# Fibrous-long Spacing Collagen in Post-partum Rat Cervix Uteri

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Fibrous-long spacings (FLS) are one of atypical or artificial forms of collagen fibrils. We analyzed occurrence of this form of interstitial collagens of cervix uteri in pregnant, nonpregnant and post-partum white Wistar rats. Light and electron microscopy were used.
Extensive collagenolysis in rat cervical stroma on the eve of labour and in close post-partum period was found. Extravasated activated eosinophils participated in this process. In the solid milieu of massive collagenolysis FLS were found. FLS were not found in nonpregnant cervix ureri. Administration of bleomycin had no influence on collagen degradation rate or on FLS formation.

**Key words:** collagen, fibrous-long spacings, rat, cervix uteri

## INTRODUCTION

Collagen is a heterogeneous and most abundant structural protein found in animal connective tissues. Many types of collagen forms very long fibrils with a characteristic axial periodic structure. Strong fibrils provide the major biomechanical scaffold for cells and tissues. Collagen fibril formation is basically a self-assembly extracellular process (1) to a large extent determined by the intrinsic properties of the collagen molecules themselves, but it is also sensitive to cell-mediated regulation. Only the molecules on fiber surface are available for interaction.

Fibrous-long spacing collagen (FLS) fibrils are collagen fibrils that display a banding with a periodicity greater than 67 nm of native collagen. FLS fibrils can be formed *in vitro* by changing the chemical assembly environment (2). Our previous study (3) has shown that FLS are formed *in vivo* in post-partum involution changes in matrices of cervix uteri in rats. Collagen degradation in this case was mostly attributed to eosinophils. The mechanism of FLS fibrillogenesis is poorly elucidated and not well studied. Until the pathological or physiological significance of FLS collagen *in vivo* has yet to be determined, clarification of its structure and occurrence are very important issues. The aim of this study was a further elucidation of FLS occurrence in post-partum cervix uteri, in the period of massive collagen degradation.

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## MATERIALS AND METHODS

### Animals

Young adult Wistar white female rats were housed in cages separately from males. Usual laboratory diet and water were available *ad libitum.* Mating opportunity was provided at the appropriate time. Pregnant rats were randomly divided into two groups containing 6 females in each. One pregnant group and an equal unmated group served as controls. The first pregnant group on the 5th day of gestation was treated by 1 mg subcutaneous injection of bleomycin, an agent known as enhancing the fibrotic process, and the 2nd group received 30 mg of D-penicillamine *per os* with the purpose to diminish collagen fibrillogenesis. One half of pregnant and nonpregnant control rats were sacrified on the 18th day of gestation and the second half 2 days after parturition. Ether anesthesia was used.

### Methods

The cervices were divided into two parts. One part was fixed in 10% neutral formalin solution, dehydrated, embedded in paraffin and cut into 5-μm-thick sections. Deparaffinized sections were stained with hematoxylin and eosin (HE).

The second part was fixed in 2% glutaraldehyde dissolved in 0.15 M phosphate buffer at pH 7.2 for 2 h, followed by postfixation in 1% osmium tetroxide for 1 h and overnight block staining in 1% uranyl acetate dissolved in 70% ethanol. The samples were embedded in Epon resin, thin-sectioned in LKB ultratome, double-stained by uranyl acetate and lead citrate, and examined on a JEM-100B JEOL electron microscope.

## RESULTS

The samples of cervix obtained from nonpregnant controls contained no eosinophil infiltrates in stroma and no unusual presence of eosinophils in blood vessel lumina. Cervix fibrillar collagen bundles had a normal structure and regular arrangement in solid milieu (Fig. 1). The dominating, not numerous cells in abundant stroma were fibroblasts. There were no evident structural signes of collagen depolymerization or collagenolysis.

At the 18th day of gestation the cervices contained moderate eosinophil infiltrates. A rather strong predominant orientation of collagen fibers characteristic of a nonpregnant cervix uteri was much less evident. Oedema was pronounced, but there was no strong evidence of collagenolysis around eosinophils or separated extruded eosinophil granules in the stroma.

On the second day after parturition an extensive halo of degraded collagen around eosinophils (Fig. 2) and around their extruded granules was seen. At that time massive collagen depolymerization as well as FLS fibrils occurred (Fig. 3). The stroma of the cervix uteri was strongly edematous and many collagen fibers were disarranged.

Administration of bleomycin evoked no dramatic changes in the structure of cervix uteri typical for the course of pregnancy and post-partum period.

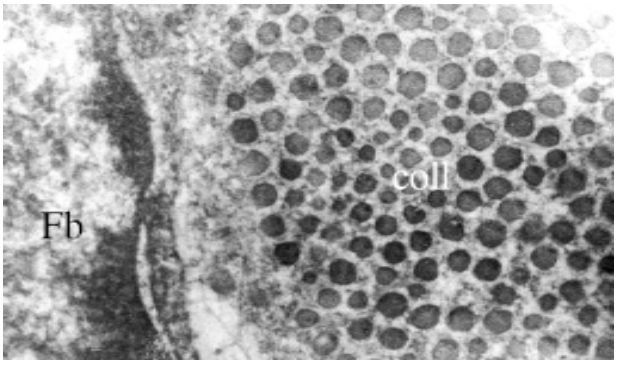


Fig. 1. Collagen stroma of cervix uteri of nonpregnant rat. ×50000. coll – collagen fibers; Fb – fibroblast

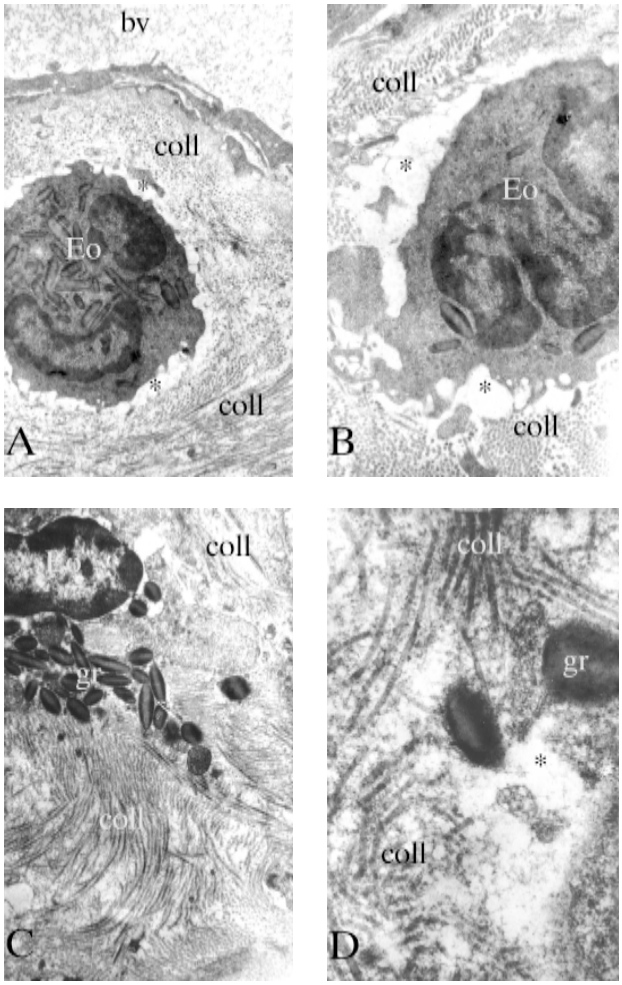


Fig. 2. Eosinophils in post-partum cervix uteri. A – collagen-free halo formation (asteriscs) around extravasated eosinophil. ×8000. B – the same in progress. ×10000. C – free eosinophil granules (arrows) among collagen fibers. x10000. D – collagen depolymerization around eosinophil granules. ×20000. Eo – eosinophil, gr – eosinophil granules, coll – collagen, bv – blood vessel

## DISCUSSION

Fibrous-long spacing collagen is a distinct ultrastructural form of collagen present in normal tissue, various tumors, and tissues degraded by bacterial collagenases *in vivo* and *in vitro* (4). An association between FLS collagen and endothelial cells in normal tissue and in certain vascular proliferations also appears to exist. Abundant FLS were found around the chondrocytes in the rabbit ear cartilage after intravenous papain administration, unmasking native collagen fibers from the normally covering proteoglycans muff (5). In experimental FLS formation *in vivo* it was found that these atypical cross-striated dark bands had about 91 nm periodicity and had longitudinally aligned filaments with a diameter of about 6.5 nm (6). FLS fibrils formed *in vitro* by

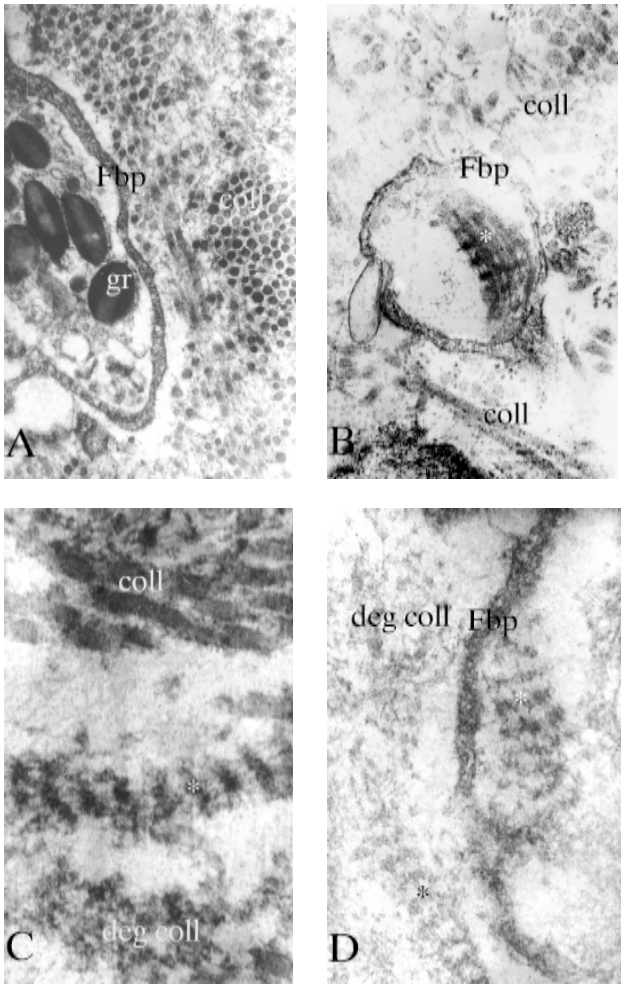


Fig. 3. Fibrous-long spacings (asteriscs) in the degrading stroma. A – eosinophil granules encircled by fibroblast processus. ×20000. B – FLS formation in fibroblast-encircled milieu. ×40000. C – FLS formation in open milieu. ×500000. D – the same in progress. ×30000. Fbp – fibroblast processus, gr – eosinophile granules, coll – collagen fibers, deg coll – degrading collagen

addition of $\alpha_1$-acid glycoprotein to an acidified solution of monomeric collagen were typically ~150 nm in diameter and had a distinct banding pattern with a 250-nm periodicity. Protofibrils were aligned along the main fibril axis. The alignment of protofibrils produced grooves along the main fibril, which were 2 nm deep and 20 nm wide (7). Examination by atomic force microscopy of the tips of FLS suggested that they grew via the merging of protofibrils to the tip, followed by the entanglement and, ultimately, by a tight packing of protofibrils. For comparison, an individual normal type I collagen molecule is a semiflexible rod ~280 nm in length and ~1 nm in diameter. A self-assembled native fibril has a banding pattern with a ~67 nm period (8).

Two major hypotheses of the origin of *in vivo* FLS fibrils have been proposed (9, 10). The first suggests that FLS fibrils are the degradation product formed by the activity of endogenous collagenases upon reticular collagen fibers. The second suggests that FLS form as a result of interactions between immature collagen microfibrils and acid mucopolysaccharides present in tissues. Evidence exists for both views (6, 11). It has been speculated that FLS fibrils are formed in tissue by disassembly of normal collagen and subsequent reassembly into FLS in presence of glycoproteins or proteoglycans (9, 10). This was not confirmed by other investigators (2, 12). The situation is complicated by the fact that the collagen superfamily of proteins includes more than 20 collagen types with altogether at least 38 distinct polypeptide chains, and more that 15 additional proteins that have collagen-like domains (13). Not all collagens have a fibrillar structure, and not all of them form atypical fibrils in the same manner, if at all (14).

Eosinophils are not only involved in the classically known effects of host defense against parasite infections and hypersensitivity reaction, but they also take part in many immune and nonimmune conditions.They are specifically involved in the activation of estrogens, glucocorticoids and other hormones (15). Eosinophil collagenase degrades in a type-specific manner some matrix collagens (16, 17). Extracellular deposition of eosinophil granules means an expression of its activation (18). It is known that during gestation and parturition the uterine cervix undergoes profound histologic changes. Eosinophil granulocytes are present in cervix uteri under different conditions (19, 20).

In our experiments, in the final stage of gestation and shortly after parturition an abundance of eosinophils in rat cervix uteri matrix was found. In the places of intense collagenolysis much of FLS collagen fibrils were observed. It was possible to suspect cooperation between eosinophils and fibroblasts in the process of FLS formation. Interestingly, bleomycin known to interfere with collagen metabolism did not influence FLS fibril occurrence in pregnant and post-partum rats cervix uteri matrix.

## CONCLUSIONS

A widespread collagenolysis in rat cervical stroma compared with nonpregnant controls was found at term. Activated eosinophils were involved in this process. In the solid milieu of intense collagenolysis many FLS fibrils were found. Bleomycin injections did not interfere with the FLS formation rate.

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**FLS TIPO KOLAGENO FIBRILĖS ŽIURKIŲ GIMDOS KAKLELYJE POGIMDYMINIU LAIKOTARPIU**

S a n t r a u k a

FLS tipo kolageno dariniai – tai atipiškos ar dirbtinės kolageno fibrilės. Mes ištyrėme FLS fibrilių atsiradimą žiurkių gimdos kaklelio stromoje prieš gimdymą ir tuoj po jo, taip pat nenėščioms žiurkėms.

Nustatėme išplitusią kolagenolizę baigiamuoju nėštumo ir ankstyvuoju pogimdyminiu laikotarpiu. Kolagenolizės procese dalyvavo aktyvuoti eozinofilai. Išplitusios kolagenolizės terpėje radome daug FLS fibrilių. Tuo tarpu nenėščių žiurkių kaklelių stromoje FLS tipo darinių neradome. Bleomicino injekcijos neturėjo įtakos FLS fibrilių formavimuisi ir kolagenolizės intensyvumui.

**Raktažodžiai:** kolagenas, FLS tipo fibrilės, žiurkės, gimdos kaklelis