REVIEW ARTICLE ISSN- 0102-9010

COLLAGEN REMODELING DURING CERVICAL RIPENING IS A KEY EVENT FOR SUCCESSFUL VAGINAL DELIVERY

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ABSTRACT

Parturition involves a complex interplay of maternal and fetal factors. An understanding of the physiological mechanisms involved in maternal adaptations would be of great benefit in the diagnosis, management, and outcome of dystocic parturition, an important problem in human health care and animal production. In this review, we consider the histofunctional changes in the uterine cervix that are essential for successful vaginal delivery and focus on work from our laboratory. The functions of the uterine cervix change considerably during pregnancy. As the uterus enlarges to accommodate the growing fetus, the cervix behaves essentially as a barrier. At term, however, the cervix softens and dilates through a process known as cervical ripening. This process is extremely complex and involves interactions between different cellular compartments and the extracellular matrix, as well as properly timed biochemical cascades, and stromal infiltration by inflammatory cells. Since the main component of the uterine cervix is connective tissue, collagen remodeling is a key event for ripening and delivery. Moreover, because of their intrinsic mechanical properties, elastic fibers may be involved in the recovery of shape immediately after parturition. Despite the advances in our knowledge of cervical ripening, the signals responsible for initiating these changes remain to be elucidated. By understanding the mechanisms involved in these changes, it should be possible to address complex issues such as cervical incompetence, pre- and post-term delivery, and proper "ripening" of the cervix in order to avoid surgical delivery.

Key words: Collagen remodeling, extracellular matrix, parturition, uterine cervix

Abbreviations: α-smooth muscle actin (α-SMA), basement membrane (BM), blood vessels (bv), cytoplasmic processes (CP), eosinophils (eos), epithelium (E), estradiol (E_2), estrogen receptor (ER), fibroblast (Fib), muscle layer (MS), myofibroblast (Myof), nucleus (N), progesterone (P_4), progesterone receptor (PR), subepithelial stroma (SS).

Parturition results from a complex interplay of maternal and fetal factors. Maternal preparation for parturition involves many events, including cervical ripening, relaxation of the interpubic joint, induction of receptors for uterine-activating agents, and the formation of gap junctions between uterine smooth muscle cells in order to coordinate myometrial contractions [15]. The most important issue in perinatology is how to predict when a patient at term or preterm will proceed to active labour. Further knowledge of uterine contractility and cervical ripening may be useful in helping to recognize when the uterus or the cervix is prepared for labour and thus select effective therapeutic strategies. An understanding of the physiological mechanisms involved in maternal adaptations would be of great benefit in the diagnosis, management, and outcome of dystocic parturition, an important problem in human health care and animal production. In this review, we consider the histofunctional changes in the uterine cervix that are essential for successful vaginal delivery and focus on work from our laboratory.

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THE UTERINE CERVIX: HISTOARCHITECTURE AND FUNCTIONS

The uterine cervix was initially thought to be merely an anatomic end of the uterus that allowed drainage of the menstrual flow (when present), sperm migration and the passage of the conceptus during delivery. However, numerous studies have shown that the cervix has an important role in the normal transport and capacitation of spermatozoa, as well as acting as a protective barrier, together with the cervical mucus, against the penetration of microorganisms and toxic substances into the uterine cavity. The cervix also serves to prevent the expulsion of the preterm conceptus. At term, however, the cervix softens and

dilates through a process known as cervical ripening [61]. This process is an active biochemical response which occurs independently of uterine contractions and is similar to an inflammatory reaction. Cervical ripening involves interactions between various cellular compartments and the extracellular matrix, as well as properly timed biochemical cascades and stromal infiltration by inflammatory cells [26,65].

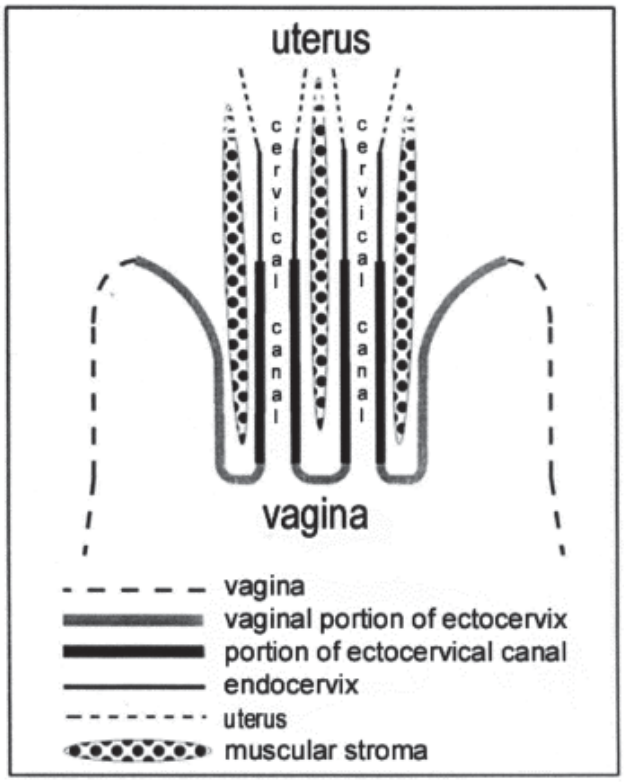


Figure 1. Schematic representation of the rat uterine cervix. As in most other species, including humans, connective tissue was the predomintat component, with smooth muscle accounting for 10-15% of the rat cervical tissue.

The uterine cervix is a complex organ that undergoes extensive histoarchitectural changes to allow its successful adaptation to the different physiological conditions mentioned above [11,33,36,71]. As shown in the organization of rat cervical tissue (Fig.1), the cervix is composed predominantly of connective tissue [9,10]. The rate and extent to which the cervix must dilate and efface to allow expulsion of the fetus(es) vary among species [6]. Since, in some species, a considerable number of muscle fibers is observed in the cervix, it was initially believed that these fibers were responsible for the physiological valve function of the cervix [57]. Although smooth muscle fibers may participate in the functional mechanisms of the uterine cervix, it is the connective tissue that confers the required mechanical properties to this segment [18]. This conclusion is reinforced by observations in species in which the muscular component is either scant or absent. Connective tissues have a predominantly mechanical function and must be able to withstand high tensile or compressional stress and to recover their shape and form when the stress is removed [50]. Collagen-containing fibers add strength to the tissues, whereas elastin and proteoglycans are essential for matrix resiliency.

During parturition, the increased amount of water present obviously helps to soften the uterine cervix, but proper dilation is dependent on changes in collagen-containing fibers and other components of the extracellular matrix [26,29,39,40,55,69]. It is, therefore, not surprising that connective tissue is the major component of the uterine cervix (Figs. 1 and 2), and that collagen fibers play an important role in the functions of this structure. Elastic fibers, the second most important fibrillar component of the cervical connective tissue, protect against rupture during dilation/parturition, thus guaranteeing the anatomical integrity and continuity between the uterus and vagina during delivery. These fibers may also be involved in the recovery of shape during the post-partum period, particularly in view of their ability to undergo reversible extension.

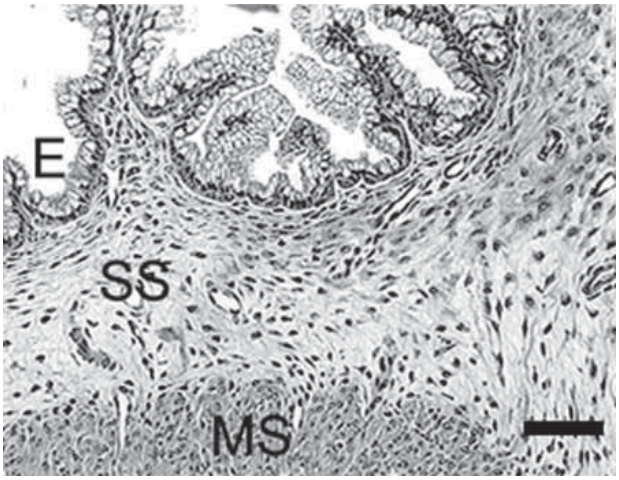


Figure 2. Histological section stained with hematoxylin-eosin showing the different tissue compartments of the guinea pig uterine cervix: The subepithelial stroma **(SS)** is the area of connective tissue from the basement membrane to the muscle layer **(MS)**. The **SS** occupies most of the cervical tissue and collagen fibers play an important role in the physiological adaptation of the organ. **E** - epithelium, **SS** - subepithelial stroma, **MS** - smooth muscular stroma. Bar = 150 μm.

CERVICAL RIPENING: INVOLVEMENT OF COLLAGENOLYSIS AND POLYMORPHONUCLEAR LEUKOCYTE INFILTRATION

The dilation and effacement (ripening) of the cervix are necessary prerequisites for a normal vaginal delivery [35]. Cervical dilation is accompanied by disruption of the ordered collagen bundles (Fig. 3A, C). The cellular response involves modifications of the extracellular matrix, with cervical stromal cells and immune cells being responsible, at least in part, for the enzymatic remodeling of fibrous connective tissue [26,37-42,47,67].

The physiological mechanisms involved in the onset of ripening at term are still unclear, although collagenase has been implicated in this process. There is good evidence that the activity of collagenase and other proteolytic enzymes increases at term [28,51]. One of the sources of these enzymes could be the infiltrating neutrophils seen in women, sheep and guinea-pigs [21,26,37,47,49] and the eosinophils seen in rats [38,40,54]. A close spatial and temporal association between the infiltration of eosinophils and collagenolysis has been observed in the cervical stroma of rats during parturition (Fig. 3C,D) [40,54].

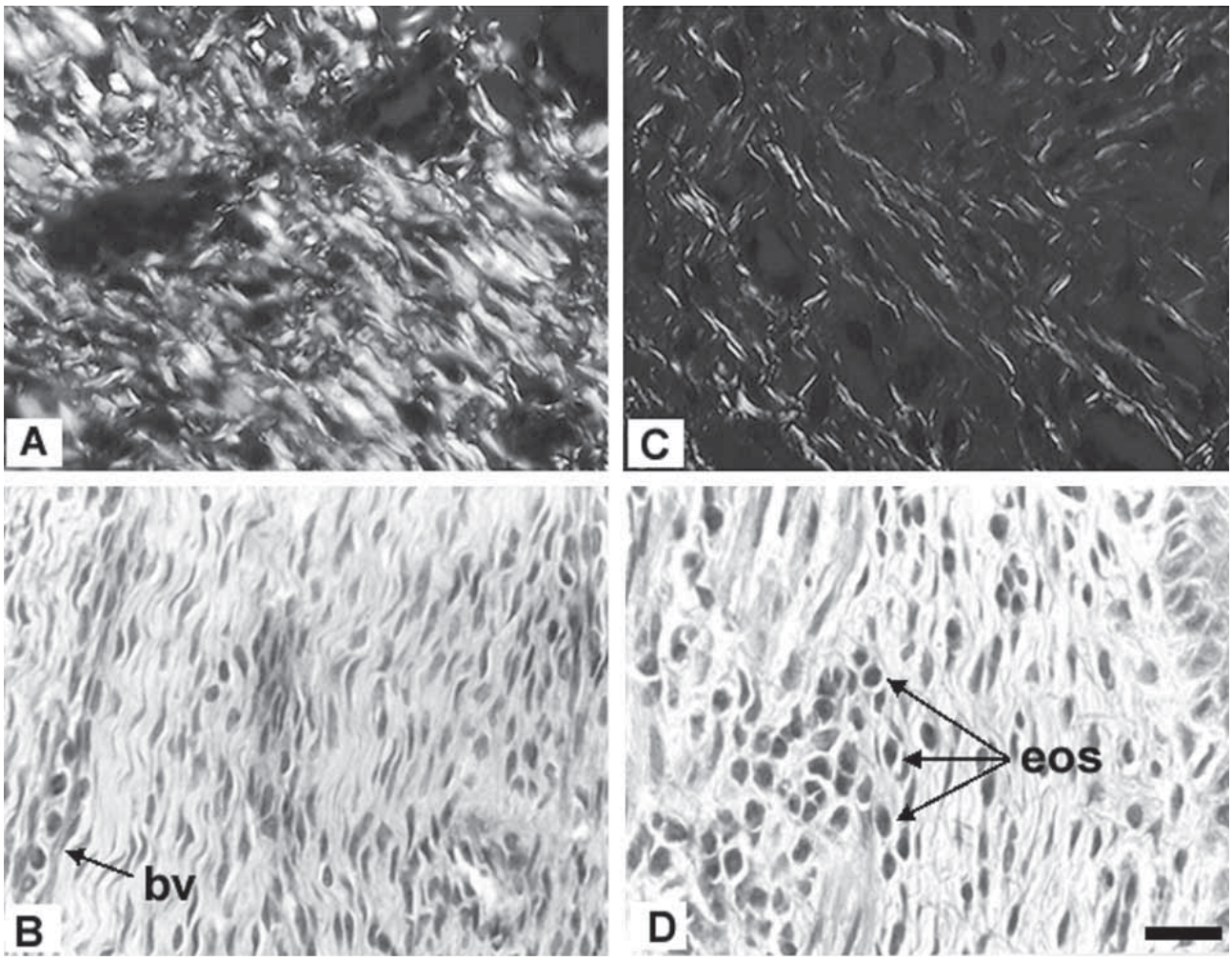


Figure 3. Photomicrographs of nonpregnant **(A** and **B)** and intrapartum **(C** and **D)** rat cervix. In the upper panel the sections were studied by the Picrosirius-polarization method, a specific procedure to detect oriented collagenous structures. All brightly birefringent structures shining against a dark background were collagenous material **(A).** Section of a nonpregnant control cervix in which collagen appears as thick bundles of continuous, densely packed fibers. Compare the regular arrangement of the collagen fibers in **(A)** with the disturbed appearance of the corroded collagenous framework seen in an intrapartum sample **(C)**. In the latter, the collagenous fibers showed the fragmented and irregular appearance characteristic of collagen remodeling. The section in the lower panel were stained with Sirius red in alkaline solution in order to detect eosinophils. In the nonpregnant cervix **(B)**, eosinophils were limited to blood vessels **(bv)** and no eosinophils were seen in the stroma. In the intrapartum sample **(D)**, a heavy eosinophilic infiltration **(eos)** was observed in the connective tissue. Bar = 35 μm.

The presence of polymorphonuclear leukocytes in cervical tissue during parturition has been confirmed by electron microscopy [21,26,37,38] and by immunohistochemistry using specific antibodies against granule-associated neutrophil enzymes or against eosinophil major basic protein [12,47]. In addition, polymorphonuclear leukocytes degranulate in the cervical stroma at term and this event coincides with the widespread collagenolysis seen in the extracellular matrix [26,38]. The changes in the organization of cervical collagen fibers have been quantified [39,40] using the picrosirius-polarization method which allows the morphometric analysis of collagen fiber organization [25,43]. This method is specific for orientated collagen molecules since only these structures show a bright birefringence [43]. Collagen fibers normally form thick bundles of densely packed, regularly arranged fibers that appear as brightly birefringent structures throughout the entire microscopic field. During collagen remodeling, collagen fibers are not dense or regularly arranged and show weak birefringence. Figure 3 shows the appearance of nonpregnant (A) and intrapartum (C) rat cervix studied with the picrosirius-polarization method. Note that the greater the collagen remodeling, the lower the birefringence.

Electron microscopic examination of human [26], rat [38] and ewe [37] cervical stroma during labor has revealed dramatic changes in the fine structure of collagen. The regular arrangement of collagen fibrils, which is typical of nonpregnant cervix, is markedly disturbed in intrapartum cervical biopsy specimens (Fig. 4A,B). Electron micrographs of collagen degradation show fragmented collagen fibrils which, in cross-section, have a ragged, irregular outline. Fibroblast are the main cell type in cervical tissue from nonpregnant rats, ewes, guinea pigs and women (Fig. 3B). In intrapartum samples there is extensive polymorphonuclear leukocyte infiltration of the cervical stroma (Fig. 3D) with few mast cells and macrophages, in addition to the fibroblasts. Light and electron microscopy have revealed a series of characteristics in the appearance and distribution of these polymorphonuclear leukocytes. The identity of polymorphonuclear leukocytes which varies among species, was confirmed by electron microscopy, immunohistochemistry and special staining techniques [52]. There is currently no explanation for the differences in the types of polymorphonuclear leukocytes that infiltrate each species or for the variations in their migration patterns. In the ewe, neutrophils migrate towards the cervical lumen since their highest number occurs in the luminal mucus [37]. These neutrophils may be responsible for preventing uterine infections associated with parturition since they make the cervical mucus less penetrable to microorganisms.

THE MODULATION OF LEUKOCYTE INFILTRATION BY ESTROGEN AND PROGESTERONE AND THE INVOLVEMENT OF RELAXIN IN COLLAGEN REMODELING

The uterine cervix is a dynamic structure with a high capacity to adapt to different, often opposing, roles during the physiological events associated with gestation, parturition and postpartum recovery. To achieve this adaptation, the cervix responds to changes in hormone levels [5,16,39,61]. Progesterone (P_4) is essential for the maintenance of pregnancy in most, if not all, eutherian mammals [62] and reduces the ability of the female to combat intrauterine bacterial infections [20,27,58]. This latter effect is thought to result, at least in part, from the P_4-dependent reduction in uterine leukocyte infiltration [2,21,63].

Parturition in rats and sheep is preceded by a fall in P_4 and an increase in estradiol (E_2) plasma levels, with both steroids being implicated in the regulation of cervical softening in sheep [30,48]. Estradiol stimulates whereas P_4 inhibits the infiltration of eosinophils in the rat cervix at term [39,40]. Neither estrogen nor P_4 alone is responsible for collagen remodeling. Rather, this response is mediated by relaxin, a hormone with a major role in promoting the growth and widespread reorganization of collagen fibers in the rat cervix [7,23,31,39]. Estradiol-induced eosinophil infiltration in the rat cervix is blocked by tamoxifen [54], an antagonist that interacts with the estrogen receptor (ER). Progesterone also antagonizes the effect of E_2. The anti-progestin RU-486 blocks the inhibitory action of P_4 on eosinophil infiltration in the cervix [54], thus suggesting that the effect of P_4 is mediated by the progestin receptor (PR). The antagonistic actions of both steroids explain the time course of the leukocyte invasion in intact pregnant rats during the last days of pregnancy. Following the decrease in P_4 levels that occurs during the last 36 h of pregnancy in the rat [45], the increased E_2 levels act through the ER promoting the massive infiltration seen in cervical tissue at term.

In addition to the serum steroid hormone levels, the level of the receptors for these mediators also play

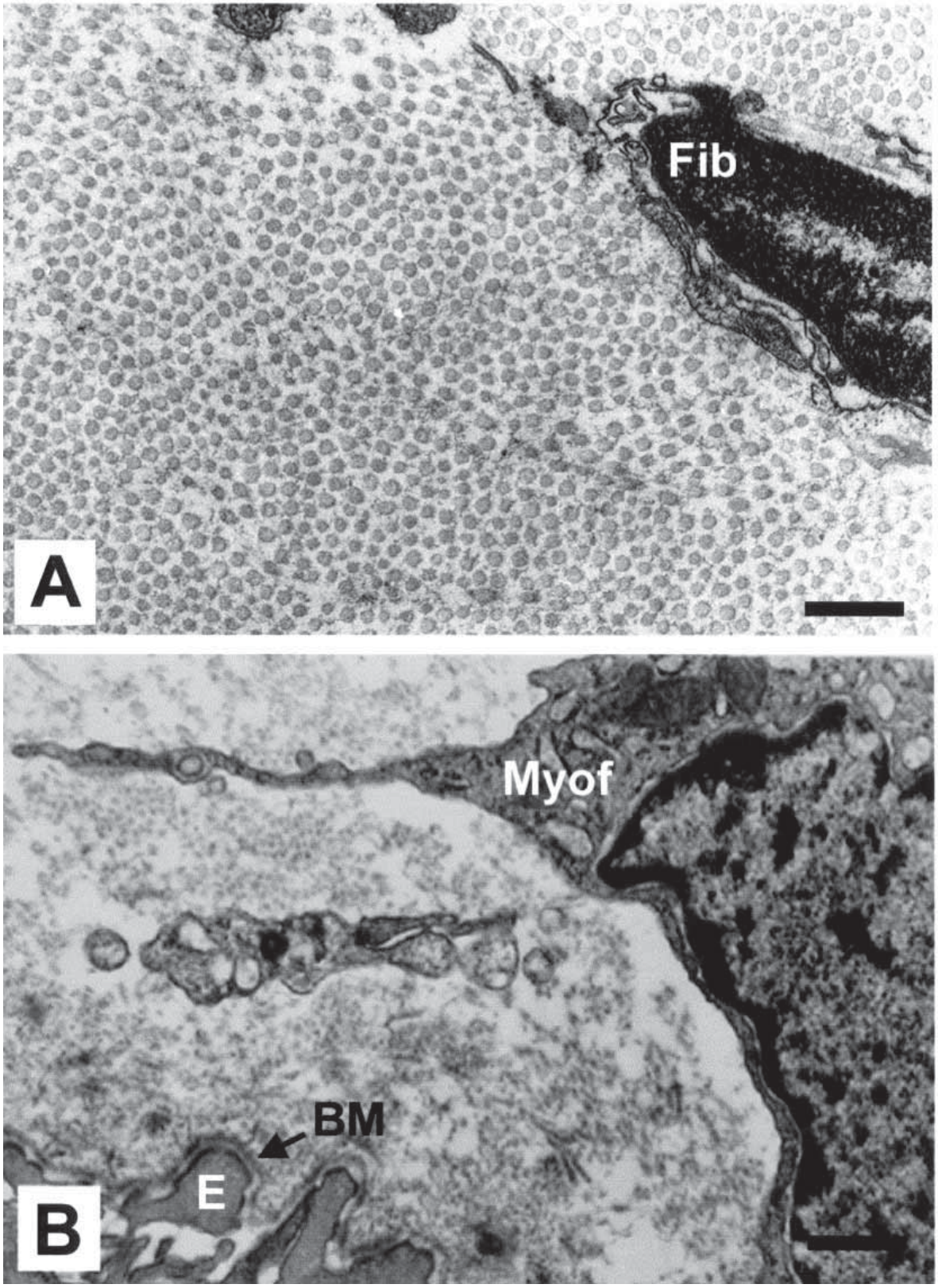


Figure 4. Photomicrographs of a nonpregnant **(A)** and intrapartum **(B)** rat cervix studied by electron microscopy. **(A)** A fibroblast **(Fib)** surrounded by collagen fibers showing the regular arrangement characteristic of nonpregnant cervical tissue. Bar = 1.3 µm. **(B)** A myofibroblast **(Myof)** and collagen fibers with a disturbed appearance and corroded framework characteristic of collagen remodeling in intrapartum cervical tisssue. A cervical epithelial cell **(E)** and the basement membrane **(BM)** are shown. Bar = 0.5 µm.

pivotal role in the histofunctional changes of the cervix. Thus, in humans and guinea pigs, in which no significant changes in the serum concentrations of either E_2 or P_4 occur immediately before parturition [68], there is a significant down-regulation of PR and ERα [56,64]. Recently, an increase in ERβ but not ERα mRNA has been reported in the human cervix at term [72]. Thus, the onset of parturition may involve changes in the responsiveness of the uterus and cervix to P_4 and E_2 through alterations in their receptor density [56].

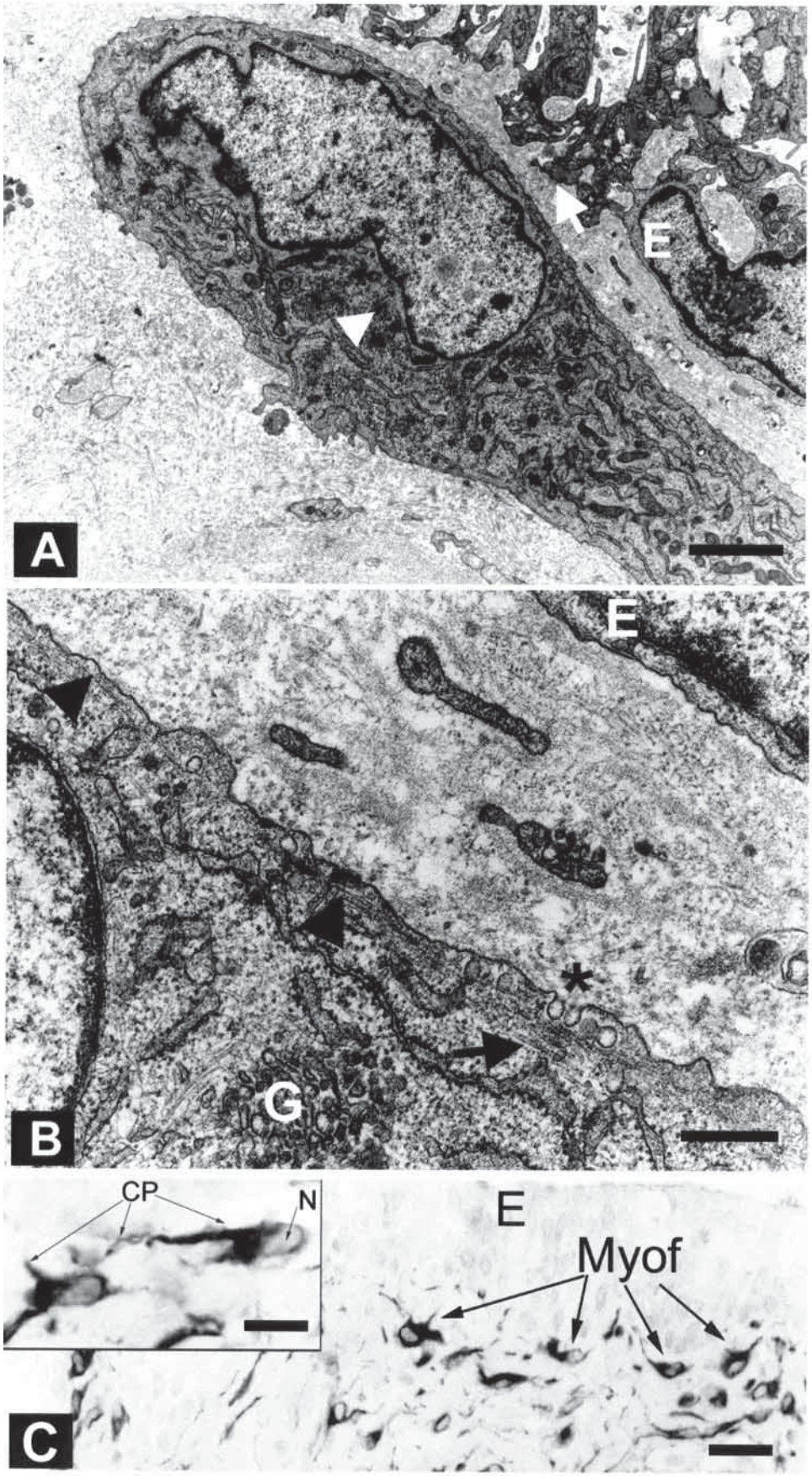
For ethical reasons, time course studies during the entire gestation cannot be done in humans, so an animal model with responses similar to those in humans would be a valuable tool for studying temporal and spatial changes in the expression of ER and PR in the uterus and cervix from mid pregnancy to early postpartum. In our laboratory, the guinea pig has been used to study the profile of ERα and PR expression and collagen remodeling (as an indicator of functional changes) in different regions of the uterus and cervix during pregnancy, parturition and postpartum [56]. Our findings indicate that, in the presence of high levels of P_4 and E_2, the diminished target organ responsiveness to P_4 before parturition may be caused by a decrease in PR levels in the subepithelium and muscular region. Alterations in PR levels could help to coordinate cervical dilatation and uterine contractions. Indeed, it has been hypothesised [8,16] that a reduction in P_4–mediated inhibition, either through a decrease in hormone production (rats, rabbits, sheep) or in hormone activity in the target organs (primates, guinea-pigs), is the major mechanism for initiating parturition. Our results [56] are consistent with the P_4 withdrawal theory of parturition.

PHENOTYPIC MODULATION OF FIBROBLASTS AND CELL TURNOVER IN THE CELLULAR COMPARTMENTS OF THE UTERINE CERVIX

As already mentioned, connective tissue cells and the extracellular matrix play important roles in cervical functions. Fibroblasts, the most common cells in the connective tissue, show marked phenotypic plasticity in architecture and biochemical composition in various physiological and pathological situations [59,60]. Changes in the cytoskeletal elements are prominent features in the morphological alterations in fibroblasts with desmin, vimentin and α-smooth muscle actin (α-SMA) frequently being expressed in specific pathways of differentiation [4,22]. The contractile machinery represented by cytoskeletal structures such as microfilaments and intermediate filaments provides characteristic ultrastructural features that are useful for defining the myofibroblast [13,14]. We have examined the ultrastructural and immunohistochemical characteristics of fibroblasts in the mucous layer of uterine cervices from rats [70] and women [44]. The differential expression of cytoskeletal proteins, and the ultrastructural features seen in both species, indicate that the resident fibroblasts seen in the mucous layer of nonpregnant cervices are replaced by a typical myofibroblastic-cell phenotype characteristic of intrapartum tissue (Figs. 4B and 5). The implications of the plasticity of fibroblastic-myofibroblastic cells in the physiological changes seen in the uterine cervix during pregnancy, labour and postpartum involution require further investigation.

In addition to the phenotypic modulation of fibroblastic cells, adaptative changes in the uterine cervix during pregnancy imply a dynamic cell turnover. In rats, the proliferation and death of epithelial and stromal cells during pregnancy, at term and in early postpartum are influenced by hormones [3,32,53,66,73]. In all stages studied, 1) ERα and PR have different patterns of expression and responses to the signals that modulate proliferation and/or apoptosis, depending on the cellular compartment, and 2) although the epithelium is the region with the highest cell turnover, the fibroblastic and muscle stroma are dynamic compartments with their own patterns of behavior [53].

Figure 5. Photomicrographs of myofibroblastic cells in the lamina propria of human uterine cervix during labor. Electron micrographs of a subepithelial myofibroblastic cell at low **(A)** and high **(B)** magnification. **(A)** The well-developed rough endoplasmic reticulum and the Golgi complex **(arrowhead)** indicate that the myofibroblast is actively involved in secretion. The luminal epithelium **(E)** and its basement membrane **(arrow)** can be seen. Bar = 2.5 μm. **(B)** A high magnification of the cell shown in **(A)**, with cell surface features characteristic of myofibroblasts, including a subplasmalemmal web of filaments **(arrowheads)** and microtubules **(arrow)** and plasmalemmal caveolae with pinocytotic vesicles **(asterisk)**. **E** - epithelium, **G** - Golgi complex. Bar = 0.4 μm. **(C)** Myofibroblastic cells **(Myof)** immunostained for desmin show a halo arround the nucleus **(N)** that extends through the cytoplasm up to the cell membrane. The inset shows cytoplasmic processes **(CP)** that are an important characteristic of myofibroblastic cells. **E** - epithelium. Bar = 25 μm, inset = 10 μm.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MAST CELLS ARE INVOLVED IN CERVICAL ANGIOGENESIS

Cervical ripening is an extremely complex process that involves interactions between different cellular compartments and the extracellular matrix, as well as properly timed biochemical cascades, and the infiltration of inflammatory cells into the stroma. Cervical ripening is an energy-dependent process that requires an adequate supply of nutrients. The vascular system and new vessel formation (angiogenesis) are critical for the cervical histo-architectural changes that are necessary for a successful vaginal delivery [24,34,Varayoud *et al*., unpublished results]. Endothelial cell proliferation assessed by BrdU incorporation and measurement of the vascular area showed a significant increase in the subepithelial and muscular cervical stroma at the end of gestation in the rat [Varayoud *et al*., unpublished results]. Although angiogenesis is essentially an endothelial cell event, other cell types and various mediators are involved in this process [17]. Studies *in vitro* and *in vivo* have implicated mast cells in angiogenesis [34,46]. We have also demonstrated that mast cells are involved in cervical angiogenesis since the inhibition of mast cell degranulation results in a significant decrease in endothelial cell proliferation and in the vascular area [Varayoud *et al*., unpublished results]. A better understanding of the regulation of angiogenesis, would allow the development of therapeutic strategies for controlling cervical function.

CERVICAL RECOVERING AFTER DELIVERY

Apoptosis is a predominant event during postpartum cervical involution and may contribute to the recovery of the uterine cervix after delivery [53]. In addition, water resorption and a decrease in the levels of some proteoglycans contribute to this process [19,29]. However, none of these events can account for the recovery in uterine cervix shape that occurs immediately after parturition. The intrinsic mechanical properties of elastic fibers suggests that these structures may be involved in cervical rigidity and in the recovery of form immediately after delivery. In agreement with this, we recently reported an increase in the elastic system fibers in the uterine cervix at the end of pregnancy [1].

CONCLUSIONS

Understanding the complex cellular and molecular biology underlying the dynamic function of the uterine cervix is a basic challenge for studies investigating the physiology of gestation and parturition. During pregnancy, extensive tissue remodeling involves both the extracellular matrix and cells of the cervical tissue. The cellular and extracellular compartments must rapidly adapt to new functional demands imposed by gestation and parturition, and then subsequently return to their original state during the period of involution. In this review, we have discussed the histofunctional features observed in the uterine cervix during gestation, parturition and early postpartum, and have stressed the key role of collagen remodeling in adaptations to different functional demands, particularly during cervical ripening. Despite advances in our knowledge of cervical ripening, the precise signals and hormonal control responsible for initiating these changes remain to be fully elucidated. If we can understand the mechanisms responsible for these changes, then we may be better able to address complex phenomena such as cervical incompetence, pre- and post-term delivery, and proper "ripening" of the cervix.

ACKNOWLEDGMENTS

This review is dedicated to the memory of Professor Gregorio S. Montes (1952-2002) who was an invaluable source of advice, assistance, and moral support in our work. Professor Montes instilled in us his passion for the impossible and introduced us to research in collagen biology. He nourished our work through his insights and valuable critiques; and this is reflected in the many papers cited which were joint efforts between his and our laboratories. All LETH members are indebted to him for the time and effort he devoted to supporting the development and expansion of our research group. We were extremely priviledged to have enjoyed his friendship.

We also thank our fellows whose work contributed to many of the results discussed in this article. The studies described here were supported by grants from the Argentine National Council for Science and Technology (CONICET), the Universidad Nacional del Litoral, SECyT-CAPES, and the Argentine National Agency for the Promotion of Science and Technology (ANPCyT).

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Received: April 30, 2003
Accepted: July 1, 2003