Uterine Remodeling During Pregnancy - Studies on the Effect of Heparin/Heparan Sulfate

Åkerud, Anna

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Anna Åkerud

Department of Experimental Medical Science, 2009

LUND UNIVERSITY
Faculty of Medicine

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To Daniel

Abstract

During pregnancy, the uterine tissues must go through a major transformation to be able to first hold the growing fetus and then, at term, expel it through the opened birth canal (cervix). It is a requirement for successful labor that there is adequate cervical softening combined with effective myometrial contractions. This is accomplished by an extensive remodeling of the extracellular matrix in both the cervix and the uterus, executed by different types of cells and mediators in a complex interplay. The overall purpose of this project was to investigate mechanisms controlling the remodeling of the uterus and cervix before parturition.

This thesis demonstrates that fibroblasts with an active pro-inflammatory phenotype are important for the transformation of the uterine cervix preceding labor. Moreover, heparan sulfate proteoglycans appear to be central for the remodeling of the cervix and uterus before parturition, as shown by the finding that heparin fragments can be used in a clinical setting to prevent protracted labor.

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Abbreviations

ECM	extracellular matrix
MMP	matrix metalloproteinase
FACIT	fibril-associated collagens with interrupted triple helices
GAG	glucosaminoglycan
TIMP	tissue inhibitor of metalloproteinase
TLR	Toll-like receptor
TIR	Toll/IL-1R homology domain
PAMP	pathogen-associated molecular pattern
LPS	lipopolysaccharide
MCP-1	monocyte chemotactic protein-1 (CCL2)
IL-8	interleukin-8 (CXCL8)
INF-γ	interferon gamma
α-SMA	α-smooth muscle actin
PBS	phosphate buffered saline

Introduction

The mechanisms and pathways of labor are still largely unknown. It is of great importance to investigate these mechanisms in order to be able to prevent preterm labor, prolonged pregnancies, and slow progress of labor. A dysfunctional and protracted labor is recognized as a major clinical problem in maternity clinics today, and it accounts for much of the maternal and neonatal morbidity[1]. A large proportion of all first-time deliveries in Scandinavia are protracted, and currently this is the most common obstetric problem in nulliparous women[1]. It has a negative effect on both the mother and the fetus, and results in increased numbers of instrumental deliveries. In the developed world, Caesarean sections for poor progress of labor contribute to at least a third of the overall rate of Caesarean sections[2-6].

A prerequisite for successful labor is adequate cervical ripening/softening combined with effective myometrial contractions. This is accomplished by an extensive remodeling of the extracellular matrix (ECM) in both the cervix and the uterus. Many factors including hormones, cytokines, matrix metalloproteinases (MMPs), and ECM components are known to regulate this remodeling.

The overall purpose of this project was to investigate the mechanisms controlling the remodeling of the uterus and cervix before parturition. In this thesis, I will discuss the importance of heparan sulfate – a proteoglycan found ubiquitously in tissues throughout the body – for uterine remodeling during pregnancy. I will also show that heparan sulfate can be used in a clinical setting to prevent protracted labor.

Background

Anatomy of the uterus

Corpus uteri

Anatomically, the corpus uteri can be divided into the upper region, called the fundus, and the distal region close to cervix, called the isthmus (Figure 1). The uterus is mainly composed of smooth muscle cells, which constitute about 70% of the tissue[7]. The uterus does not, however, only consist of smooth muscle cells: a substantial part of the tissue is made up of ECM. The ECM surrounds the muscle fibers, facilitating the cell-cell and cell-matrix communication that is crucial for achievement of effective contractions during labor[8]. Even though the proportion of muscle fibers is reduced gradually along the uterus[9], the isthmus is still dominated by smooth muscle cells, making it an anatomical part of the corpus uteri.

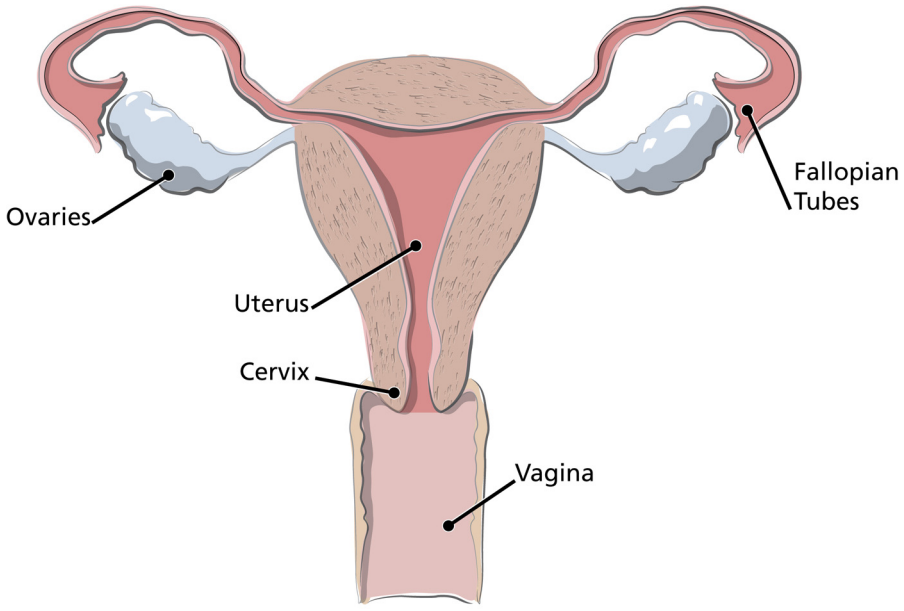


Figure 1. Anatomy of the human uterus.

Cervix uteri

Cervix means neck in Latin, and this is the small, cylindrical narrow part that leads from the uterus to the vagina (Figure 1). It has a vital function in pregnancy, where it must stay closed and firm during most of the pregnancy but must undergo extensive remodeling at term to allow passage of the fetus through the birth canal. The cervix is dominated by fibrous connective tissue, which gives it its tensile strength. More than 85% of the non-pregnant cervix consists of ECM, where collagens are the major component[7]. Only about 4-10% of the cervical tissue consists of smooth muscle cells[7].

Fetal membranes

The fetal membranes are the amnion and the chorion. The major ECM components that provide tensile strength and elastic recoil in the fetal membranes include collagens, elastin, fibronectin, and laminins. Collagen types I and III, together with small amounts of collagen types V, VI, and VII, provide the major tensile strength[10]. Proteoglycans, mainly biglycan, decorin, and hyaluronan, are also found in the fetal membranes[11].

Connective tissue

The fibrous connective tissue of the uterus is a highly organized network of ECM molecules with cells sparsely distributed within it. The network provides tissues with mechanical support and tensile strength and forms the framework for the vertebrate body. The predominant cells in fibrous tissues are fibroblasts, but immune cells such as lymphocytes, granulocytes, and macrophages may also infiltrate. The ECM provides an environment that regulates cell behavior by influencing survival, migration, and the ability of cells to interact with other cells and effector molecules. The major components of the ECM are collagens, proteoglycans, hyaluronan, and other glycoproteins. In the next section there is a brief overview of these components.

Collagens

Collagens are the most abundant proteins in mammals[12] and they constitute the molecular scaffold of the body, providing strength and support for the connective tissue. To date, about 28 types of collagens have been described in the literature, all of which have at least one domain composed of three polypeptide chains wound around each another to form a triple helix. The three polypeptide chains are packed tightly into a collagen superhelix stabilized by hydrogen bonds, which makes it very resistant to pro-

teolytic degradation. The collagens are categorized into different subgroups according to their molecular structure, interactions, and type of polymerization. The fibril-forming collagens are the main collagens found in connective tissues and consist of collagen types I, II, III, V, and XI. About 90% of the total collagen mass consists of fibrillar collagens, where collagen I is quantitatively the most abundant. The non-fibrillar-forming collagens are types IV, VI, VII, VIII, X, and XIII. These collagens bind to the surface of fibrillar collagens or are present at the cell membrane. The fibril-associated collagens with interrupted triple helices (FACIT) consist of type IX, XII, XIV, XIX, and XXI, and appear to connect fibril-forming collagens to other ECM molecules[13-16]. Type IX, XV, and XVIII collagens can also be considered to be proteoglycans, as collagen type IX and XV contain chondroitin sulfate chains[16,17] and collagen type XVIII contain heparan sulfate chains[18].

Proteoglycans

Proteoglycans are a highly heterogeneous superfamily of molecules that all consist of a protein core substituted with one or several glycosaminoglycan (GAG) side chains. Based on the domain structure of the protein core, proteoglycans can be divided into basement membrane proteoglycans, hyalectans, and small leucine-rich proteoglycans[19]. Basement membrane proteoglycans and small leucine-rich proteoglycans carry few GAG chains whereas hyalectans can carry up to a hundred individual GAG chains. The GAG chains are linear polysaccharides composed of alternating disaccharides. Proteoglycans are found in a variety of tissues and are synthesized by all types of mammalian cells. They can be found intracellularly, at cell surfaces, and in the ECM where they interact with many other ECM components, mainly through their GAG chains[20,21]. The GAGs can be divided into four distinct groups depending on their structure: chondroitin/dermatan sulfate, keratan sulfate, hyaluronan, and heparan sulfate/heparin (Figure 2). During biosynthesis of the proteoglycans, a series of epimerization and sulfation reactions orchestrated by numerous enzymes modifies the GAG chains. This is the basis of the vast structural diversity of the proteoglycans. Except in hyaluronan, all GAG side chains are sulfated to a varying degree, giving the proteoglycans a negative charge. The highly charged GAG chains allow the proteoglycans to be molecularly promiscuous, binding many different proteins including growth factors, extracellular proteases, protease inhibitors, cytokines, cell receptors, and other ECM molecules. As a result, the proteoglycans are involved in various aspects of cell-cell or cell-ECM interactions[22,23].

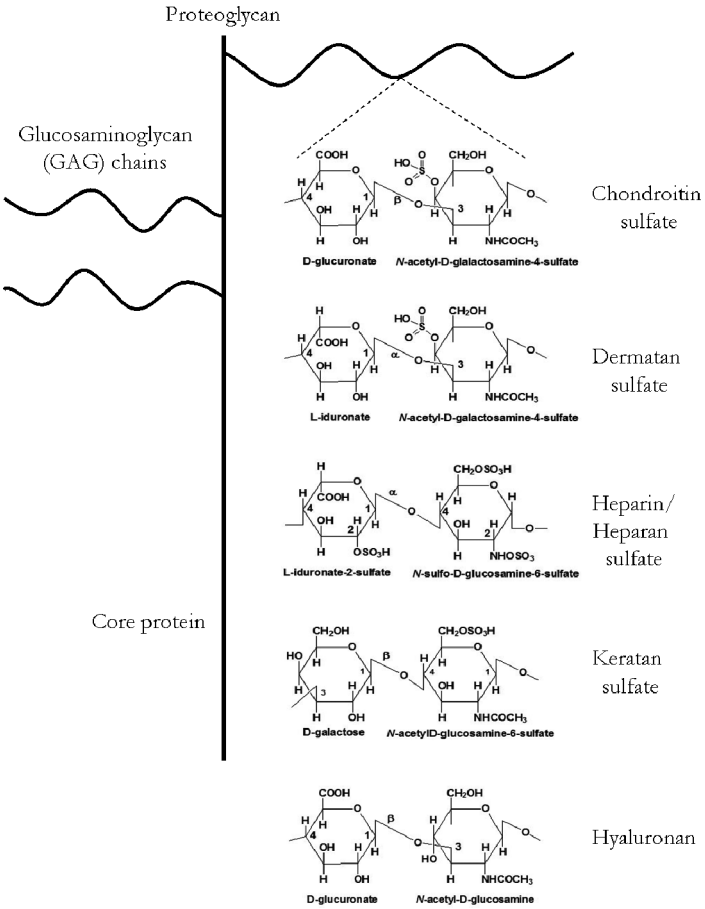


Figure 2. Disaccharide composition of the GAG chains. Dermatan sulfate is distinguished from chondroitin sulfate by the presence of iduronic acid. Heparin is a highly sulfated form of heparan sulfate. Hyaluronan is a non sulfated GAG, devoid of a protein core.

Other ECM proteins

Apart from collagens and proteoglycans, the ECM contains several other proteins that are important for the organization, maintenance, and function of ECM. One example is elastin, which is a protein found in elastic fibers throughout the body. Analysis of the human cervix suggests that elastin is localized in specific regions of the uterine cervix and is not dispersed throughout the connective tissue stroma[24]. Fibronectin is another ECM protein; it regulates a variety of cell activities through direct interactions with cell surface integrin receptors. Fetal fibronectin is produced by fetal cells and it is found at the border of the fetal sack and the uterine lining. Fetal fibronectin has been implicated in labor and attempts have been made to use it as a diagnostic test to predict preterm labor[25-27]. Together with collagen IV, laminin, another protein found in the ECM, forms

the substrate of basement membranes. In humans, there is a cyclic expression of laminin and type IV collagen in basement membrane structures of the endometrium, reflecting the endometrial epithelial remodeling during the menstrual cycle[28]. Tenascin is an ECM glycoprotein involved in embryogenesis, carcinogenesis, and wound healing. In human reproductive tissues, it has been implicated to play a role in the development of endometriosis[29]. It also affects cell proliferation and its expression is upregulated in cervical and endometrial carcinomas[30-32].

Matrix metalloproteinases (MMPs)

The ECM undergoes constant turnover, with both matrix degradation and synthesis occurring simultaneously in a highly organized manner, which preserves the matrix function. Turnover of the ECM is a biological problem since collagens, which make up a large part of the ECM, are highly resistant to proteolytic degradation. There are at least two groups of enzymes involved in this process. These are the MMPs and the tissue inhibitors of metalloproteinases (TIMPs). MMPs are a large family of zinc- and calcium-dependent endopeptidases, and to date 23 members have been characterized in humans. MMPs can be divided into at least four subclasses – collagenases, gelatinases, stromelysins, and membrane-bound MMPs – depending on their substrate preference and structural features. It is becoming increasingly apparent, however, that these divisions are rather artificial as a number of MMPs do not fit into any of the four sub-classes. The collagenases are capable of degrading fibrillar collagens into distinct 3/4- and 1/4-fragments. The main collagenases are MMP-1, MMP-8, MMP-13, and MMP-18, but MMP-2 and MMP-14 have also been shown to cleave fibrillar collagen. Gelatinases (MMP-2 and MMP-9) mainly degrade type IV and V collagens, elastin, and gelatin. The stromelysins are mostly involved in degradation of ECM proteins such as proteoglycans, laminin, and fibronectin. Well-recognized members of this group are MMP-3, MMP-10, and MMP-11. The MMPs are initially synthesized as proenzymes, with a propeptide domain that must be removed before the enzyme becomes active. The TIMPs can bind an MMP in both its inactive and its active form, and the balance between MMPs and TIMPs is essential in tissue homeostasis.[33,34]

Fibroblasts in the connective tissue

The main cell type found in connective tissue is the fibroblast. The major function of fibroblasts is to maintain the structural integrity of the connective tissue by continuously producing and secreting ECM molecules, but fibroblasts also have the capacity to produce cytokines and MMPs[35-37] (paper I). Fibroblasts are morphologically heterogeneous, with diverse appearances depending on their location and activity[38-40]. Fibroblasts are generally elongated and have a large and well-developed rough endoplasmic reticulum and Golgi apparatus, since they constantly produce bulky ECM molecules. Fibroblasts play a crucial role in remodeling of the ECM, which occurs both under normal conditions such as wound healing and pregnancy and during many pathological processes such as fibrosis, cancer, and rheumatic diseases[38,41-44]. Fibroblasts

can be activated or deactivated by a number of factors in the tissue, such as cytokines, growth factors, and components of the ECM. Activated spindle-shaped fibroblast-like cells such as myofibroblasts are found in tissues that undergo remodeling[38-40]. The origin of these activated fibroblast-like cells is not known. Several possibilities exist, including proliferation from resident mesenchymal cells, transition from epithelial and endothelial cells to fibroblasts, and recruitment of circulating fibroblast-like cells that are derived from bone-marrow stem cells (fibrocytes)[45].

Remodeling and inflammation

In all major remodeling events – both physiological, such as wound healing and pregnancy, and pathological, such as cancer, fibrosis, and atherosclerosis – the matrix changes are accompanied by inflammation. Remodeling of uterine tissues during pregnancy is associated with high levels of pro-inflammatory cytokines and infiltrating immune cells. In the following sections, a brief overview of the human immune system, of immune cells important in uterine remodeling during pregnancy, and of Toll–like receptors (TLRs) is given.

Adaptive & innate immunity

The human immune system can be divided into the adaptive (specific) response and the innate (non-specific) response. The adaptive immune system is only found in vertebrates. It is triggered by antigens and has the ability to form an immunological memory, which allows the adaptive immune system to mount faster and stronger attacks each time the antigen is encountered. Innate immunity, on the other hand, is the major system of host defense against pathogens in nearly all other living organisms[46]. It functions as a non-specific but immediate sensor of pathogens or tissue injury. Innate immunity relies on a large family of pattern recognition receptors, which detect distinct evolutionarily conserved structures on pathogens or endogenous components. Many cells in the body are capable of initiating an innate immune response. The response is very rapid, since all the transcription factors are transcribed and present in the cytosol prior to exposure.

Toll-like receptors (TLRs)

The most well-known family of pattern recognition receptors is the TLRs. These are all membrane-spanning glycoproteins that are characterized by an extracellular domain that contains varying numbers of leucine-rich repeat motifs and an intracellular signaling domain homologous to that of the IL-1 receptor, termed the Toll/IL-1R homology (TIR) domain[47,48]. The TLRs were first discovered in *Drosophila melanogaster* where they are necessary for embryonic dorso-ventral development and are essential for the

fly's immunity to fungal infections[49]. Human homologs were soon discovered, and to date 10 different functional TLR receptors have been described, all recognizing different structurally conserved pathogen-associated molecular patterns (PAMPs) (Table 1). However, not only bacterial and viral components but also endogenous molecules have recently been shown to activate various TLRs. For example, TLR4 can be triggered by gram-negative bacterial lipopolysaccharides (LPSs) and also several endogenous ligands from the ECM, such as hyaluronan[50], biglycan[51], fibronectin[52], and heparan sulfate[53].

Table 1. Endogenous and exogenous ligands for TLRs.

Human TLRs	**Endogenous ligands**	**Exogenous ligands**
TLR 1	-	Triacyl lipopeptides
TLR 2	Heat shock protein 70 HMBG1 Serum amyloid A	Glycolipids Zymosan Glycoproteins Lipoteichoic acid Numerous others
TLR 3	-	Double-stranded RNA Polyinosinic:polycytidylic acid
TLR 4	Heat shock proteins Fibrinogen Heparan sulfate Biglycan Fibronectin Hyaluronan HMBG1 Numerous others	Lipopolysaccharide Heat shock proteins
TLR 5	-	Flagellin
TLR 6	-	Diacyl lipopeptides
TLR 7	-	Single-stranded DNA Imidazoquinoline Synthetic compounds
TLR 8	-	Single-stranded DNA Synthetic compunds
TLR 9	-	Unmethylated CpG DNA
TLR 11	-	Profilin

The TLRs signal via two main pathways, the MyD88-dependent pathway and the MyD88-independent pathway (Figure 3). All TLRs except TLR3 are capable of signaling through the MyD88-dependent pathway, inducing NFκB nuclear translocation and transcription of pro-inflammatory cytokines. TLR4 and TLR3 can signal through the MyD88-independent pathway, which is mediated by TRIF. This pathway mainly mediates type I IFN responses through activation and nuclear translocation of the transcription factor IRF3.[54]

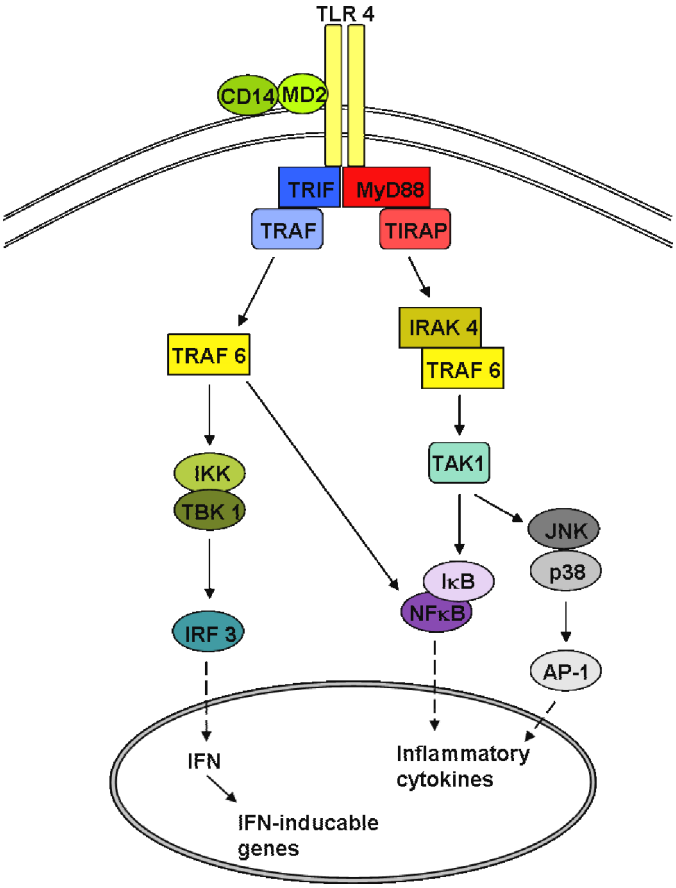


Figure 3. The MyD88-dependent and MyD88-independent signaling pathways for TLR4.

The expression of TLRs has been extensively studied and established in different cells of the immune system, including dendritic cells, monocytes, macrophages, and neutrophils, illustrating the role of TLRs in modulating inflammatory responses.

TLRs in the female reproductive tract

The precise mechanism of the onset of parturition remains unknown. The inflammatory response of uterine tissues and fetal membranes to microbes and endogenous agents is thought to play a major role in premature labor and normal labor at term[55]. Thus, a possible role for TLRs in the initiation of both preterm and term labor cannot be neglected. A small number of studies have been published showing expression of TLRs in gestation-associated tissues. TLRs 1-9 are expressed in the ovaries, the fallopian tubes, the uterine endometrium, the cervix, and the ectocervix[56-59]. Transcripts of the signaling adapter molecule MyD88 and the accessory molecules CD14 and MD2 are also present in all the tissues listed above[58]. Furthermore, expression of mRNA for

TRIF and other TLR signaling proteins has been detected in the placenta and uterus[59]. Besides ligand binding, recruitment of the intracellular adaptor molecule MyD88 is required for most TLR-mediated signaling[60]. MD2 and CD14 are necessary for efficient recognition of LPS by TLR4[61,62], and TRIF is necessary for MyD88-independent signaling by TLR3 and TLR4. Thus, the tissues of the female reproductive tract possess the full complement of adaptor and accessory molecules required for optimal TLR signaling. Expression of TLRs has also been demonstrated in primary epithelial cell cultures from different reproductive tissues[63-65]. A microarray study comparing the uterine cervix transcriptome before and after spontaneous term parturition has shown upregulation of TLR2, TLR4, and TLR6 in patients after spontaneous labor. TLR3 and TLR5 were found to be significantly downregulated in cervical tissue after spontaneous labor. The significant decrease in expression of TLR3 and TLR5 could be confirmed by conventional quantitative PCR whereas the apparent upregulation of TLR2 and TLR4 could not be confirmed[66].

Infiltrating inflammatory cells

Mononuclear phagocytes (monocytes/macrophages)

Monocytes are a type of white blood cell (leukocyte) that circulates in the bloodstream for about one to three days and then typically moves into tissues throughout the body. In the tissues, monocytes mature into different types of specific resident macrophages or dendritic cells. In response to pro-inflammatory signals, circulating monocytes quickly migrate to sites of infection/inflammation and differentiate into macrophages and dendritic cells to elicit an immune response. Monocytes are capable of destroying foreign substances in the body through phagocytosis. Monocytes are also able to clear inflamed areas of malfunctioning or infected endogenous cells by killing cells bound to specific antibodies. This mechanism is termed antibody-mediated cell-mediated cytotoxicity[67]. Monocytes are recruited from the circulation by CCL2, also known as monocyte chemotactic protein-1 (MCP-1). Studies have shown that protein concentrations of MCP-1 in the cervix are significantly increased during both preterm and term labor[68]. Also, histological analysis of the cervix at term has shown infiltration of macrophages[69,70]. However, even when the woman is not pregnant the reproductive tract contains a resident population of macrophages, but the numbers fluctuate throughout the menstrual cycle[71-73]. Locally acting inflammatory mediators and steroid hormones regulate the recruitment of leukocytes during the menstrual cycle[74-76].

Neutrophils

Neutrophils are the most abundant cells of the innate immune system. They are found in the circulation but respond quickly to inflammatory signals by migrating into the site of inflammation. When entering the inflamed tissue, they act as phagocytes, start the production of reactive oxygen species, and release inflammatory mediators and antimicrobial substances[77,78]. Neutrophil migration toward the site of inflammation is orchestrated by cytokines. Neutrophils are exquisitely sensitive to small differences in

cytokine concentration, and migrate towards the cytokine source. This phenomenon is called chemotaxis and is facilitated by proteoglycans such as heparan sulfate, which bind and sequester IL-8[79,80]. The major neutrophil-attracting molecule in humans is interleukin-8 (IL-8/CXCL8) but interferon-gamma (IFN-γ) is also active in this respect. Several studies have shown that IL-8, and subsequently neutrophils, are involved in the final cervical ripening at term[69,81-86]. Neutrophils are also present in all tissues of the female reproductive tract under normal healthy conditions[87]. The lumen of the vagina is not sterile and is colonized by a mixture of commensal microorganisms. Despite this, the greatest numbers of neutrophils are found in the fallopian tubes and become progressively less through the lower regions of the reproductive tract[72].

Lymphocytes

The three major types of lymphocytes are T cells, B cells, and natural killer cells. Natural killer cells are a part of the innate immune system, whereas B cells and T cells are the major cellular components of the adaptive immune response. T cells, B cells, and natural killer cells can be found throughout the human female reproductive tract[71,72]. In early pregnancy (first trimester), most of the lymphocytes found in the cervix are different subsets of T cells, but B cells are also present. At term, tissue-bound leukocytes are present in the human cervix at higher density than during the first trimester[88].

Tissue remodeling during pregnancy and labor

Uterine remodeling

During pregnancy, the uterus is transformed into a large muscular organ that is able to accommodate the fetus, the placenta, and the amniotic fluid. This is facilitated by major tissue remodeling and hypertrophy of smooth muscle cells[89]. During labor, the uterine myometrium is converted from a tissue with relatively low connectivity between individual smooth muscle cells (myocytes) into one with extensive physical connections. The physical connections occur through pores (gap junctions) formed by connexin-43. This connectivity allows the changes in membrane potential in individual myocytes to travel to neighboring cells and give rise to extensive waves of depolarization and contraction over large areas of the uterus. These coordinated muscle contractions are necessary for successful parturition. Although the uterus is mainly a muscular organ, the uterine connective tissue is important for regulation and formation of the myometrium during pregnancy and at term. Various ECM components such as chondroitin sulfate proteoglycans, dermatan sulfate proteoglycans, heparin, fibronectin, and hyaluronan are known to regulate gap junction synthesis and function[90-95]. Low expression of the heparan sulfate proteoglycan syndecan-3 in uterine tissues is associated with a dysfunctional and protracted labor[8]. The localization of syndecan-3 suggests that there is a functional interaction between connexin 43 and Syndecan-3[8]. Non-pregnant uter-

ine tissues contain considerable amounts of proteoglycans. The predominant one is the chondroitin/dermatan sulfate proteoglycan decorin, but the chondroitin/dermatan sulfate proteoglycan biglycan and heparan sulfate proteoglycans are also found[96]. During pregnancy and parturition, changes occur in the composition of the ECM in the uterus. At term, the concentration of decorin and biglycan are considerably lower than in non-pregnant tissue[97]. At labor there is an increase in heparan sulfate proteoglycans: expression of syndecan-3 especially is upregulated and localized to smooth muscle cells[98]. Also, the amount of collagen changes during pregnancy. At term pregnancy, the collagen concentration is reduced to about 50% in the corpus uteri[99]. The biological role of ECM components in the uterus during pregnancy is to provide a scaffold for the smooth muscle cells and, during labor, to enable effective contractions. In order to generate a tissue with suitable mechanical properties, a gradual remodeling of the ECM during pregnancy is necessary[9,99].

Cervical remodeling

The cervix is mainly composed of fibrous connective tissue with vast amounts of collagen fibers surrounded by highly hydrated proteoglycans. Collagen type I is the predominant form of collagen. It accounts for 70% – and collagen type III makes up around 30% – of all collagen fibers[100,101]. The cervix has a dual structural function: during most of the pregnancy it has to stay firm and closed to allow the growing fetus to develop normally, and at term the cervix must soften and dilate to let the fetus pass. The cervical remodeling in preparation for parturition begins early in pregnancy. It is a slow, progressive process that can be divided into 4 overlapping phases: softening, ripening, dilatation, and – after labor – repair (involution)[102].

The first phase, softening, is characterized by increased tissue hydration[103-106], collagen solubility[100,103-107], and tissue compliance[106] without any loss of structural integrity. Already at a gestational age of 10 weeks, the total collagen concentration has decreased to about 70% of the non-pregnant cervix. Also, the total amount of proteoglycans decreases throughout pregnancy[103].

At the end of pregnancy, the cervix undergoes an accelerated remodeling phase termed cervical ripening. This phase is characterized by an extensive degradation of the ECM mediated by infiltrating immune cells, and a further change in the composition of proteoglycans. The final cervical ripening that occurs just before or at onset of labor is an inflammatory process with a rapid elevation of the pro-inflammatory cytokines IL-8, IL-6, IL-1β, and MCP-1[68,69,82-84,108,109]. This promotes an influx of immune cells, predominantly neutrophils and macrophages[69,70,88,108,110,111]. The activated neutrophils secrete proteolytic enzymes such as MMP-2, MMP-8, MMP-9, and leukocyte elastase[36,82,112], but resident fibroblasts and smooth muscle cells are also a source of matrix-degrading enzymes[36,82,113]. These proteolytic enzymes are necessary for the final breakdown of the collagen network, which ensures increased tissue compliance[99,103,104,114,115]. The proteoglycan composition of non-pregnant cervical tissue is characterized by a high proportion of the small proteoglycan decorin. The non-pregnant cervix also contains small amounts of versican, biglycan, heparan sulfate proteoglycans, and a large

keratan sulfate proteoglycan[116,117]. During pregnancy, there is a gradual reduction in the total amount of proteoglycans combined with a severalfold increase in metabolic turnover of these components[107,117]. Decorin and biglycan are significantly reduced at full term compared to the corresponding tissues in the non-pregnant state, whereas levels of versican and heparan sulfate proteoglycans increase in cervical tissue at term[107,117,118]. Versican bind hyaluronan and together they attract water. It is thought that the accumulation of water molecules in the interstitial compartment results in an increased pressure that promotes dispersion and prevents aggregation of the collagen fibrils, thus facilitating cervical remodeling.

When the cervical ECM has become sufficiently degraded, uterine contractions initiate the third phase of cervical remodeling: the cervical dilatation. When the cervix is fully dilated, the child can pass through the birth canal. Immediately after delivery, an extensive recovery phase starts and the cervix quickly regains its non-pregnant properties. Instantly, the production of collagens and proteoglycans spikes and within a few days the concentration of proteoglycans is almost the same as in non-pregnant cervix[107].

The mechanisms that initiate and regulate the remodeling of ECM during pregnancy and parturition are still largely unknown. It is likely that progesterone and estrogen are important for the remodeling of the human cervix[119-121], but many other factors such as prostaglandins[122], nitric oxide[123], and fetal fibronectin[25,124] have also been suggested to play an important role in cervical remodeling.

Membrane rupture

Another important event in parturition is maturation and rupture of the fetal membranes. It is well known that the fetal membranes weaken a few days before labor. The weakening of the membranes is associated with a change in the biomechanical properties of the tissue. The amounts of collagen and decorin are reduced, and there is an increase in hyaluronan and biglycan[125]. The rupture of the membrane is facilitated by a pro-inflammatory process similar to that in the cervix, with elevated levels of IL-8, TNF-α, IL-6, and IL-1β[108,126-128]. This is associated with a significant increase in the production of several MMPs such as MMP-8[129] and MMP-9[130-133] in the placenta, fetal membranes, and amniotic fluid at the onset of labor. The sources of cytokines and MMPs are infiltrating leukocytes[69], but fibroblasts and epithelial cells also secrete pro-inflammatory cytokines. The matrix-degrading enzymes cause further weakening of the placental membranes, thereby facilitating membrane rupture.

Consequences of a dysfunctional remodeling

Disturbances in the remodeling of the cervix and uterus during pregnancy can cause preterm labor[132], protracted labor, or arrest of labor[8,9]. Protracted labor affects both the mother and the fetus negatively and results in increased numbers of instrumental deliveries[1,134]. Lack of progress in labor is a predominent reason for Caesarean delivery, and more than 50% of all unplanned Caesarean sections in nulliparous women are due to this type of obstetric complication[3,5]. Two treatments for protracted labor are currently used in clinical practice, oxytocin and prostaglandin E_2. Oxytocin is an endogenous hormone formed in the hypothalamus and stored as a precursor in the neurohypophysis. During parturition, oxytocin is secreted from the posterior pituitary gland. It stimulates contractions in the uterus, which is extremely sensitive to oxytocin at the end of pregnancy. Oxytocin has been used in obstetrics since 1911, to trigger and increase uterine contractions[135]. As a pharmacological drug it is one of the most important treatments in obstetric practice. Prostaglandins are endogenous substances that are synthesized within the human fetal membranes and decidua, and function by ripening the cervix and contracting the myometrium. Prostaglandin E_2 is given cervically when ripening of the cervix is incomplete[136]. Prostaglandins have been used for cervical ripening and induction of labor since the 1970s. The exact mechanism remains to be determined, but the effects of prostaglandin E_2 are clearly pro-inflammatory in some way. Although both oxytocin and prostaglandin E_2 have facilitated the treatment of women with protracted labor, the rate of Caesarean sections is on the increase throughout the world[137-139].

An improved understanding of the mechanisms and pathways controlling remodeling during pregnancy and labor is crucial for the task of preventing protracted labor.

Aims of the Study

As discussed previously in this thesis, the uterine remodeling process preceding labor is a complex interplay between different cell types and mediators. The overall purpose of this project was to investigate the mechanisms controlling remodeling of the uterus and cervix before parturition.

In particular, the following was investigated:

- Cervical ripening is accomplished by an extensive remodeling of the ECM. Thus, primary fibroblast cultures established from human cervices obtained at different stages of pregnancy were studied to investigate their role in cervical remodeling.

- Studies of the uterine and cervical ECM have shown considerable changes in heparan sulfate proteoglycan distribution during normal and dysfunctional labor. We hypothesized that heparan sulfate/heparin is important for a normal labor.

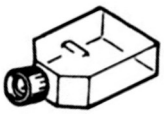
- During cervical ripening, extensive degradation of the ECM takes place, mediated by infiltrating immune cells. We hypothesized that heparan sulfate/heparin induces cervical inflammation by triggering the innate immune response via TLR4.

Materials and Methods

In this thesis, several models for studying cervical remodeling were used. In papers I, II, and IV different *in vitro* models, namely primary fibroblast cultures established from human cervices obtained at different stages of pregnancy and uterine smooth muscle tissue from pregnant women, were used. Paper III involves a retrospective study from medical records on pregnant women, and paper V involves an *in vivo* study using mice.

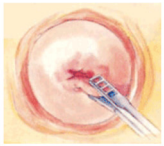
A brief overview of the study design and methods used is given below. For detailed information about the different techniques used, the reader is referred to the individual articles.

Cervical fibroblast cultures
(paper I, II, IV)



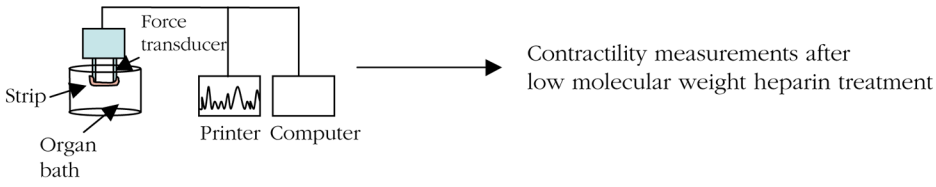
- Immunohistochemistry (α-SMA, prolyl-4-hydroxylase, Syndecan 1-4, Glypican 1, Perlecan)
- Western blots (α-SMA, prolyl-4-hydroxylase)
- IMMULITE and ELISA (IL-6, IL-8, MMP-1, MMP-3)
- Proteomics (2D-gel electrophoresis → MALDI-TOF)

Cervical biopsies
(paper I)

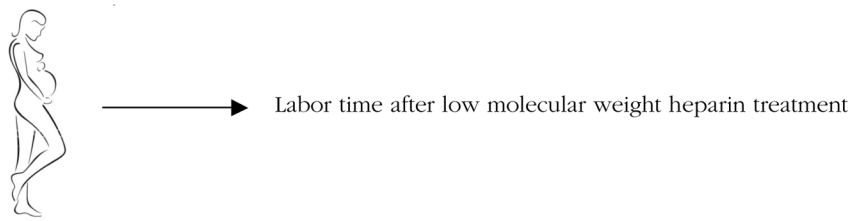


→ Immunohistochemistry (α-SMA, prolyl-4-hydroxylase, IL-8)

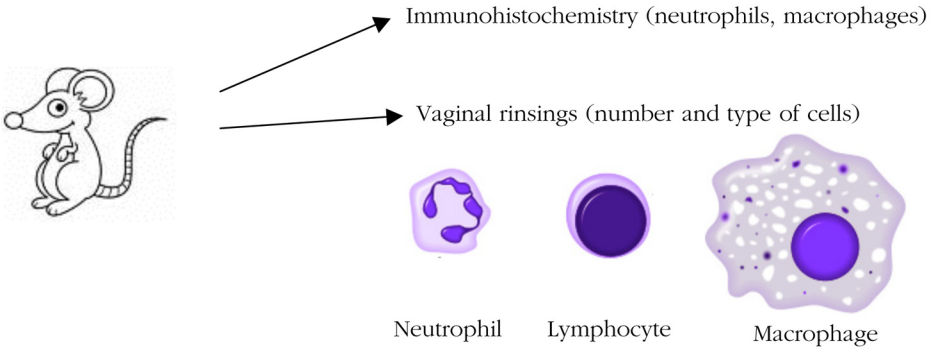
Uterine smooth muscle strips
(paper IV)



Pregnant women
(paperIII)



Wildtype and TLR4$^{-/-}$ mice
(paper V)



Results

Paper I

The importance of fibroblasts in remodelling of the human uterine cervix during pregnancy and parturition.

The main cell type found in cervical (connective) tissue is the fibroblast. Although many cell types are involved in cervical remodeling prior to parturition, fibroblasts play a crucial role in remodeling of the ECM, which occurs throughout pregnancy. The aim of this study was to examine primary fibroblast cultures established from human cervices obtained at different stages of pregnancy to investigate their role in cervical remodeling. Fibroblast cultures were established from cervices of non-pregnant woman, woman after 37 weeks of pregnancy, and woman directly after partus.

The amounts of prolyl-4-hydroxylase, a key enzyme in the biosynthesis of collagen, and α-smooth muscle actin (α-SMA), a marker of myofibroblast differentiation, were quantified by western blot. During pregnancy, significant reductions in both proteins are noted. The same pattern was seen on immunostaining of the fibroblasts.

The cultures were screened for cytokine and MMP production since cervical ripening is associated with high levels of both cytokines and MMPs. Levels of IL-6, IL-8, MMP-1, and MMP-3 were all significantly upregulated in cell cultures established from partal women.

Global proteome analyses of the three types of fibroblasts were also conducted. 2D gel electrophoresis followed by mass spectrometry showed that the expression of 59 proteins was changed significantly in cultures of partal donors.

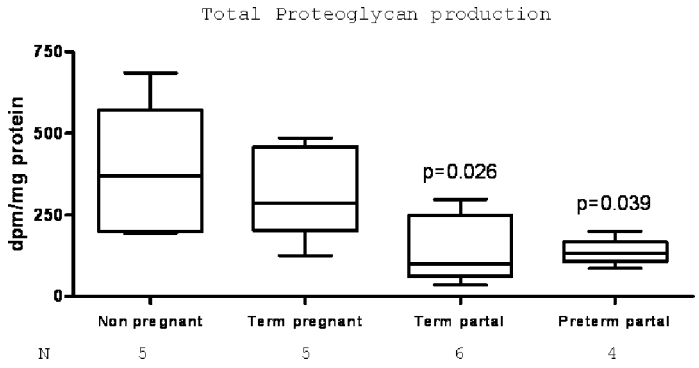
In conclusion, fibroblast cell cultures established from different stages of cervical ripening are phenotypically different and express properties typical of the *in vivo* situation they are derived from. This demonstrates the presence of different types of fibroblasts during the process of cervical ripening. The cells are phenotypically stable for at least eight passages in culture, showing that the differences are not due to any extrinsic cytokine or hormonal influence. This suggests that fibroblasts with different properties are recruited into the tissue from the circulation or differentiate from resident progenitor cells.

Paper II

Differences in heparan sulfate production in cervical fibroblast cultures from women undergoing term and preterm delivery.

Extensive remodeling of the human cervical connective tissue occurs throughout pregnancy, with a reduction in the total concentration of both collagen and proteoglycans. The aim of this study was to investigate the proteoglycan production in human cervical fibroblasts from different stages of pregnancy. Fibroblast cultures were established from cervical biopsies taken from non-pregnant women, spontaneously preterm delivered women (week 25-35), term pregnant women undergoing elective Caesarian sections (weeks 37–38), and women within 20 minutes of spontaneous term vaginal delivery (week ≤39). We hypothesized that the profound changes in proteoglycan production in the cervix would also be seen in corresponding cervical fibroblasts.

Proteoglycan production was measured as incorporation of radioactive sulfate into the GAG chains. The labeled proteoglycans were purified by ion-exchange chromatography and separated by gel electrophoresis. Proteoglycan production was reduced by 50% in fibroblast cultures obtained from term and preterm women in labor. In comparison to equivalent control cultures from non-pregnant women, this decline was significant.



The level of versican remained unchanged whereas the amounts of decorin and biglycan dropped in cultures from pregnant women. The levels of heparan sulfate proteoglycans increase significantly during pregnancy. The heparan sulfate proteoglycan perlecan increases by about 60% in cultures from pregnant donors. The levels of other heparan sulfate proteoglycans also have a tendency to increase in cultures from pregnant women, and in preterm cell cultures the increase is statistically significant. By immunolabeling, heparan sulfate proteoglycans were localized to cell membranes and to intracellular compartments.

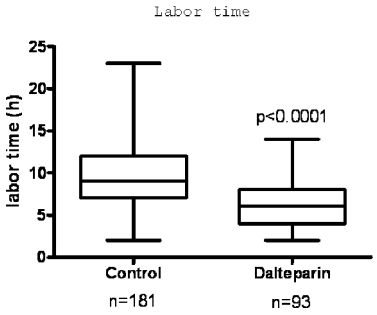
To conclude, the changes in total proteoglycan production in the cervix of a pregnant woman can also be seen in corresponding cervical fibroblasts. Term partal cells and preterm partal cells secrete significantly more heparan sulfate proteoglycans than their non-pregnant counterparts, which suggests that heparan sulfate proteoglycans have a role in cervical ripening.

Paper III

Does low molecular weight heparin shorten term labor?

Protracted labor is characterized by slow progression of labor, and is a common indication for Caesarean section, which is associated with increased maternal and neonatal morbidity. Studies of the uterine and cervical ECM have shown that there are considerable changes in heparan sulfate proteoglycan distribution during normal and dysfunctional labor. This, taken together with the clinical notion that pregnant women treated with low molecular weight heparins are less likely to suffer from protracted labor, led us to investigate whether low molecular weight heparin influences the parturition process. Thus, a retrospective study was performed to assess the effects of the low molecular weight heparin dalteparin on labor time. Dalteparin-treated women showed a 30% shorter labor time than matched untreated controls. No differences in the neonatal outcome could be detected.

In conclusion, the low molecular weight heparin substance dalteparin significantly shortens parturition time, which may reduce the number of operative interventions due to protracted labor.



Paper IV

Low molecular weight heparin stimulates myometrial contractility and cervical remodeling in vitro.

In paper III, we showed that women on prophylactic low molecular weight heparins have a 30% shorter parturition time than matched controls. The aim of this study was to investigate the mechanism behind this effect. We hypothesized that heparin derivatives were able to influence both myometrial contractility and cervical ripening. We also wanted to investigate whether the anticoagulative motif in heparin is necessary for the outcome. To test the effect on myometrial contractility, we used smooth muscle strips from biopsies obtained at elective Caesarean sections and measured contractility *in vitro*. The effects on cervical ripening were assessed by measuring IL-8 secretion in cervical fibroblasts cultured from explants of cervical biopsies obtained at delivery.

Myometrial smooth muscle strips pretreated with low molecular weight heparins showed increased contractile activity compared to untreated smooth muscle strips. Treatment of cervical fibroblasts with dalteparin yielded increased IL-8 production in the higher dose range. Treatment with low-anticoagulant heparin fragments resulted in

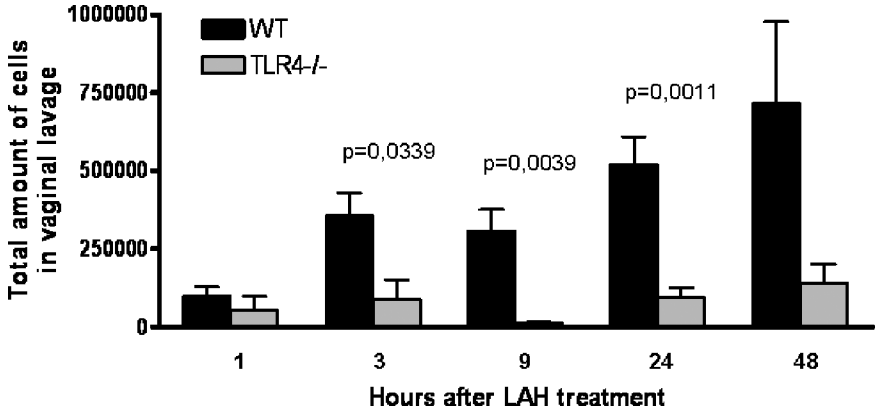
a dose-dependent stimulation of IL-8 production, which was significant already at 0.1 µg/ml.

In conclusion, the *in vitro* data indicate that myometrial contractility and cervical ripening are influenced by heparin derivatives. The anticoagulant motif is not necessary for the effects. These findings suggest a possible new therapy for the obstetric problem of protracted labor.

Paper V

Heparin fragments induce cervical inflammation by attracting immune cells through Toll-like receptor 4.

During cervical ripening, an extensive degradation of the ECM takes place, mediated by infiltrating immune cells. The aim of this study was to investigate the underlying mechanism behind the effect of heparin on parturition. We hypothesized that heparin induces cervical inflammation, a hallmark of cervical ripening, by triggering the innate immune response via TLR4. Low-anticoagulant heparin fragments or phosphate buffered saline (PBS) were applied into the vaginas of wild-type or TLR4 knockout mice. The extent of local inflammation was determined after 1, 3, 9, 24, and 48 hours by evaluation of morphology, of neutrophil and macrophage infiltration, and of the number of inflammatory cells in vaginal lavage. Low-anticoagulant heparin fragments induced a local inflammatory response in wild-type mice, visualized as a rapid infiltration of neutrophils and macrophages into the cervix and vagina. There were no signs of inflammation in Toll-like receptor 4 knockout mice.



In conclusion, our findings show that there is a TLR4 receptor-mediated innate immune response in vaginal/cervical cells induced by heparin fragments.

General Discussion

Human labor is a complicated process in which the softening and dilatation of the cervix have to be combined with forceful and synchronized myometrial contractions for a successful parturition. It would be of great value to have a better understanding of the mechanisms involved in the progression of human labor at term, in order to prevent preterm labor, prolonged pregnancies, or slow progress of labor. The number of instrumental interventions has risen during the past few years. Today, around 18% of all women who give birth in Sweden[140] and more than 30% of all women who give birth in the USA[141] do so by Caesarean section. One of the most frequent indications for an emergency Caesarean section is poor progress of labor, which is also called dystocia[2-4,6]. Protracted labor is linked to increased maternal and neonatal morbidity[1,134] and is caused by an insufficient remodeling of the uterine connective tissue[8,99]. Thus, the work presented in this thesis focused on the mechanisms that control the remodeling of the uterus and cervix before parturition.

The initiation and regulation of labor in humans have been remarkably elusive. One reason for this is the fact that human parturition is a distinctly human event – animal models can give only limited insights. Variations on the theme of parturition in mammals are considerable. For example, parturition in sheep is initiated by processes involving the fetal pituitary-adrenal axis[142] whereas parturition in goats depends on dissolution of the maternal corpus luteum[143]. Genomic analyses have revealed that more than 98% of human and chimpanzee DNA sequences are shared[144-146], but one of the greatest differences between the two species occur in genes related to reproduction[147]. This demonstrates the necessity to study human pregnancy, despite the ethical and practical difficulties in conducting studies that involve women in labor.

In three of the five papers included in this thesis, tissue samples from the uterine cervix were used to establish fibroblast cell cultures. The tissue samples were taken from non-pregnant women, term pregnant women undergoing elective Caesarian sections, and women within 20 minutes of spontaneous vaginal delivery either at term (week 39-41) or preterm (weeks 25–35). As profound remodeling occurs after delivery[107], questions often arise as to whether biopsies taken directly after delivery represent cervical ripening or involution. However, our results showed cell characteristics typical of cervical ripening. Fibroblast cell cultures derived from cervices of women directly after partus secrete high amounts of both MMPs and pro-inflammatory cytokines, have a low expression of prolyl 4-hydroxylase (an enzyme important in the biosynthesis of col-

lagen), and produce low amounts of proteoglycans. All of these findings correlate with the cervical ripening seen *in vivo*.

Fibroblast cell cultures established from cervices at different stages of pregnancy are phenotypically diverse, and this difference in cell behavior persists over at least eight passages, demonstrating that the differences are not due to any external signals such as cytokines or hormones. This reflects the necessity for a set of fibroblasts with different properties to promote degradation of the ECM in preparation for a successful parturition. This can either be achieved by recruitment of fibroblast progenitor cells from the circulation or differentiation from progenitors on site. During both normal and pathological remodeling, for instance during wound healing[148], asthma[149,150], idiopathic pulmonary fibrosis[151] or cancer[152], progenitor fibroblasts (fibrocytes) are recruited from the circulation and can differentiate into inflammatory fibroblasts. The fate of the inflammatory fibroblasts after partus is uncertain, but one possibility is that they undergo apoptosis. As yet unpublished data from our group show that caspase-3, a protease important in cellular apoptosis, is upregulated in cervical tissues taken after a vaginal delivery.

It has been known for some time that the levels of heparan sulfate proteoglycans increase both in the cervix and the uterus during pregnancy[97,98,107,117,118]. Our results indicate that this increase is important for a functional labor. We have shown that treatment with heparin fragments significantly shortens parturition time in pregnant women. The mechanisms involved in this effect are most likely enhanced myometrial contractility combined with an increase in secretion of pro-inflammatory cytokines, giving improved cervical ripening. The heparin-induced secretion of pro-inflammatory cytokines is dependent on a functional TLR4. Local application of heparin fragments in the vagina/cervix in wild-type mice give rise to an influx of both neutrophils and macrophages into the cervical/vaginal tissue. When mice devoid of TLR4 were treated with heparin fragments no signs of inflamation were seen (paper V). It is tempting to speculate that heparan sulfate is the body's natural drug or medicine to facilitate labor. A potential starting point in the late cervical remodeling is leakage of fetal fibronectin from the amniotic fluid, which happens a few days before labor[25,153]. The fetal fibronectin can act as a pro-inflammatory signal, causing surrounding cells to produce interleukins that attract immune cells[52,154,155]. The invading immune cells represent a readily available source of proteolytic enzymes, which, when released, cause shedding of heparan sulfate proteoglycans from cell surfaces, thus producing heparan sulfate proteoglycan fragments[156]. The soluble heparan sulfate fragments signal through TLR4, which leads to secretion of pro-inflammatory cytokines. Infiltrating neutrophils and monocytes attracted by the chemotactic gradient release MMPs, which degrade the ECM further. This creates a positive feedback loop, which explains the accelerated ripening of the cervix seen at term.

The mechanism behind the contractile effects of heparin fragments shown in paper IV has not been elucidated at this point and can only be speculated upon. Initial experiments conducted at our lab have shown that addition of heparan sulfate to fibroblast cell cultures gives rise to a rapid increase in cytosolic calcium concentrations, detected

by the fluorescent dye Fluo-4. The source of the calcium is not known at present, but several possibilities exist. Heparan sulfate may influence the voltage-gated Ca^{2+}-transport channels in the cell membrane or may affect receptors on the sarcoplasmic reticulum membrane (i.e. IP_3-induced Ca^{2+} release or Ca^{2+}-induced Ca^{2+} release via ryanodine receptors)[157]. Previous studies have shown that the heparan sulfate proteoglycan syndecan-3 co-localizes with the gap junction protein connexin 43 in the human uterus during labor, which suggests that there may be a functional interaction between the two proteins[8]. Yet another possibility is the novel TLR4 signaling pathway involving Ca^{2+}, cAMP, and CAMP response element binding protein recently discovered in human bladder epithelial cells[158].

In conclusion, our data suggest that heparan sulfate has an important role in human labor, since heparin fragments reduce the parturition time in nulliparous women by 30%. To make use of this highly interesting effect, a heparin molecule devoid of the anticoagulant motif has been constructed by controlled periodate oxidation followed by alkaline elimination and reduction (paper III-V). This substance has passed toxicity tests and phase I clinical studies and is now in a phase II clinical study to test its effect on protracted labor.

Summary and Conclusions

Based on our finding we conclude that:

- Fibroblast cell cultures established from cervices at different stages of ripening are phenotypically different, and express properties typical of the *in vivo* situation they are derived from.
- Cervical fibroblasts established from pregnant women secrete more heparan sulfate proteoglycans than their non-pregnant counterparts, suggesting that heparan sulfate has a role in cervical ripening.
- Heparin fragments given as injections significantly shorten parturition time in pregnant women. Possible underlying mechanisms are enhanced myometrial contractility and increased secretion of pro-inflammatory cytokines, giving improved cervical ripening.
- Heparin fragments induce a TLR4-mediated innate immune response in mice, giving a molecular explanation for the increase in pro-inflammatory cytokines seen after heparin treatment.

In summary, our data suggest that fibroblasts with an active pro-inflammatory phenotype have an important role in the transformation of the uterine cervix preceding labor. Moreover, heparan sulfate proteoglycans appear to be central for the remodeling of the cervix and uterus before parturition, as shown by the finding that heparin fragments can be used in the clinical setting to prevent protracted labor.

Future Perspectives

The findings presented in this thesis pave the way for several exciting new ideas and experiments.

Regarding our finding that cervical fibroblast cultures established from different stages of pregnancy differ in phenotype, it would be highly interesting to find out where these different clones of fibroblasts originate from. Are they recruited from the circulation or do they differentiate from progenitors, epithelial cells or fibroblasts on site? To try to answer this question, we could label cells for different progenitor markers. The difficulty would be to get tissue samples from the right time frame. When do these fibroblast clones appear? It is possible to get tissue samples at abortions, at term and preterm labor, and at elective Caesarean sections (term and preterm). Outside of these time points, the prospect of getting tissue samples is very limited.

This thesis has concentrated on the importance of sufficient uterine remodeling during pregnancy and the importance of heparan sulfate in accomplishing this. The mechanism involved in the effect of heparin/heparan sulfate on cervical ripening appears to be TLR4-dependent. Knockout mice for different proteins in the two main TLR4 signal transduction pathways (MyD88-dependent and MyD88-independent) are available, and studies to elucidate the signaling pathways in greater detail are planned. To find out how heparin/heparan sulfate enhance uterine contractility, it would be important to establish whether heparin/heparan sulfate stimulation leads to changes in intracellular calcium and hence force generation or whether it affects the sensitivity of the contractile machinery to calcium, a phenomenon called calcium sensitization[159,160]. To test this we could isolate uterine smooth muscle cells and load them with the calcium indicator Fluo-4 to visualize and measure changes in intracellular calcium upon heparin/heparan sulfate stimulation. We could further dissect the mechanism underlying changes in cytosolic calcium by using specific pharmacological blockers of Ca^{2+} entry channels.

Another interesting idea would be to examine the quantity and pattern of heparan sulfate proteoglycan in cervical biopsies from women with a dysfunctional labor. It is known that an imbalance in the production and distribution of the heparan sulfate proteoglycan Syndecan-3 in uterine tissues is associated with protracted labor[8]. To date, no such study has been conducted using cervical tissue.

We have previously tried to study the structure-function relationship of heparan sulfate using transfected HEK 293 $TLR4^{+/+}$ cells, but with no success. It could be predicted that heparan sulfate uses a co-receptor for its binding to TLR4, and that this molecule is absent *in vitro*. To overcome this obstacle, a structure-function relationship study could

be conducted using mice, or perhaps one could try to use other cell lines that express TLR4 (and hopefully the putative co-receptor).

To try to find out which type of cell that reacts to heparin in mice, heparin fragments coupled to the fluorescent molecule rhodamine have been constructed. Our initial experiments suggested that the epithelial cells lining the uterocervical cavity bind the heparin-rhodamine construct. This was no surprise; however, some fluorescence was also detected in the stroma below the epithelial cells, indicating that the heparin-rhodamine molecules are taken up through the epithelium. To resolve which cells these are (fibroblasts, macrophages, or others), co-localization studies will have to be carried out.

In the future, we hope to be able to control uterine remodeling and thereby prevent dysfunctional and protracted labor by giving pregnant women heparin fragments.

Summary in Swedish

Under graviditet och förlossning genomgår vävnaden i livmodern och livmoderhalsen stora förändringar. Allt eftersom fostret växer expanderar livmodern och blir större medan livmoderhalsen förblir fast och stängd. I slutet av graviditeten, några dagar innan förlossningen, går livmoderhalsen från att vara fast och sluten till att bli mjuk och kan då öppnas så mycket att barnet kan passera. Denna process brukar kallas utmognad av livmoderhalsen och är en inflammatorisk reaktion med höga nivåer av inflammatoriska molekyler. Dessa molekyler rekryterar inflammatoriska celler från blodcirkulationen in till livmoderhalsens vävnad. Cellerna som kommer in i vävnaden bryter ner livmoderhalsens bindväv vilket förändrar sammansättningen i vävnaden och gör livmoderhalsen mjuk och elastisk. När sedan den aktiva förlossningen sätter igång kommer täta och kraftiga kontraktioner i livmodern pressa ut barnet genom livmoderhalsen.

Förändringarna i vävnaden är nödvändiga för att en förlossning ska kunna ske. Sker detta för tidigt leder det till en prematur förlossning. Om utmognaden av livmoderhalsen å andra sidan inte är tillräcklig leder det till en långdragen förlossning med ökad risk för ett akut kejsarsnitt. Upp emot 30% av alla förstföderskor i Skandinavien genomlider idag utdragna och långsamma förlossningar. Målet med denna avhandling har varit att undersöka hur förändringarna i livmodern och livmoderhalsen sker samt vad som styr vävnadsförändringen.

Vi har visat att fibroblaster (en bindvävs-producerande cell) tagna från livmoderhalsen hos gravida kvinnor har inflammatoriska egenskaper, vilket motsvarande celler tagna från icke-gravida kvinnor saknar. Dessa celler tillverkar också mer av en bindvävsmolekyl som heter heparansulfat. En närbesläktad molekyl till heparansulfat som kallas heparin har länge använts i den västerländska skolmedicinen för att förhindra att blodproppar täpper till blodkärl. Vi har undersökt hur lång förlossningstid kvinnor som har behandlats med heparin har och jämfört med obehandlade kvinnors förlossningstid. Vi upptäckte att heparinbehandlade kvinnor i genomsnitt har en 30% kortare förlossning. Orsaken till detta tycks vara att heparin påverkar livmodern att kontrahera kraftigare samt att utmognaden av livmoderhalsen påskyndas. Mekanismen bakom den påskyndade utmognaden av livmoderhalsen vid heparinbehandling involverar en cellbunden receptor som kallas Toll-like receptor 4. När heparinmolekylen binder till denna receptor går en signal genom cellen som börjar tillverka och utsöndra inflammatoriska molekyler. Detta sätter igång rekryteringen av inflammatoriska celler in till livmoderhalsens vävnad. Dessa inflammatoriska celler bryter i sin tur ned vävnaden.

Denna upptäckt har lett till att en heparinmolekyl nu testas i kliniska försök på gravida kvinnor för att se om man kan använda den som ett läkemedel för att förhindra utdragna förlossningar.

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