.e. isosorbidmononitrate (ISM), sodium nitroprusside (SNP)) applied vaginally or intracervically induce human cervical ripening and the onset of labour. These donors cause the same morphological alterations in cervical tissue as are seen in connection with spontaneous ripening at term (Ledingham *et al.* 2001; Thomson *et al.* 1997) (Ekerhovd et al. 2002; Piccinini et al. 2003). Thus, NO has been proposed to be the key mediator of preterm cervical ripening (Facchinetti et al. 2005), (Chwalisz and Garfield 1998). Our demonstration that the cervical expression of

mRNA encoding for all three NO isozymes of NO synthase is higher in women experiencing preterm labour than at term labour supports this hypothesis.

Why is higher expression of mediators of cervical remodelling required in connection with preterm parturition than at term? One explanation could be that when PTL begins, the collagen is more tightly cross-linked and the cervix stiffer and less ripened than in the case of normal term labour. Thus, achievement of the same morphological changes and finally, of a fully ripened cervix, requires higher levels of mediators of ECM degradation.

Unlike the cytokines analyzed and PGDH, the cervical levels of bNOS and iNOS mRNA did not differ between labour and non-labour, in other words between ripe and unripe cervices. Only the level of eNOS mRNA increased significantly during transition from the unripe to the cervical status favourable for labour. This observation indicates that eNOS plays a crucial role in the very final stages of ripening, just prior to the onset of labour, both term and preterm.

Earlier studies on eNOS trafficking in the cell have revealed that the inactive form of this enzyme is normally bound to the plasma membrane in a quiescent state. Upon activation by agonists, eNOS is translocated from the plasma membrane to the cytoplasmatic compartment of the cell. Proteins such as NOSTRIN, NOSIP, P13 kinase and HSP90 have been shown to be important in regulation of this activation, although it is still not known exactly how these proteins regulate eNOS trafficking. For example, oestradiol activates eNOS via the P13 kinase pathway (Ortiz and Garvin 2003), (Gorodeski 2000a; Gorodeski 2000b; Gorodeski 2000c; Gorodeski and Haens 2003). Thus, the localisation of eNOS can potentiate or attenuate its physiological effects, so that this enzyme does not need to be present in high amounts in a tissue before its activity is required. However, this is simply a hypothesis, since we analyzed the mRNA expression and not the protein levels.

Interestingly, immunohistochemical staining for NOS in the cervix was most intense for bNOS. Perhaps bNOS is involved in the process underlying neurogenic inflammation?

Recently, cervical ripening has been discussed in terms of a neurogenic inflammation. It is well known that almost complete denervation of the corpus uteri occurs during pregnancy and parturition (Bryman et al. 1987), (Morizaki et al. 1989), (Alm et al. 1988). On the other hand, the cervix is densely innervated, throughout pregnancy and labour and at term rich peptidergic innervation of this tissue has been demonstated (Stjernholm et al. 2000), (Collins et al. 2002), (Collins et al. 2002), (Bryman et al. 1987), (Stjernholm et al. 1999).

The peripheral nervous system has been shown to be actively involved in the initiation and control of inflammatory processes (Yellon et al. 2003). The distal ends of afferent peptidergic nerve fibres can participate in cell communicating in such a way as to give rise to a proinflammatory process. These extremely thin nerve endings (0.2 -1 μm) contain noiciceptors which are activated by mechanical, chemical and/or heat stimuli associated with tissue injury.

Neuropeptides such as Substance P (SP) secreted by the neuronal cells into synovial joints directly stimulate prostaglandin production and activate immunomodulatory cells, including macrophages and lymphocytes. These effects initiate in turn the inflammatory cascade resulting in the production of cytokines and other pro-inflammatory substances. Furthermore, this inflammatory process activates the noiciceptors and elevates the production of substances, such as prostaglandins, that enhances the sensibility, of these receptors in a process referred to as sensitisation (Hansson 1998).

One way to interrupt this neuronally induced inflammatory process is to reduce the release of neuropeptides from the free nerve endings. Both endogenous opiods and opiods administered peripherally, such as morphine, attenuate the inflammatory process in this manner. In addition, by activating receptors on lymphocytes, CRH and certain cytokines can stimulate the release of endogenously produced opiods and thereby help to diminish inflammation (Hansson 1998). Animals' studies on rodents have revealed the presence of neurons expressing NOS in the reproductive tract, most abundantly in the cervix, with clusters in the ganglion of Frankenhäuser. Pressure exerted on these ganglions by the head of the foetus during parturition stimulates uterine contractions and promotes labour (Buhimschi et al. 1996).

The mechanisms initiating the onset of cervical ripening and labour at term are still poorly understood, despite decades of research. If no underlying bacterial infection evokes the onset of preterm labour, what, then, is the mechanism involved? Throughout the years the involvement of a foetal signal, a paternal signal relayed in some way by the foetus, a reaction of the mothers´ immune system to the foetus and other factors has been proposed, but none of these has been confirmed.

The more recent development of molecular biological techniques, have made it possible to investigate the hypothesis that certain women have a genetic predisposition to deliver preterm on a molecular level (Wang et al. 2001b), (Dizon-Townson 2001), (Macones et al. 2004; Nguyen et al. 2004; Romero et al. 2002b). The challenge with these new techniques is, on the basis of the massive amount of information acquired, to decide which genes to investigate further in detail, and how to demonstrate their possible significance in the physiology of parturition. Most likely, any genetic predisposition for PTB is not the result of a perturbation in a single gene. Genes encoding oxytocin or its receptor, mutations resulting in inadequate or defective collagen synthesis, giving rise to ECM disorders, and other as yet unidentified genes may all predispose to PTB (Dizon-Townson 2001). In this context ethnic variations in genotype and/or allele frequencies may exist and should always be considered.

Polymorphisms in several of the genes regulating cytokine production have been described. Such polymorphisms are can be associated with increased susceptibility to certain infectious diseases and increased severity of autoimmune disease (Simhan et al. 2003), (Wilson et al. 1995). For instance genetic hyper-immune responsiveness to an infectious agent (e.g., *Group B-streptococci*) may result in overproduction of inflammatory cytokines such as TNF-α and IL-1 (Macones et al. 2004), (Dizon-Townson 2001).

Maternal carriers of the rarer allele of the TNF-2 gene are at significantly increased risk for spontaneous PTB compared to those carrying the more common allele, especially when a bacterial vaginosis infection is present (Macones et al. 2004). Accordingly, Dizon-Townson raised the hypothesis that potential susceptibility genes are in fact epiphenomena – i. e., the activity of their protein product can be perturbed, but only after another factor, (e.g., infection) has already set into motion the cascade of events leading to preterm cervical ripening and onset of labour (Dizon-Townson 2001).

If true this might explain why our study women did not reveal any differences in the levels of TNF-α.

There are dramatic racial differences in the distributions of allelic variants. PTB is significantly more common among black Afroamerican women, irrespective of socioeconomic status or educational level. Genetic regulation of IL-6 production is known to influence susceptibility to human diseases, especially those of an infectious or inflammatory nature. Promotor-174 polymorphism in the IL-6 gene is associated with PTB, being less frequent among women undergoing PTB after less than 34 weeks of gestation. Possession of this allelic variant has been shown to provide protection against immunological rejection, probably because less IL-6 is produced. The frequency of this mutation in the IL-6 gene in Afroamerican women is dramatically less than among caucasian women (Simhan et al. 2003). Our study did not reveal any differences in the production of IL-6 by women in preterm and term labour. Could this be due to the fact that we included only women without infections?

Today, any key role for prostaglandins in the cervical ripening is being questioned, since the underlying mechanisms appear to be more complex than previously believed (Kelly 1994). Cervical remodelling occurs prior to labour and independently of uterine contractions or increases in prostaglandin levels. Moreover, reduction of the prostaglandin synthesis, with COX inhibitors does not block block antiprogestin-induced (RU 486, Mifepristone) cervical ripening (Garfield et al. 1998) . There may be more important factors and indeed, our present study supports the hypothesis that NO is the key mediator of final cervical ripening.

In summary, our study has shed new light on the mechanisms underlying initiation of preterm cervical ripening and onset of labour. As is the case at term, preterm cervical ripening appears to involve an inflammatory reaction, as reflected in cervical levels of PGDH, COX-2 and the pro-inflammatory cytokines IL-6, IL-8, MCP-1, RANTES and TNF-α, as well as in serum indicators of inflammation; the level of CRP and WBC count, which may reflect the local changes in the cervix. We believe that this inflammation may be a normal physiological adaptation to the onset of labour. Furthermore, our findings, especially with respect to eNOS indicate that NO has a special role to play in preterm cervical ripening, a role which it does not play at term.

These findings may also be relevant to postterm pregnancy and abnormal labour, since alterations in the same mediators that induce PTL, may inhibit normal labour and lead to postterm pregnancy.

6 CONCLUSIONS

- A crucial role of NO in the preterm cervical ripening seems to exist and supports the role of NO as the final mediator of the preterm cervical ripening process.

- The process of preterm cervical ripening and remodelling appear to resemble those occurring at term.

- The inflammation observed may well be a normal physiological adaptation to the onset of labour.

- The increases in the serum of white blood cell count and levels of C- reactive protein associated with labour, irrespective of the gestational age of the foetus, may in the future prove to be valuable in monitoring active labour work.

- Local application of prostaglandin is as effective and safe a procedure for inducing preterm labour as it is for inducing term labour.

- The frequency of extensive postpartum bleeding (>1000ml) is four-fold higher in connection with postterm pregnancy than with normal term labour.

7 FUTURE PERSPECTIVES

These studies shed new light on the highly complicated physiological processes regulating spontaneous preterm cervical ripening and onset of labour in the absence of infection. In summary, the available data support the hypothesis that preterm ripening is an inflammatory process, as is cervical ripening at term. In my opinion, the most interesting differences observed here are those in cervical levels of NO and serum WBC counts and levels of CRP.

The expression of the different isozymes of NO synthase and their involvement in preterm cervical ripening has to date been largely unexplored. Our findings that the levels of the mRNA ´s encoding all three isozymes are significantly increased in connection with preterm, in comparison to term labour, as well as that the level of eNOS mRNA is significantly lower in connection with non-labour than to labour, motivates complementary studies on the levels of the NOS proteins themselves. The intense immunohistochemical staining for bNOS, indicative of a coupling to neurogenic inflammation, also requires further investigations.

The clear differences in serum white blood cell counts and the level of C-reactive protein between women undergoing and not undergoing labour were striking and somewhat unexpected. It would be of great value to perform a randomised prospective study designed to compare these parameters in women giving birth preterm and term, with and without labour, both before and after partus. In addition validating our present findings, such a study could hopefully contribute to improved prediction and treatment of threatening preterm labour.

8 REFERENCES

Abrahams C, Katz M. 2002. A perspective on the diagnosis of preterm labor. J Perinat Neonatal Nurs 16(1):1-11.

Alm P, Lundberg LM, Wharton J, Polak JM. 1988. Ontogenetic development of the guinea pig uterine innervation. An immunohistochemical study of different neuronal markers, neuropeptides and S-100 protein. Histochemistry 90(1):19-24.

Aronica SM, Katzenellenbogen BS. 1993. Stimulation of estrogen receptor-mediated transcription and alteration in the phosphorylation state of the rat uterine estrogen receptor by estrogen, cyclic adenosine monophosphate, and insulin-like growth factor-I. Mol Endocrinol 7(6):743-52.

Athayde N, Romero R, Maymon E, Gomez R, Pacora P, Araneda H, Yoon BH. 1999. A role for the novel cytokine RANTES in pregnancy and parturition. Am J Obstet Gynecol 181(4):989-94.

Bao S, Rai J, Schreiber J. 2001. Brain nitric oxide synthase expression is enhanced in the human cervix in labor. J Soc Gynecol Investig 8(3):158-64.

Barclay CG, Brennand JE, Kelly RW, Calder AA. 1993. Interleukin-8 production by the human cervix. Am J Obstet Gynecol 169(3):625-32.

Bennett P, Allport V, Loudon J, Elliott C. 2001. Prostaglandins, the fetal membranes and the cervix. Front Horm Res 27:147-64.

Berkowitz GS, Lapinski RH, Lockwood CJ, Florio P, Blackmore-Prince C, Petraglia F. 1996. Corticotropin-releasing factor and its binding protein: maternal serum levels in term and preterm deliveries. Am J Obstet Gynecol 174(5):1477-83.

Black S, Kushner I, Samols D. 2004. C-reactive Protein. J Biol Chem 279(47):48487-90.

Bryman I, Norstrom A, Dahlstrom A, Lindblom B. 1987. Immunohistochemical evidence for preserved innervation of the human cervix during pregnancy. Gynecol Obstet Invest 24(2):73-9.

Buhimschi I, Ali M, Jain V, Chwalisz K, Garfield RE. 1996. Differential regulation of nitric oxide in the rat uterus and cervix during pregnancy and labour. Hum Reprod 11(8):1755-66.

Calder AA, Embrey MP. 1975. Comparison of intravenous oxytocin and prostaglandin E2 for induction of labour using automatic and non-automatic infusion techniques. Br J Obstet Gynaecol 82(9):728-33.

Calder AA, Embrey MP, Tait T. 1977. Ripening of the cervix with extra-amniotic prostaglandin E2 in viscous gel before induction of labour. Br J Obstet Gynaecol 84(4):264-8.

Casey ML, Cox SM, Beutler B, Milewich L, MacDonald PC. 1989. Cachectin/tumor necrosis factor-alpha formation in human decidua. Potential role of cytokines in infection-induced preterm labor. J Clin Invest 83(2):430-6.

Challis JR, Lye SJ, Gibb W. 1997. Prostaglandins and parturition. Ann N Y Acad Sci 828:254-67.

Challis JR, Lye SJ, Gibb W, Whittle W, Patel F, Alfaidy N. 2001. Understanding preterm labor. Ann N Y Acad Sci 943:225-34.

Challis JR, Sloboda DM, Alfaidy N, Lye SJ, Gibb W, Patel FA, Whittle WL, Newnham JP. 2002. Prostaglandins and mechanisms of preterm birth. Reproduction 124(1):1-17.

Challis JR, Smith SK. 2001. Fetal endocrine signals and preterm labor. Biol Neonate 79(3-4):163-7.

Challis JRG. 2000. Mechanism of parturition and preterm labor. Obstet Gynecol Surv 55(10):650-60.

Chatziantoniou C, Boffa JJ, Ardaillou R, Dussaule JC. 1998. Nitric oxide inhibition induces early activation of type I collagen gene in renal resistance vessels and glomeruli in transgenic mice. Role of endothelin. J Clin Invest 101(12):2780-9.

Chauhan SP, Magann EF, Scott JR, Scardo JA, Hendrix NW, Martin JN, Jr. 2003. Cesarean delivery for fetal distress: rate and risk factors. Obstet Gynecol Surv 58(5):337-50.
Chwalisz K, Garfield RE. 1997. Regulation of the uterus and cervix during pregnancy and labor. Role of progesterone and nitric oxide. Ann N Y Acad Sci 828:238-53.
Chwalisz K, Garfield RE. 1998. Nitric oxide as the final metabolic mediator of cervical ripening. Hum Reprod 13(2):245-8.
Collins JJ, Usip S, McCarson KE, Papka RE. 2002. Sensory nerves and neuropeptides in uterine cervical ripening. Peptides 23(1):167-83.
Copper RL, Goldenberg RL, Das A, Elder N, Swain M, Norman G, Ramsey R, Cotroneo P, Collins BA, Johnson F et al. 1996. The preterm prediction study: maternal stress is associated with spontaneous preterm birth at less than thirty-five weeks' gestation. National Institute of Child Health and Human Development Maternal-Fetal Medicine Units Network. Am J Obstet Gynecol 175(5):1286-92.
Cork BA, Tuckerman EM, Li TC, Laird SM. 2002. Expression of interleukin (IL)-11 receptor by the human endometrium in vivo and effects of IL-11, IL-6 and LIF on the production of MMP and cytokines by human endometrial cells in vitro. Mol Hum Reprod 8(9):841-8.
Creasy RK. 1991. Preventing preterm birth. N Engl J Med 325(10):727-9.
Csapo AI, Knobil E, van der Molen HJ, Wiest WG. 1971. Peripheral plasma progesterone levels during human pregnancy and labor. Am J Obstet Gynecol 110(5):630-2.
Danforth DN. 1954. The distribution and functional activity of the cervical musculature. Am J Obstet Gynecol 68(5):1261-71.
Danforth DN, Veis A, Breen M, Weinstein HG, Buckingham JC, Manalo P. 1974. The effect of pregnancy and labor on the human cervix: changes in collagen, glycoproteins, and glycosaminoglycans. Am J Obstet Gynecol 120(5):641-51.
Denison FC, Calder AA, Kelly RW. 1999. The action of prostaglandin E2 on the human cervix: stimulation of interleukin 8 and inhibition of secretory leukocyte protease inhibitor. Am J Obstet Gynecol 180(3 Pt 1):614-20.
Denison FC, Riley SC, Elliott CL, Kelly RW, Calder AA, Critchley HO. 2000. The effect of mifepristone administration on leukocyte populations, matrix metalloproteinases and inflammatory mediators in the first trimester cervix. Mol Hum Reprod 6(6):541-8.
Dizon-Townson DS. 2001. Preterm labour and delivery: a genetic predisposition. Paediatr Perinat Epidemiol 15 Suppl 2:57-62.
Dudley DJ, Collmer D, Mitchell MD, Trautman MS. 1996. Inflammatory cytokine mRNA in human gestational tissues: implications for term and preterm labor. J Soc Gynecol Investig 3(6):328-35.
Ekerhovd E, Brannstrom M, Weijdegard B, Norstrom A. 2000. Nitric oxide synthases in the human cervix at term pregnancy and effects of nitric oxide on cervical smooth muscle contractility. Am J Obstet Gynecol 183(3):610-6.
Ekerhovd E, Weijdegard B, Brannstrom M, Mattsby-Baltzer I, Norstrom A. 2002. Nitric oxide induced cervical ripening in the human: Involvement of cyclic guanosine monophosphate, prostaglandin F(2 alpha), and prostaglandin E(2). Am J Obstet Gynecol 186(4):745-50.
Ekman G, Forman A, Marsal K, Ulmsten U. 1983a. Intravaginal versus intracervical application of prostaglandin E2 in viscous gel for cervical priming and induction of labor at term in patients with an unfavorable cervical state. Am J Obstet Gynecol 147(6):657-61.
Ekman G, Malmstrom A, Uldbjerg N, Ulmsten U. 1986. Cervical collagen: an important regulator of cervical function in term labor. Obstet Gynecol 67(5):633-6.
Ekman G, Perssen PH, Ulmsten U, Wingerup L. 1983b. 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 113:173-5.

Ekman G, Uldbjerg N, Malmstrom A, Ulmsten U. 1983c. Increased postpartum collagenolytic activity in cervical connective tissue from women treated with prostaglandin E2. Gynecol Obstet Invest 16(5):292-8.
Ekman G, Uldbjerg N, Wingerup L, Ulmsten U. 1983d. Intracervical instillation of PGE2-gel in patients with missed abortion or intrauterine fetal death. Arch Gynecol 233(4):241-5.
Ekman G, Ulmsten U, Wingerup L. 1983e. Intracervical application of PGE2-gel combined with early intravenous infusion of oxytocin for induction of term labor in women with unripe cervix. Arch Gynecol 234(1):61-5.
Ekman-Ordeberg G, Malmstrom A. 1998. [Remodelling of connective tissue of the cervix during pregnancy. An important process for labor onset and progress]. Lakartidningen 95(38):4097-100.
Ekman-Ordeberg G, Stjernholm Y, Wang H, Stygar D, Sahlin L. 2003. Endocrine regulation of cervical ripening in humans--potential roles for gonadal steroids and insulin-like growth factor-I. Steroids 68(10-13):837-47.
El-Bastawissi AY, Williams MA, Riley DE, Hitti J, Krieger JN. 2000. Amniotic fluid interleukin-6 and preterm delivery: a review. Obstet Gynecol 95(6 Pt 2):1056-64.
Esplin MS, Romero R, Chaiworapongsa T, Kim YM, Edwin S, Gomez R, Gonzalez R, Adashi EY. 2003. Amniotic fluid levels of immunoreactive monocyte chemotactic protein-1 increase during term parturition. J Matern Fetal Neonatal Med 14(1):51-6.
Facchinetti F, Venturini P, Blasi I, Giannella L. 2005. Changes in the cervical competence in preterm labour. Bjog 112 Suppl 1:23-7.
Farb HF, Arnesen M, Geistler P, Knox GE. 1983. C-reactive protein with premature rupture of membranes and premature labor. Obstet Gynecol 62(1):49-51.
Farina L, Winkelman C. 2005. A review of the role of proinflammatory cytokines in labor and noninfectious preterm labor. Biol Res Nurs 6(3):230-8.
Finn CA. 1986. Implantation, menstruation and inflammation. Biol Rev Camb Philos Soc 61(4):313-28.
Foulon W, Van Liedekerke D, Demanet C, Decatte L, Dewaele M, Naessens A. 1995. Markers of infection and their relationship to preterm delivery. Am J Perinatol 12(3):208-11.
Frydman R, Lelaidier C, Baton-Saint-Mleux C, Fernandez H, Vial M, Bourget P. 1992. Labor induction in women at term with mifepristone (RU 486): a double-blind, randomized, placebo-controlled study. Obstet Gynecol 80(6):972-5.
Garcia-Velasco JA, Arici A. 1999. Chemokines and human reproduction. Fertil Steril 71(6):983-93.
Garfield RE, Saade G, Buhimschi C, Buhimschi I, Shi L, Shi SQ, Chwalisz K. 1998. Control and assessment of the uterus and cervix during pregnancy and labour. Hum Reprod Update 4(5):673-95.
Giannoulias D, Patel FA, Holloway AC, Lye SJ, Tai HH, Challis JR. 2002. Differential changes in 15-hydroxyprostaglandin dehydrogenase and prostaglandin H synthase (types I and II) in human pregnant myometrium. J Clin Endocrinol Metab 87(3):1345-52.
Gibb W. 1998. The role of prostaglandins in human parturition. Ann Med 30(3):235-41.
Gibb W, Challis JR. 2002. Mechanisms of term and preterm birth. J Obstet Gynaecol Can 24(11):874-83.
Gibbs RS, Eschenbach DA. 1997. Use of antibiotics to prevent preterm birth. Am J Obstet Gynecol 177(2):375-80.
Goldenberg RL. 2002. The management of preterm labor. Obstet Gynecol 100(5 Pt 1):1020-37.
Goldenberg RL, Hauth JC, Andrews WW. 2000. Intrauterine infection and preterm delivery. N Engl J Med 342(20):1500-7.
Goldenberg RL, Iams JD, Mercer BM, Meis PJ, Moawad AH, Copper RL, Das A, Thom E, Johnson F, McNellis D et al. 1998. The preterm prediction study: the value of new vs standard risk factors in predicting early and all spontaneous preterm births. NICHD MFMU Network. Am J Public Health 88(2):233-8.

Gorodeski GI. 2000a. cGMP-dependent ADP depolymerization of actin mediates estrogen increase in cervical epithelial permeability. Am J Physiol Cell Physiol 279(6):C2028-36.
Gorodeski GI. 2000b. NO increases permeability of cultured human cervical epithelia by cGMP-mediated increase in G-actin. Am J Physiol Cell Physiol 278(5):C942-52.
Gorodeski GI. 2000c. Role of nitric oxide and cyclic guanosine 3',5'-monophosphate in the estrogen regulation of cervical epithelial permeability. Endocrinology 141(5):1658-66.
Gorodeski GI, Haens G. 2003. Nitric oxide regulation of permeability in human cervical and vaginal epithelial cells and in human endothelial cells. Curr Pharm Des 9(5):411-8.
Grammatopoulos D, Dai Y, Chen J, Karteris E, Papadopoulou N, Easton AJ, Hillhouse EW. 1998. Human corticotropin-releasing hormone receptor: differences in subtype expression between pregnant and nonpregnant myometria. J Clin Endocrinol Metab 83(7):2539-44.
Granstrom L, Ekman G, Ulmsten U. 1990. Myometrial activity after local application of prostaglandin E2 for cervical ripening and term labor induction. Am J Obstet Gynecol 162(3):691-4.
Granstrom L, Ekman G, Ulmsten U, Malmstrom A. 1989. Changes in the connective tissue of corpus and cervix uteri during ripening and labour in term pregnancy. Br J Obstet Gynaecol 96(10):1198-202.
Hagberg H, Mallard C, Jacobsson B. 2005. Role of cytokines in preterm labour and brain injury. Bjog 112 Suppl 1:16-8.
Hagberg H, Wennerholm UB. 2000. [Spontaneous premature birth: physiopathology, predictors and management. The frequency is constant--early detection can improve therapeutic possibilities]. Lakartidningen 97(4):301-6, 308-10.
Hansson P. 1998. Noiciceptiv och neurogen smärta. Uppkomstmekanismer och behandlingsstrategier. Pharmacia & Upjohn booklet.
Hertelendy F, Zakar T. 2004. Prostaglandins and the myometrium and cervix. Prostaglandins Leukot Essent Fatty Acids 70(2):207-22.
Higby K, Suiter CR. 1999. A risk-benefit assessment of therapies for premature labour. Drug Saf 21(1):35-56.
Hillhouse EW, Grammatopoulos D, Milton NG, Quartero HW. 1993. The identification of a human myometrial corticotropin-releasing hormone receptor that increases in affinity during pregnancy. J Clin Endocrinol Metab 76(3):736-41.
Hirst JJ, Teixeira FJ, Zakar T, Olson DM. 1995. Prostaglandin endoperoxide-H synthase-1 and -2 messenger ribonucleic acid levels in human amnion with spontaneous labor onset. J Clin Endocrinol Metab 80(2):517-23.
Hjelm AM, Barchan K, Malmstrom A, Ekman-Ordeberg GE. 2002. Changes of the uterine proteoglycan distribution at term pregnancy and during labour. Eur J Obstet Gynecol Reprod Biol 100(2):146-51.
Hjelm Cluff A, Malmstrom A, Tingaker B, David G, Ekman-Ordeberg G. 2005. Normal labor associated with changes in uterine heparan sulfate proteoglycan expression and localization. Acta Obstet Gynecol Scand 84(3):217-24.
Iams JD. 2003. The epidemiology of preterm birth. Clin Perinatol 30(4):651-64.
Ianaro A, O'Donnell CA, Di Rosa M, Liew FY. 1994. A nitric oxide synthase inhibitor reduces inflammation, down-regulates inflammatory cytokines and enhances interleukin-10 production in carrageenin-induced oedema in mice. Immunology 82(3):370-5.
Ignarro LJ, Buga GM, Wood KS, Byrns RE, Chaudhuri G. 1987. Endothelium-derived relaxing factor produced and released from artery and vein is nitric oxide. Proc Natl Acad Sci U S A 84(24):9265-9.
Jacobsson B, Mattsby-Baltzer I, Andersch B, Bokstrom H, Holst RM, Nikolaitchouk N, Wennerholm UB, Hagberg H. 2003. Microbial invasion and cytokine response in amniotic fluid in a Swedish population of women with preterm prelabor rupture of membranes. Acta Obstet Gynecol Scand 82(5):423-31.
Jones RL, Kelly RW, Critchley HO. 1997. Chemokine and cyclooxygenase-2 expression in human endometrium coincides with leukocyte accumulation. Hum Reprod 12(6):1300-6.

Junqueira LC, Zugaib M, Montes GS, Toledo OM, Krisztan RM, Shigihara KM. 1980. Morphologic and histochemical evidence for the occurrence of collagenolysis and for the role of neutrophilic polymorphonuclear leukocytes during cervical dilation. Am J Obstet Gynecol 138(3):273-81.
Kayem G, Dallot E, Ferre F, Cabrol D. 2003. Effect of amniotic fluid upon prostaglandin E2 and I2 production by cultured human myometrial cells. Eur J Obstet Gynecol Reprod Biol 108(2):152-6.
Kayisli UA, Mahutte NG, Arici A. 2002. Uterine chemokines in reproductive physiology and pathology. Am J Reprod Immunol 47(4):213-21.
Keelan JA, Blumenstein M, Helliwell RJ, Sato TA, Marvin KW, Mitchell MD. 2003. Cytokines, prostaglandins and parturition--a review. Placenta 24 Suppl A:S33-46.
Keelan JA, Sato T, Mitchell MD. 1997. Interleukin (IL)-6 and IL-8 production by human amnion: regulation by cytokines, growth factors, glucocorticoids, phorbol esters, and bacterial lipopolysaccharide. Biol Reprod 57(6):1438-44.
Keirse MJ. 1992. Therapeutic uses of prostaglandins. Baillieres Clin Obstet Gynaecol 6(4):787-808.
Keirse MJ. 1993. Prostaglandins in preinduction cervical ripening. Meta-analysis of worldwide clinical experience. J Reprod Med 38(1 Suppl):89-100.
Keirse MJ. 1995. New perspectives for the effective treatment of preterm labor. Am J Obstet Gynecol 173(2):618-28.
Keirse MJ, Turnbull AC. 1975. Metabolism of prostaglandins within the pregnant uterus. Br J Obstet Gynaecol 82(11):887-93.
Kelly RW. 1994. Pregnancy maintenance and parturition: the role of prostaglandin in manipulating the immune and inflammatory response. Endocr Rev 15(5):684-706.
Kelly RW. 2002. Inflammatory mediators and cervical ripening. J Reprod Immunol 57(1-2):217.
Kelly RW, King AE, Critchley HO. 2001. Cytokine control in human endometrium. Reproduction 121(1):3-19.
Kirschenbaum A, Liotta DR, Yao S, Liu XH, Klausner AP, Unger P, Shapiro E, Leav I, Levine AC. 2000. Immunohistochemical localization of cyclooxygenase-1 and cyclooxygenase-2 in the human fetal and adult male reproductive tracts. J Clin Endocrinol Metab 85(9):3436-41.
Kjellen L, Lindahl U. 1991. Proteoglycans: structures and interactions. Annu Rev Biochem 60:443-75.
Knowles RG, Moncada S. 1994. Nitric oxide synthases in mammals. Biochem J 298 (Pt 2):249-58.
Korita D, Itoh H, Sagawa N, Yura S, Yoshida M, Kakui K, Takemura M, Nuamah MA, Fujii S. 2004. Cyclic mechanical stretching and interleukin-1alpha synergistically up-regulate prostacyclin secretion in cultured human uterine myometrial cells. Gynecol Endocrinol 18(3):130-7.
Kornman L, Jacobs V, Hodgson RP, Godfrey J, Dunlevy L, Tyler JP, Baird PJ, Hudson CN. 1988. Chorioamnionitis: how useful is the determination of C-reactive protein? Aust N Z J Obstet Gynaecol 28(1):45-8.
Kovacs EJ, DiPietro LA. 1994. Fibrogenic cytokines and connective tissue production. Faseb J 8(11):854-61.
Kuhnert M, Strohmeier R, Stegmuller M, Halberstadt E. 1998. Changes in lymphocyte subsets during normal pregnancy. Eur J Obstet Gynecol Reprod Biol 76(2):147-51.
Kurki T. 1998. A survey of etiological mechanisms and therapy of preterm labor. Acta Obstet Gynecol Scand 77(2):137-41.
Ledingham MA, Denison FC, Kelly RW, Young A, Norman JE. 1999. Nitric oxide donors stimulate prostaglandin F(2alpha) and inhibit thromboxane B(2) production in the human cervix during the first trimester of pregnancy. Mol Hum Reprod 5(10):973-82.
Ledingham MA, Thomson AJ, Greer IA, Norman JE. 2000a. Nitric oxide in parturition. Bjog 107(5):581-93.
Ledingham MA, Thomson AJ, Lunan CB, Greer IA, Norman JE. 2001. A comparison of isosorbide mononitrate, misoprostol and combination therapy for first

trimester pre-operative cervical ripening: a randomised controlled trial. Bjog 108(3):276-80.
Ledingham MA, Thomson AJ, Young A, Macara LM, Greer IA, Norman JE. 2000b. Changes in the expression of nitric oxide synthase in the human uterine cervix during pregnancy and parturition. Mol Hum Reprod 6(11):1041-8.
Lemancewicz A, Urban R, Urban J, Skotnicki M, Kretowska M, Sierakowski S. 2001. Evaluation of interleukin concentrations in amniotic fluid in preterm and term parturition and in oligohydramnios. Med Sci Monit 7(5):924-7.
Liggins GC. 1978. Ripening of the cervix. Semin Perinatol 2(3):261-71.
MacLean MA, Wilson R, Thomson JA, Krishnamurthy S, Walker JJ. 1992. Immunological changes in normal pregnancy. Eur J Obstet Gynecol Reprod Biol 43(3):167-72.
Macones GA, Parry S, Elkousy M, Clothier B, Ural SH, Strauss JF, 3rd. 2004. A polymorphism in the promoter region of TNF and bacterial vaginosis: preliminary evidence of gene-environment interaction in the etiology of spontaneous preterm birth. Am J Obstet Gynecol 190(6):1504-8; discussion 3A.
Mahendroo MS, Porter A, Russell DW, Word RA. 1999. The parturition defect in steroid 5alpha-reductase type 1 knockout mice is due to impaired cervical ripening. Mol Endocrinol 13(6):981-92.
Marvin KW, Keelan JA, Eykholt RL, Sato TA, Mitchell MD. 2002. Use of cDNA arrays to generate differential expression profiles for inflammatory genes in human gestational membranes delivered at term and preterm. Mol Hum Reprod 8(4):399-408.
Maul H, Longo M, Saade GR, Garfield RE. 2003. Nitric oxide and its role during pregnancy: from ovulation to delivery. Curr Pharm Des 9(5):359-80.
Mitchell MD, Trautman MS, Dudley DJ. 1993. Cytokine networking in the placenta. Placenta 14(3):249-75.
Morizaki N, Morizaki J, Hayashi RH, Garfield RE. 1989. A functional and structural study of the innervation of the human uterus. Am J Obstet Gynecol 160(1):218-28.
Nakatsuka M, Habara T, Kamada Y, Tada K, Kudo T. 2000. Elevation of total nitrite and nitrate concentration in vaginal secretions as a predictor of premature delivery. Am J Obstet Gynecol 182(3):644-5.
Nguyen DP, Genc M, Vardhana S, Babula O, Onderdonk A, Witkin SS. 2004. Ethnic differences of polymorphisms in cytokine and innate immune system genes in pregnant women. Obstet Gynecol 104(2):293-300.
North RA, Whitehead R, Larkins RG. 1991. Stimulation by human chorionic gonadotropin of prostaglandin synthesis by early human placental tissue. J Clin Endocrinol Metab 73(1):60-70.
Ortiz PA, Garvin JL. 2003. Trafficking and activation of eNOS in epithelial cells. Acta Physiol Scand 179(2):107-14.
Osmers RG, Adelmann-Grill BC, Rath W, Stuhlsatz HW, Tschesche H, Kuhn W. 1995. Biochemical events in cervical ripening dilatation during pregnancy and parturition. J Obstet Gynaecol 21(2):185-94.
Pacora P, Romero R, Maymon E, Gervasi MT, Gomez R, Edwin SS, Yoon BH. 2000. Participation of the novel cytokine interleukin 18 in the host response to intra-amniotic infection. Am J Obstet Gynecol 183(5):1138-43.
Palmer RM, Rees DD, Ashton DS, Moncada S. 1988. L-arginine is the physiological precursor for the formation of nitric oxide in endothelium-dependent relaxation. Biochem Biophys Res Commun 153(3):1251-6.
Papanicolaou DA, Wilder RL, Manolagas SC, Chrousos GP. 1998. The pathophysiologic roles of interleukin-6 in human disease. Ann Intern Med 128(2):127-37.
Patel FA, Clifton VL, Chwalisz K, Challis JR. 1999. Steroid regulation of prostaglandin dehydrogenase activity and expression in human term placenta and chorio-decidua in relation to labor. J Clin Endocrinol Metab 84(1):291-9.
Piccinini F, Fano RA, Volpe A, Facchinetti F. 2003. Ripening of the cervix with sodium nitroprusside in nonpregnant women. J Soc Gynecol Investig 10(7):438-42.

Porter TF, Fraser AM, Hunter CY, Ward RH, Varner MW. 1997. The risk of preterm birth across generations. Obstet Gynecol 90(1):63-7.
Pschirrer ER, Monga M. 2000. Risk factors for preterm labor. Clin Obstet Gynecol 43(4):727-34.
Rath W, Winkler M, Kemp B. 1998. The importance of extracellular matrix in the induction of preterm delivery. J Perinat Med 26(6):437-41.
Robertson SA, Mayrhofer G, Seamark RF. 1996. Ovarian steroid hormones regulate granulocyte-macrophage colony-stimulating factor synthesis by uterine epithelial cells in the mouse. Biol Reprod 54(1):183-96.
Romero R, Chaiworapongsa T, Kuivaniemi H, Tromp G. 2004. Bacterial vaginosis, the inflammatory response and the risk of preterm birth: a role for genetic epidemiology in the prevention of preterm birth. Am J Obstet Gynecol 190(6):1509-19.
Romero R, Espinoza J, Chaiworapongsa T, Kalache K. 2002a. Infection and prematurity and the role of preventive strategies. Semin Neonatol 7(4):259-74.
Romero R, Gomez R, Chaiworapongsa T, Conoscenti G, Kim JC, Kim YM. 2001. The role of infection in preterm labour and delivery. Paediatr Perinat Epidemiol 15 Suppl 2:41-56.
Romero R, Kuivaniemi H, Tromp G. 2002b. Functional genomics and proteomics in term and preterm parturition. J Clin Endocrinol Metab 87(6):2431-4.
Romero R, Mazor M, Sepulveda W, Avila C, Copeland D, Williams J. 1992. Tumor necrosis factor in preterm and term labor. Am J Obstet Gynecol 166(5):1576-87.
Romero R, Oyarzun E, Mazor M, Sirtori M, Hobbins JC, Bracken M. 1989. Meta-analysis of the relationship between asymptomatic bacteriuria and preterm delivery/low birth weight. Obstet Gynecol 73(4):576-82.
Rorie DK, Newton M. 1967. Histologic and chemical studies of the smooth muscle in the human cervix and uterus. Am J Obstet Gynecol 99(4):466-9.
Ruiz RJ, Fullerton J, Dudley DJ. 2003. The interrelationship of maternal stress, endocrine factors and inflammation on gestational length. Obstet Gynecol Surv 58(6):415-28.
Saji F, Samejima Y, Kamiura S, Sawai K, Shimoya K, Kimura T. 2000. Cytokine production in chorioamnionitis. J Reprod Immunol 47(2):185-96.
Salamonsen LA, Woolley DE. 1999. Menstruation: induction by matrix metalloproteinases and inflammatory cells. J Reprod Immunol 44(1-2):1-27.
Salvemini D, Misko TP, Masferrer JL, Seibert K, Currie MG, Needleman P. 1993. Nitric oxide activates cyclooxygenase enzymes. Proc Natl Acad Sci U S A 90(15):7240-4.
Sanborn BM, Kuo HS, Held B. 1978. Estrogen and progestogen binding site concentrations in human endometrium and cervix throughout the menstrual cycle and in tissue from women taking oral contraceptives. J Steroid Biochem 9(10):951-5.
Sangha RK, Walton JC, Ensor CM, Tai HH, Challis JR. 1994. Immunohistochemical localization, messenger ribonucleic acid abundance, and activity of 15-hydroxyprostaglandin dehydrogenase in placenta and fetal membranes during term and preterm labor. J Clin Endocrinol Metab 78(4):982-9.
Schwalm H, Dubrauszky V. 1966. The structure of the musculature of the human uterus--muscles and connective tissue. Am J Obstet Gynecol 94(3):391-404.
Sennstrom M. 2000. Cervical Ripening - an inflammatory process involving cytokines, metalloproteinases adn foetal fibronectin. PhD thesis. [Medical thesis]. Stockholm: Karolinska Institute.
Sennstrom MB, Brauner A, Bystrom B, Malmstrom A, Ekman G. 2003. Matrix metalloproteinase-8 correlates with the cervical ripening process in humans. Acta Obstet Gynecol Scand 82(10):904-11.
Sennstrom MB, Ekman G, Westergren-Thorsson G, Malmstrom A, Bystrom B, Endresen U, Mlambo N, Norman M, Stabi B, Brauner A. 2000. Human cervical ripening, an inflammatory process mediated by cytokines. Mol Hum Reprod 6(4):375-81.

Sennstrom MK, Brauner A, Lu Y, Granstrom LM, Malmstrom AL, Ekman GE. 1997. Interleukin-8 is a mediator of the final cervical ripening in humans. Eur J Obstet Gynecol Reprod Biol 74(1):89-92.
Siegel I, Gleicher N. 1981. Peripheral white blood cell alterations in early labor. Diagn Gynecol Obstet 3(2):123-6.
Simhan HN, Krohn MA, Roberts JM, Zeevi A, Caritis SN. 2003. Interleukin-6 promoter -174 polymorphism and spontaneous preterm birth. Am J Obstet Gynecol 189(4):915-8.
Slattery MM, Morrison JJ. 2002. Preterm delivery. Lancet 360(9344):1489-97.
Smith LP, Nagourney BA, McLean FH, Usher RH. 1984. Hazards and benefits of elective induction of labor. Am J Obstet Gynecol 148(5):579-85.
Speroff L, Glass R, Kase N. 1989. Clinical gynecologic endocrinology and infertility. 4TH EDN. WILLIAMS& WILKINS, USA(4TH EDITION):317-350.
Splichal I, Trebichavsky I. 2001. Cytokines and other important inflammatory mediators in gestation and bacterial intraamniotic infections. Folia Microbiol (Praha) 46(4):345-51.
Steinborn A, Gunes H, Roddiger S, Halberstadt E. 1996. Elevated placental cytokine release, a process associated with preterm labor in the absence of intrauterine infection. Obstet Gynecol 88(4 Pt 1):534-9.
Stevens MY, Challis JR, Lye SJ. 1998. Corticotropin-releasing hormone receptor subtype 1 is significantly up-regulated at the time of labor in the human myometrium. J Clin Endocrinol Metab 83(11):4107-15.
Stjernholm Y, Sahlin L, Akerberg S, Elinder A, Eriksson HA, Malmstrom A, Ekman G. 1996. Cervical ripening in humans: potential roles of estrogen, progesterone, and insulin-like growth factor-I. Am J Obstet Gynecol 174(3):1065-71.
Stjernholm Y, Sahlin L, Malmstrom A, Barchan K, Eriksson HA, Ekman G. 1997. Potential roles for gonadal steroids and insulin-like growth factor I during final cervical ripening. Obstet Gynecol 90(3):375-80.
Stjernholm Y, Sennstrom M, Granstrom L, Ekman G, Johansson O. 1999. Protein gene product 9.5-immunoreactive nerve fibers and cells in human cervix of late pregnant, postpartal and non-pregnant women. Acta Obstet Gynecol Scand 78(4):299-304.
Stjernholm Y, Sennstrom M, Granstrom L, Ekman G, Liang Y, Johansson O. 2000. Neurochemical and cellular markers in human cervix of late pregnant, postpartal and non-pregnant women. Acta Obstet Gynecol Scand 79(7):528-37.
Stygar D, Wang H, Vladic YS, Ekman G, Eriksson H, Sahlin L. 2001. Co-localization of oestrogen receptor beta and leukocyte markers in the human cervix. Mol Hum Reprod 7(9):881-6.
Stygar D, Wang H, Vladic YS, Ekman G, Eriksson H, Sahlin L. 2002. Increased level of matrix metalloproteinases 2 and 9 in the ripening process of the human cervix. Biol Reprod 67(3):889-94.
Teixeira FJ, Zakar T, Hirst JJ, Guo F, Sadowsky DW, Machin G, Demianczuk N, Resch B, Olson DM. 1994. Prostaglandin endoperoxide-H synthase (PGHS) activity and immunoreactive PGHS-1 and PGHS-2 levels in human amnion throughout gestation, at term, and during labor. J Clin Endocrinol Metab 78(6):1396-402.
Thomson AJ, Lunan CB, Cameron AD, Cameron IT, Greer IA, Norman JE. 1997. Nitric oxide donors induce ripening of the human uterine cervix: a randomised controlled trial. Br J Obstet Gynaecol 104(9):1054-7.
Thomson AJ, Lunan CB, Ledingham M, Howat RC, Cameron IT, Greer IA, Norman JE. 1998. Randomised trial of nitric oxide donor versus prostaglandin for cervical ripening before first-trimester termination of pregnancy. Lancet 352(9134):1093-6.
Thomson AJ, Telfer JF, Young A, Campbell S, Stewart CJ, Cameron IT, Greer IA, Norman JE. 1999. Leukocytes infiltrate the myometrium during human parturition: further evidence that labour is an inflammatory process. Hum Reprod 14(1):229-36.
Torbe A, Czajka R. 2004. Proinflammatory cytokines and other indications of inflammation in cervico-vaginal secretions and preterm delivery. Int J Gynaecol Obstet 87(2):125-30.

Tschugguel W, Schneeberger C, Lass H, Stonek F, Zaghlula MB, Czerwenka K, Schatten C, Kaider A, Husslein P, Huber JC. 1999. Human cervical ripening is associated with an increase in cervical inducible nitric oxide synthase expression. Biol Reprod 60(6):1367-72.
Tucker J, McGuire W. 2004. Epidemiology of preterm birth. Bmj 329(7467):675-8.
Turnbull AC, Patten PT, Flint AP, Keirse MJ, Jeremy JY, Anderson AB. 1974. Significant fall in progesterone and rise in oestradiol levels in human peripheral plasma before onset of labour. Lancet 1(7848):101-3.
Uldbjerg N, Ekman G, Herltoft P, Malmstrom A, Ulmsten U, Wingerup L. 1983a. Human cervical connective tissue and its reaction to prostaglandin E2. Acta Obstet Gynecol Scand Suppl 113:163-6.
Uldbjerg N, Ekman G, Malmstrom A, Olsson K, Ulmsten U. 1983b. Ripening of the human uterine cervix related to changes in collagen, glycosaminoglycans, and collagenolytic activity. Am J Obstet Gynecol 147(6):662-6.
Uldbjerg N, Malmstrom A, Ekman G, Ulmsten U. 1985. The integrity of cervical collagen during pregnancy and labor. Gynecol Obstet Invest 20(2):68-73.
Uldbjerg N, Ulmsten U, Ekman G. 1983c. The ripening of the human uterine cervix in terms of connective tissue biochemistry. Clin Obstet Gynecol 26(1):14-26.
Ulmsten U, Wingerup L, Andersson KE. 1979. Comparison of prostaglandin E2 and intravenous oxytocin for induction of labor. Obstet Gynecol 54(5):581-4.
Vaisanen-Tommiska M, Nuutila M, Aittomaki K, Hiilesmaa V, Ylikorkala O. 2003. Nitric oxide metabolites in cervical fluid during pregnancy: further evidence for the role of cervical nitric oxide in cervical ripening. Am J Obstet Gynecol 188(3):779-85.
Vaisanen-Tommiska M, Nuutila M, Ylikorkala O. 2004. Cervical nitric oxide release in women postterm. Obstet Gynecol 103(4):657-62.
Van Meir CA, Ramirez MM, Matthews SG, Calder AA, Keirse MJ, Challis JR. 1997. Chorionic prostaglandin catabolism is decreased in the lower uterine segment with term labour. Placenta 18(2-3):109-14.
Wang H, Stjernholm Y, Ekman G, Eriksson H, Sahlin L. 2001a. Different regulation of oestrogen receptors alpha and beta in the human cervix at term pregnancy. Mol Hum Reprod 7(3):293-300.
Wang X, Zuckerman B, Kaufman G, Wise P, Hill M, Niu T, Ryan L, Wu D, Xu X. 2001b. Molecular epidemiology of preterm delivery: methodology and challenges. Paediatr Perinat Epidemiol 15 Suppl 2:63-77.
Westergren-Thorsson G, Norman M, Bjornsson S, Endresen U, Stjernholm Y, Ekman G, Malmstrom A. 1998. Differential expressions of mRNA for proteoglycans, collagens and transforming growth factor-beta in the human cervix during pregnancy and involution. Biochim Biophys Acta 1406(2):203-13.
Wilson AG, di Giovine FS, Duff GW. 1995. Genetics of tumour necrosis factor-alpha in autoimmune, infectious, and neoplastic diseases. J Inflamm 45(1):1-12.
Winkler M. 2003. Role of cytokines and other inflammatory mediators. BJOG 110 Suppl 20:118-23.
Winkler M, Fischer DC, Ruck P, Marx T, Kaiserling E, Oberpichler A, Tschesche H, Rath W. 1999. Parturition at term: parallel increases in interleukin-8 and proteinase concentrations and neutrophil count in the lower uterine segment. Hum Reprod 14(4):1096-100.
Winkler M, Kemp B, Fischer DC, Maul H, Hlubek M, Rath W. 2001. Tissue concentrations of cytokines in the lower uterine segment during preterm parturition. J Perinat Med 29(6):519-27.
Winkler M, Rath W. 1999. Changes in the cervical extracellular matrix during pregnancy and parturition. J Perinat Med 27(1):45-60.
Wiqvist I, Norstrom A, Wiqvist N. 1986. Induction of labor by intra-cervical PGE2 in viscous gel. Mechanism of action and clinical treatment routines. Acta Obstet Gynecol Scand 65(5):485-92.
Yellon SM, Mackler AM, Kirby MA. 2003. The role of leukocyte traffic and activation in parturition. J Soc Gynecol Investig 10(6):323-38.
Yoon BH, Romero R, Moon JB, Shim SS, Kim M, Kim G, Jun JK. 2001. Clinical significance of intra-amniotic inflammation in patients with preterm labor and intact membranes. Am J Obstet Gynecol 185(5):1130-6.

Yoshida M, Sagawa N, Itoh H, Yura S, Korita D, Kakui K, Hirota N, Sato T, Ito A, Fujii S. 2001. Nitric oxide increases matrix metalloproteinase-1 production in human uterine cervical fibroblast cells. Mol Hum Reprod 7(10):979-85.
Young A, Thomson AJ, Ledingham M, Jordan F, Greer IA, Norman JE. 2002. Immunolocalization of proinflammatory cytokines in myometrium, cervix, and fetal membranes during human parturition at term. Biol Reprod 66(2):445-9.
Young RC, Hession RO. 1999. Three-dimensional structure of the smooth muscle in the term-pregnant human uterus. Obstet Gynecol 93(1):94-9.
Zakar T, Olson DM, Teixeira FJ, Hirst JJ. 1996. Regulation of prostaglandin endoperoxide H2 synthase in term human gestational tissues. Acta Physiol Hung 84(2):109-18.