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DIFFERENTIAL DISTRIBUTION OF ELASTIC SYSTEM FIBERS IN THE PUBIC SYMPHYSIS OF MICE DURING PREGNANCY, PARTUM AND POST-PARTUM

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ABSTRACT
The expansion of the pubic symphysis during pregnancy in some rodent species depends on the growth of the interpubic ligament, primarily through the biosynthesis of extracellular matrix. Although there is a significant metabolism of elastin in the female reproductive tract during pregnancy, little is known of the architectural and ultrastructural aspects of the elastic system fibers in the mouse pubic symphysis. In this study, the main characteristics of the elastic system of the mouse pubic symphysis during pregnancy, partum and post-partum were determined by light and electron microscopy. A distinct arrangement of microfibrils, elastin deposition and development of the thin elastic fiber network was observed during pregnancy and after delivery. The elastic system fibers of the extracellular matrix formed a supporting framework that uniformly distributed stress in order to provide adequate interpubic resilience during delivery. These changes support a role for elastic system fibers in symphyseal maturation and reconstruction during pregnancy and after delivery.

Key words: Elastic system fibers, ligament, pubic symphysis, ultrastructure

INTRODUCTION

The pelvis serves as a support for the vertebral column (in bipeds) and for articulation with the lower limbs. This structure is formed by the innominate bones, the sacrum, the coccyx and the joints and ligaments which unite them. In females, the internal diameter of the pelvis is intimately related to delivery, since the pelvic width limits the maximum size of the fetus compatible with normal parturition [19,34,47]. During pregnancy, structural modifications of the pelvic girdle occur in many mammalian species, including rodents, bats and humans, to enable both animal deambulation and safe passage of the fetuses at birth [14,19,39].

The pubic joint is formed by the union of the medial ends of the two pubic bones. The structure of the pubic joint varies among species, between sexes and undergoes varying degress of adaptation during pregnancy [1,14,16,34]. In rats and male guinea pigs, the bones are covered by caps of hyaline cartilage that blend in the middle [15,34]. In young, fertile female guinea pigs and mice (both sexes), the bones are also covered by caps of hyaline cartilage joined by a disk of fibrocartilaginous tissue [22,23,26, 37,44,45]. According to Crelin [13,16], in male bats "the ends of the coxal bones at the joint are less than 1 mm apart and capped with hypertrophic hyaline cartilage that is continuous with a compact lamina of fibroelastic cartilage in the center of the joint". However, in adult female, "the ends of the coxal bones at the pubic joint are interconnected by an interpubic ligament". During pregnancy, the interpubic ligament in bats undergoes minimal enlargement; whereas no structural change is seen in rats [34]; in mice and guinea pigs, however, a ligament appears in the middle of the joint [14,22,23,38].

The structural modifications of the pelvic symphysis involve hormonally regulated adaptations of the connective tissue mediated by estrogen and relaxin [12,15,41,42,44,45]. The pubic symphyses of mice and guinea pigs are classical models for study of the connective tissue and the transformations induced by hormones [39].

As in the uterine cervix [25,28], the collagen-containing fibers and glycosaminoglycans of the interpubic ligament undergo major alterations close to term to become malleable and distensible in order to accommodate the conceptus during delivery [43,46,48,49,51].

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Connective tissues consist of fibrous components, mainly collagenous and elastic system fibers, proteoglycans, glycosaminoglycans, water and minerals, and have a predominantly mechanical function. This tissue can withstand high tensile or compressional stress and can recover their form after stress [5,32,33]. Collagen-containing fibers provide strength whereas elastin and proteoglycans contribute to matrix resiliency. In mammals, mature elastic fibers have a central core of elastin surrounded by microfibrils (10-12 nm in diameter) which show an electron-dense tubular profile in cross-section [8,17,18,20,30-32].

The elastic properties of elastic system fibers in mammals are determined essentially by elastin [18,36]. Oxytalan fibers which consist solely of microfibrils without elastin, do not elongate under mechanical stress [10,35], a finding consistent with the fact that these fibers occur in areas where tissues, such as the bronchial mucosa [3], dermo-epidermal junction [11], tendons [5,6] and the ciliary zonule [10], are exposed to mechanical stress. Elaunin fibers, which contain both microfibrils and small amounts of elastin, display elastic properties intermediate to those of elastic and oxytalan fibers [9,31].

The architectural alterations during late pregnancy in mice are similar to those in guinea pigs [7,37, 38,48]. Histologically, they involve the "separation" of the pubic symphysis (gradual growth of the interpubic fibrous connective tissue) and the "relaxation" (breakdown or reorganization) of the connective tissue [44,45].

Numerous studies have shown that the hormonally regulated development of the mouse interpubic ligament consists of several distinct changes. During the last week of pregnancy the physiological changes involve: a) growth of a ligament, characterized by the deposition of coarse, densely packed collagenous fibers that are very well organized along the major axis of the symphysis, b) tissue remodeling and collagen degradation through the action of proteases (mainly metalloproteases), c) an increase in water-retaining extracellular matrix glycosaminoglycans, such as hyaluronate, that allow extra-hydration, that increases the pliancy of the tissue, and d) partial resorption of the medial ends of the pubic bones and cartilage caps [39,46,48,49,51].

While there is considerable histological and biochemical information regarding the symphyseal extracellular matrix of the pubic joint in mice, little is known of the involvement of elastic system fibers in the rapid and dramatic transformations that occur in symphyseal tissue during pregnancy. The presence of elastic system fibers in the pubic joint has been described only in rats [34] and free-tailed bats [13,16], although their arrangement and probable role in the increase in ligament length during delivery were not discussed. There is a large discrepancy between the width of the birth canal and the size of the free-tailed bats fetus. On the other hand, the increase in size length of the interpubic ligament during pregnancy is not very large. Thus, considerable distention of this structure must occur during delivery. Because of their reversible distensibility, elastic system fibers probably have a fundamental role in the function of the interpubic ligament in bats [13,16].

Since the pubic symphysis of mice is a classic model for studying connective tissue, and since no studies have examined the elastic system fibers of this structure in mice, we have examined the structure and organization of these in the pubic joint of virgin mice, as well as during pregnancy and the post-partum period.

MATERIAL AND METHODS

Virgin female Swiss mice 3 months old and overweight 25 g were obtained from the central animal house at UNICAMP. The mice were housed at 25 ± 2°C on 12 h light/dark cycle and had free access to standard rodent chow and tap water.

To obtain pregnant mice, virgin females were housed overnight with males. The presence of vaginal plug the following morning indicated successful mating and this day was considered the first day of pregnancy (D1). Delivery occurred 19 days after mating.

Samples of pubic symphysis were harvested from pregnant mice on days 12, 15, 17 and 18, immediately after delivery on day 19, and on the first (PP1), second (PP2), third (PP3), fourth (PP4) and fifth (PP5) days post-partum. Cycling virgin mice in estrus were used as controls. Estrus was determined by vaginal smears according to Shorr [40].

Tissue sample collection

For light microscopy, five mice were used for each experimental group, and three mice per group were studied for electron microscopy. The mice were sacrificed under deep anesthesia, as recommended elsewhere [21]. The pubic symphyses were dissected and fixed *in situ* with the appropriate fixative for 10 min. The specimens were then removed and immediately immersed in the same fixative.

Light microscopy

Specimens were fixed in 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4) for 24 h at 4°C, decalcified in 7% EDTA with 2% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4) for 5 days at 4°C, dehydrated in a graded ethanol series, embedded in paraffin and sectioned coronally at 7 μm intervals.

Serial sections were mounted on slides coated with poly-L-lysine (0.1% w/v in water; Sigma Chemical, St. Louis, MO, USA.) and dried for 24 h at 37°C.

The sections were stained with hematoxylin-eosin and Weigert's resorcin-fuchsin with prior oxidation. Oxidation was done using 10% aqueous oxone (monopersulphate compound, Du Pont, Wilmington, DE, USA) [31]. All three types of elastic system fibers (oxytalan, elaunin and the elastic fiber proper) are stained when the tissue is oxidized prior to staining with Weigert's resorcin-fuchsin [4,32].

Transmission electron microscopy

Small fragments of interpubic tissue were fixed in 2.5% glutaraldehyde dissolved in 0.1 M sodium cacodylate buffer (pH 7.2) containing 0.1% tannic acid for 2 h at room temperature, followed by post-fixation in 1% osmium tetroxide for 1 h at 4°C, and overnight block staining in 0.5% aqueous uranyl acetate. The samples were embedded in Epon resin, thin-sectioned in a Leica-UCT ultratome, double-stained with uranyl acetate and lead citrate, and examined with a LEO 906 electron microscope.

RESULTS

Light microscopy of coronal sections stained with hematoxylin and eosin showed that the pubic symphysis of mature cycling virgin and early pregnant (D12) mice consisted of a fibrocartilaginous disc lying between the hyaline cartilage-covered articular surfaces of the pubic bones. A layer of perichondrial tissue containing numerous fibroblasts covered the entire symphysis. Cartilage caps covered the bones and the chondrocytes were arranged along the osseous curvature. The medial fibrocartilaginous disk joined the two caps to fill all of the intervening spaces (Fig. 1).

The morphology of the pubic symphysis in virgin mice was quite similar to that of D12 pregnant females. From D15 on, a rapid proliferation of connective tissue cells with resorption of the pubic bones was observed. By D15 a ligament was present in the middle of the fibrocartilaginous disk, and gradually grew until D19. During this period, the collagen containing fibers changed from densely packed, thick fibers to thin fibers separated by large amounts of non-fibrous material containing highly hydrated glycosaminoglycans, mainly hyaluronic acid.

After delivery, the adaptations that occurred in the collagen-containing fibers and proteo- and glycosaminoglycans gradually restored the anatomy of the joint. However, the complete morphology of the articulation had still not been restored by this time. In the first days after delivery, the collagen-containing fibers partially regained their dense organization and islands of fibrocartilage were present in the middle of these fibers by the fifth day post-partum (PP5).

Staining with Weigert's resorcin-fuchsin after oxidation with oxone showed that the three types of fibers in the elastic system -oxytalan, elaunin and the elastic fiber proper- were present in all of the mice studied. In virgin and early pregnant mice (D12), a few very thin elastic system fibers were observed in the perichondrium, parallel to the major axis of the joint, and in the fibrocartilaginous disk, where they formed a meshwork that was oriented obliquely relative to the major axis of the joint (Fig. 2). Ultrastruc-

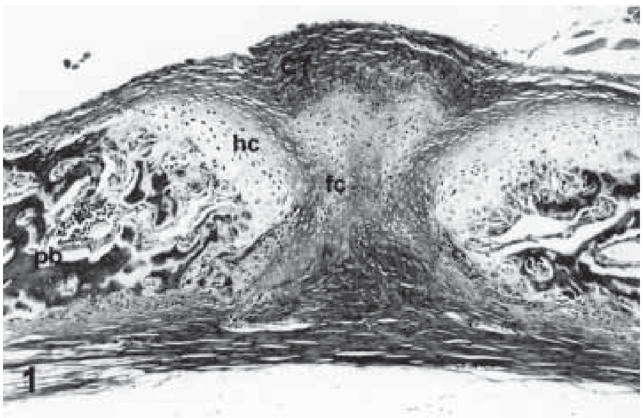


Figure 1. Coronal section through the pubic symphysis of a D12 pregnant mouse. The section was stained with hematoxylin and eosin. Note the symphyseal medial ends of the pubic bones (**pb**) with hyaline caps (**hc**) and the fibrocartilage (**fc**). The sheet of connective tissue (**CT**) forming the perichondrium can be seen in the upper region. Bar = 50 µm.

Figure 2. Serial sections of the pubic symphysis on D12 of pregnancy stained with Weigert's resorcin-fuchsin after oxidation. The detail of the hyaline cap (**hc**) and fibrocartilaginous disk shows a small number of thin fibers of the elastic system stained in black (**arrowheads**). Note the fibers arranged obliquely relative to the direction of the pubic bone separation. Bar = 20 µm.

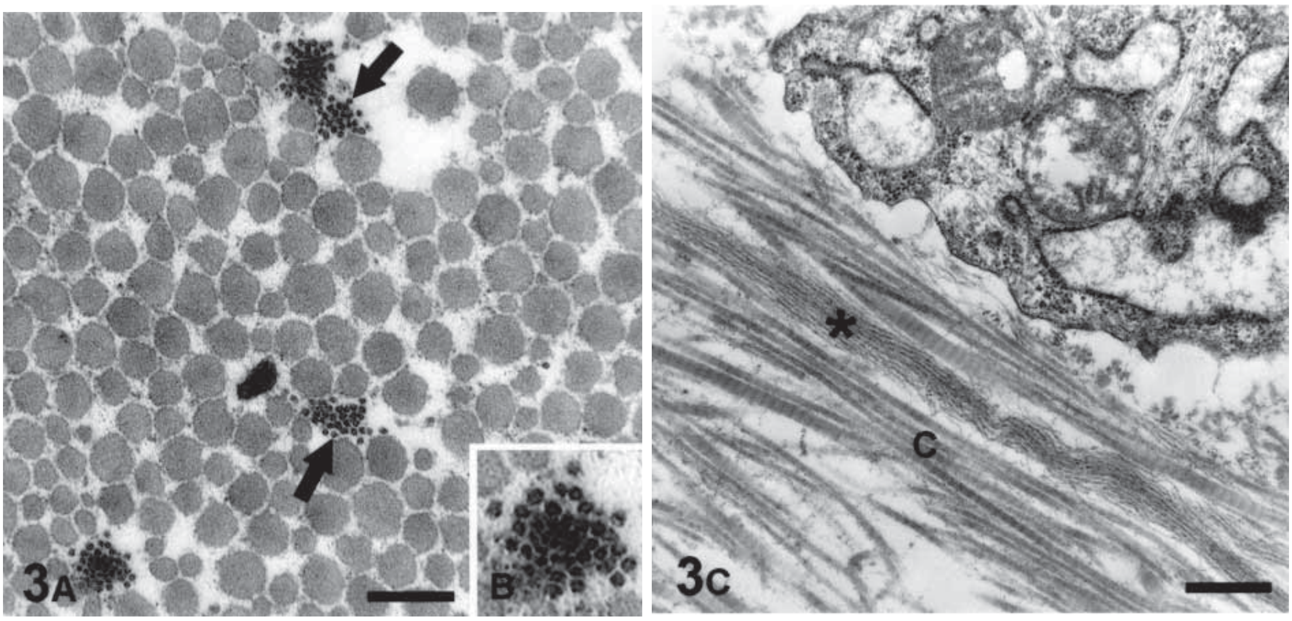


Figure 3. Transmission electron micrographs of a thin, logitudinal section of the pubic symphysis on D12: **A** is a cross-section of fibers of the elastic system and shows the same pattern observed in virgin mice. These fibers are composed exclusively of microfibrils (**arrows**). **B**, a higher magnification of A. **C**, shows a longitudinal section of an oxytalan fiber (**asterisk**) in close contact with thin collagen fibrils (**C**) in the fibrocartilage. The microfibrils are arranged in a slightly undulated manner parallel to the long axis of the pubic symphysis. Bars = l 300 nm (A), 200 nm (B) and 400 nm (C).

tural examination showed all of the fibers present in the fibrocartilage were oxytalan fibers (formed only by microfibrils), while in the perichondrium some elaunin fibers (formed by microfibrils with patches of amorphous elastin) were occasionally observed among the oxytalan fibers (Fig. 3).

On D15, a ligament was observed in the middle of the fibrocartilaginous tissue. The whole joint was formed by the medial ends of the pubic bones, each covered by caps of hyaline cartilage, and also by "caps" of fibrocartilage interconnected by the interpubic ligament. A distinction between the ligament and the perichondrium was not possible until the fifth day post-partum (Fig. 4). Elastic system fibers were observed in the fibrocartilage (with the same distribution as in D12) and the ligament [thin fibers, oriented obliquely relative to the major axis of the

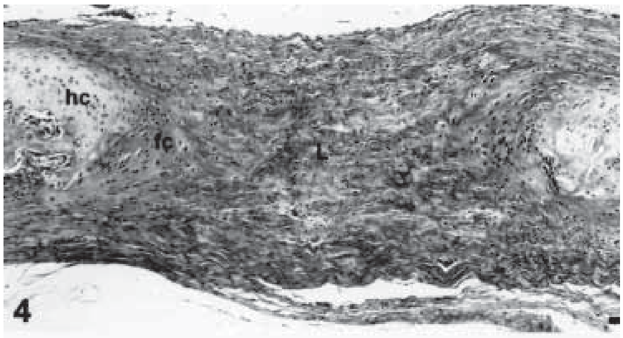
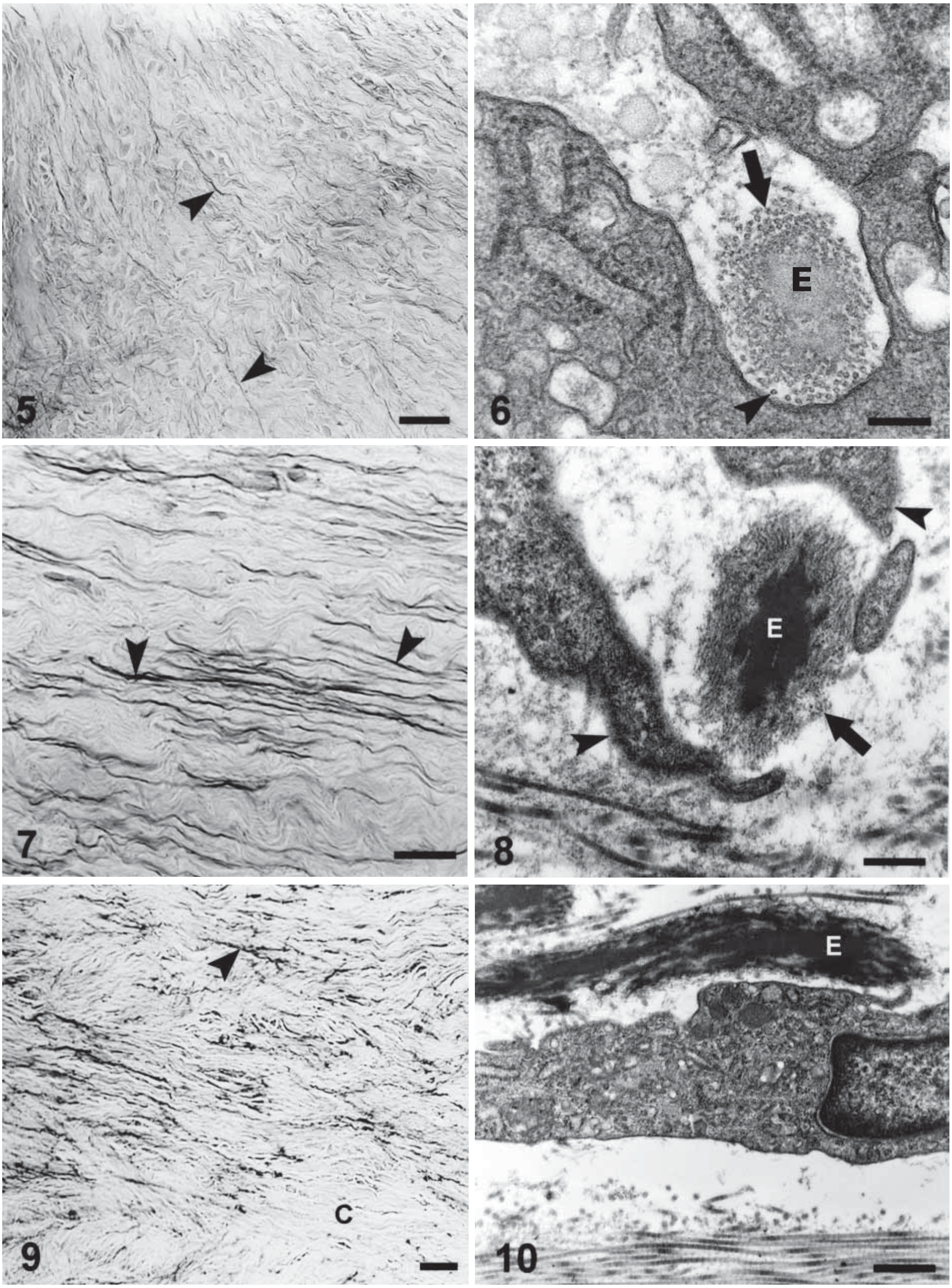


Figure 4. Transverse section of the pubic symphysis on D15 of pregnancy. The section was stained with hematoxylin-eosin. The whole ligament (**L**) is seen, as well as the hyaline cartilage caps (**hc**) and small parts of the pubic bones. Note that fibrocartilage (**fc**) is still present in the transition zone between the cartilage and the interpubic ligament. The interpubic ligament consisted of highly organized connective tissue formed by thick collagen fibers assembled into a bundle and all arranged in the same direction (i.e., parallel to the long axis of the articulation). Bar = 50 µm.

Figure 5. Photomicrograph of the mouse interpubic ligament on D15. The ligament was stained with Weigert's resorcin-fuchsin after oxidation. Note the numerous slightly thickened fibers of the elastic system (**arrowheads**) aligned preferentially with the long axis of collagen fibers and the ligament. Bar = 20 µm. **Figure 6.** Transmission electron micrograph of a thin cross-section of pubic symphysis on D15, showing an elaunin fiber (**arrow**) containing a bundle of microfibrils (**arrowhead**) arranged in parallel and interspersed with patches of amorphous, homogeneous material (elastin – **E**). Bar = 100 nm. **Figure 7.** Coronal section of a mouse interpubic ligament at parturition (D19). The section was stained with Weigert's resorcin-fuchsin after oxidation. Note the thick fibers (**arrowheads**) of the elastic system aligned with each other and with the long axis of the ligament. Bar = 20 µm. **Figure 8.** Transmission electron micrograph of a thin cross-section of the pubic symphysis of the mouse during parturition (D19). Note the long cellular processes (**arrowheads**) involving the elaunin fiber (**arrow**) rich in elastin (**E**). Bar = 300 nm. **Figure 9.** Coronal section of a mouse interpubic ligament on the 5th day post-partum (PP5). The section was stained with Weigert's resorcin-fuchsin after oxidation. The fibers of the elastic system are seen interwoven with collagen (**C**). Thin elastic fibers (**arrowhead**) are only slightly stained. Bar = 20 µm. **Figure 10.** Transmission electron micrograph of a thin cross-section of mouse pubic symphysis on the 5th day post-partum (PP5) showing ultrastructural aspects of elaunin fibers sectioned obliquely. These fibers consisted of elastin (**E**) associated with microfibrils. Although the amount of elastin was high, there was no fusion, as shown by the interrupted spaces within the fibers. Bar = 700 nm.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joint, and longer than those present in the fibrocartilage (Fig. 5)]. Ultrastructurally the elastic system fibers observed in the fibrocartilage, ligament and perichondrium were mostly of the elaunin type, with a small amount of oxytalan fibers (Fig. 6).

Although the length of the ligament increased up to D18, the elastic system fibers showed the same distribution and pattern as on D15. On D19, immediately after delivery, the joint consisted of the bone ends, very thin caps of hyaline cartilage, fibrocartilage "caps" and the ligament. The elastic system fibers were unchanged in the fibrocartilage, while in the ligament they were parallel to each other and to the major axis of the joint (Figs. 7 and 8).

After delivery, the ligament underwent progressive involution, mainly related to the initial repacking of the collagenous network followed by its simultaneous resorption and replacement by fibrocartilage. From PP1 to PP5, the elastic system fibers in the ligament reassumed their oblique distribution, and were mostly of the elaunin type (Figs. 9 and 10).

DISCUSSION

The pubic joint plays a role in various transformations involving the sexual organs during pregnancy. These hormonally regulated adaptations are species-specific and are determined mostly by the size of the pelvis and of the fetus. The pubic joint of mice and guinea pigs is frequently used as a physiological model to study the synthesis and degradation of connective tissue, and can be manipulated by administering different hormones and their combinations [39,41,42]. The elastic system fibers of this joint are synthesized during embryogenesis but have a very low turnover [24,30]. Therefore, any model that allows the study of their synthesis in a physiological manner over a short period of time is very attractive.

The presence of a ligament in the middle of the pubic symphysis of mice, as observed here, suggested that there was considerable disproportional fetal-pelvic growth in these animals that required a relatively large increase in the diameter of the pelvic girdle. A similar ligament is also present in the pubic joint of female free-tailed bats [13,16]. In this case, the ligament does not grow much during pregnancy but is highly distended during delivery (around 15 times its length) since the size of the fetus greatly exceeds the diameter of the birth canal. Elastic system fibers are present in the pubic ligament of these bats and are involved in restoring the size of the pubic gap immediately after delivery. In contrast, in mice, an increase in the size of the birth canal through growth of the ligament alone is insufficient to allow the passage of the fetus, even when stretched during pregnancy [43].

Elastic system fibers were observed in sections of pubic joint stained with Weigert's resorcin-fuchsin. These fibers were present in the central fibrocartilage (oxytalan fibers only) and perichondrium (mostly oxytalan, with very few elaunin fibers) of virgin and D12 pregnant mice, and were also seen in the ligament by the 15th day of pregnancy, mainly as elaunin fibers. From D15 onwards, the fibers in the fibrocartilage were also of the elaunin type.

Oxytalan fibers are unstretchable because they contain no elastin. In non-pregnant female mice, the pubic joint requires only minimal movement and is subject mainly to compressional forces, which probably explains the presence of only oxytalan fibers in these animals. The development of the ligament is quite fast since it is absent on D12 but present on D15. The elastic system fibers present on the 15th day are mostly of the elaunin type, both in the fibrocartilage and in the ligament. It is possible that the same stimulus for the deposition of the ligament may have induced the production and deposition of elastin in oxytalan fibers already present in the fibrocartilage. The observation that the deposition of elastin in the elastic system fibers of the fibrocartilage and ligament ceased in the elaunin fiber phase suggested that the elasticity needed for the pelvic girdle to function can be provided by these fibers.

As stated above, collagen-containing fibers are responsible for the strength of connective tissues whereas elastic system fibers, proteoglycans, glycosaminoglycans and water contribute to tissue resiliency. The amount and organization of these components gives each connective tissue its own biomechanical properties. The elastic system fibers add reversible extensibility to tissues [17,18, 29,30,33,50].

A comparison of the uterine cervix and the pubic joint during pregnancy and delivery showed that the former remained closed during pregnancy to keep the conceptus within the uterus, but became malleable and distensible during labour and delivery, to allow passage of the fetus [2,25,27,28]. The pubic joint enlarges during pregnancy, while still allowing the animal to walk. During delivery, the joint expands further to provide a sufficiently large birth canal for the passage of the fetus, but never prevents the animal from walking, even immediately after delivery. The connective tissue of the uterine cervix and of the pubic joint, particularly the pubic ligament, undergoes extensive transformations. The collagen-containing fibers are re-arranged and there is a shift in the amount and quality of proteoglycans that results in hyperhydration of the tissue and increased resilience [7,43,48,51].

Although these extensive transformations occur very close to delivery, the form and most of the rigidity of the uterine cervix are regained within 4 h post-partum; the immediate closure of the cervical lumen is probably mediated by elastic system fibers [2]. Likewise, the elastic system fibers of the fibrocartilage and pubic ligament could be responsible for restoring the size of the pubic gap to normal immediately after delivery.

The elastic system fibers may help to maintain the anatomical integrity of the ligament during the first hours after delivery, when the collagen, proteoglycan and water re-arrangements have not yet been completed. However, by the first day post-partum, the collagen-containing fibers of the ligament had almost completely regained the dense packing and high organization seen on D18 [14,22,23,43].

The elastic system fibers were arranged obliquely relative to the major axis of the joint, both in the fibrocartilage and ligament up until D18. On D19, immediately after delivery, these fibers appeared parallel to the major axis of the joint in the ligament. This probably resulted from the traction imposed on the joint by the passage of the fetus, which pulled the bony ends apart. After stretching during birth, the ligament returned to its pre-partum size and the fibers resumed their oblique arrangement.

The changes observed here contribute to our understanding of the microstructural transformations that occur in the elastic system fibers of the interpubic ligament of pregnant mice. Each new arrangement of this system of fibers represents a dynamic adaptation of the tissue to the forces acting upon it. In the interpubic ligament, these fibers provide a mechanism for the smooth transfer of forces while efficiently protecting the birth canal. The parallel arrangement of the fibers in the ligament relative to the major axis of the joint reflects a very efficient design that maintains the joint's integrity, strength and elasticity throughout late pregnancy. The architecture of the elastic system fibers in the pubic symphysis of pregnant mice suggested that changes in the extracellular matrix were partly responsible for the increase in elastin expression during remodeling. These filamentous extracellular matrix structures may help to transmit or stabilize the forces generated during pregnancy.

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REFERENCES

1. Basset EG, Phillipps DSM (1955) Changes in the pelvic region of the ewe during pregnancy and parturition. *N. Z. Vet. J. 3*, 20-25.
2. Battlehner CN, Caldini EG, Pereira JCR, Luque EH, Montes GS (2003) How to measure the increase in the elastic system fibres in the lamina propria of the uterine cervix of pregnant rats. *J. Anat. (in press).*
3. Böck P, Stockinger L (1984) Light and electron microscopic identification of elastic, elaunin and oxytalan fibers in human tracheal and bronchial mucosa. *Anat. Embryol.* **170**, 145-153.
4. Caldini EG, Caldini N, De-Pasquale V, Strocchi R, Guizzardi S, Ruggeri A, Montes GS (1990) Distribution of elastic system fibers in the rat tail tendon and its associated sheaths. *Acta Anat.* **139**, 341-348.
5. Carvalho HF, Lino Neto J, Taboga SR (1994) Microfibrils: neglected components of pressure-bearing tendons. *Anat. Anz.* **176**, 155-159.
6. Carvalho HF, Vidal BC (1995) The elastic system of a pressure-bearing tendon of the bullfrog *Rana catesbeiana. Anat. Anz.* **177**, 397-404.
7. Chihal HJ, Espey LL (1973) Utilization of the releaxed symphysis pubis of guinea pigs for clues to mechanism of ovulation. *Endocrinology* **93**, 1441-1445.
8. Cleary EG, Gibson MA (1983) Elastin-associated microfibrils and microfibrillar proteins. *Int. Rev. Connect. Tissue Res.* **10**, 97-109.
9. Cotta-Pereira G, Del-Caro LM, Montes GS (1984) Distribution of elastic system fibers in hyaline and fibrous cartilages of the rat. *Acta Anat.* **119**, 80-85.
10. Cotta-Pereira G, Iruela-Arispe ML (1989) Extracellular matrix: functional significance of oxytalan, elaunin and elastic fibers. *Prog. Clin. Biol. Res.* **295**, 101-107.
11. Cotta-Pereira G, Rodrigo FG, Bittencourt-Sampaio S (1976) Oxytalan, elaunin and elastic fibers in the human skin. *J. Invest. Dermatol.* **66**, 143-148.
12. Crelin ES (1954) The effect of estrogen and relaxin on intact and transplanted pubic symphyses in mice. *Anat. Rec.* **118**, 380-381.
13. Crelin ES (1969a) Interpubic ligament, elasticity in pregnant free-tailed bat. *Science* **164**, 81-82.

14. Crelin ES (1969b) The development of bony pelvis and its changes during pregnancy and parturition. *Trans. N. Y. Acad. Sci.* **31**, 1049-1058.
15. Crelin ES, Brightman MW (1957) The pelvis of the rat, its response of estrogen and relaxin. *Anat. Rec.* **128**, 467-484.
16. Crelin ES, Newton EV (1969) The pelvis of the free-tailed bat: sexual dimorphism and pregnancy changes. *Anat. Rec.* **164,** 349-357.
17. Debelle L, Alix AJP (1999) The structures of elastins and their function. *Biochimie* **81**, 981-994.
18. Debelle L, Tamburro AM (1999) Elastin: molecular description and function. *Int. J. Biochem. Cell Biol.* **31**, 261-272.
19. Gamble JG, Simmons SC, Freedman M (1986) The symphysis pubis. Anatomic and pathologic considerations. *Clin. Orthop.* **203,** 261-272.
20. Gawlik Z (1965) Morphological and morphochemical properties of the elastic system in the motor organ of man. *Folia Histochem. Cytochem.* **3**, 233-251.
21. Guidelines for the case of laboratory animals in transit (1993) *Lab. Anim.* **27**, 93-107.
22. Hall K (1947) The effects of pregnancy and relaxin on the histology of the pubic symphysis in the mouse. *J. Endocrinol.* **5***,* 174-182.
23. Horn EH (1960) Interpubic ligament regression in relaxin-treated virgin and primiparous, post-partum mice. *Endocrinology* **67**, 668-673.
24. Hurle JM, Corson G, Daniels K, Reiter RS, Sakai LY, Solursh M (1994) Elastin exhibits a distinctive temporal and spatial pattern of distribution in the developing chick limb in association with the establishment of the cartilaginous skeleton. *J. Cell Sci.* **107**, 2623-2634.
25. Junqueira LCU, Zugaib M, Montes GS, Toledo OMS, Krisztan RM, Shigihara KM (1980) Morphological and histochemical evidence for the occurrence of collagenolysis and for the role of neutrophilic polymorphonuclear leukocytes during cervical dilatation. *Am. J. Obstet. Gynecol.* **138**, 273-281.
26. Kroc RL, Steinetz BG, Beach VL (1958) The effects of estrogens, progestagens, and relaxin in pregnant and nonpregnant laboratory rodents. *Ann. N. Y. Acad. Sci.* **75**, 942-980.
27. Leppert PC (1995) Anatomy and physiology of cervical ripening. *Clin. Obstet. Gynecol.* **38**, 267-279.
28. Ludmir J, Sehdev HM (2000) Anatomy and physiology of the uterine cervix. *Clin. Obstet. Gynecol.* **43**, 433-439.
29. Mecham RP, Davis EC (1994) Elastic fiber structure and assembly. In*: Extracellular Matrix Assembly and Structure* (Yurchenco PD, Birk DE, Mecham RP, eds). pp. 281-314. Academic Press: San Diego.
30. Mecham RP, Heuser JE (1991) The Elastic Fiber. In: *Cell Biology of Extracellular Matrix* (Hay ED, ed.). pp.79-101. Plenum Press: New York.
31. Montes GS (1992) Distribution of oxytalan, elaunin and elastic fibres in tissues. *Cienc. Cult.* **44**, 224-233.
32. Montes GS (1996) Structural biology of the fibres of the collagenous and elastic systems. *Cell Biol. Int.* **20***,*15-27.
33. Oakes BW, Bialkower B (1977) Biomechanical and ultrastructural studies on the elastic tendon from the domestic fowl. *J. Anat.* **123**, 369-387.
34. Ortega HH, Joazeiro PP, Muñhoz-de-Toro MM, Luque EH, Montes GS (2001) Differential distribution of the fibres of the collagenous and elastic systems and of glycosaminoglycans in the rat pubic joint. *J. Submicrosc. Cytol. Pathol.* **33,** 463-472.
35. Ross R (1973) The elastic fiber. A review. *J. Histochem. Cytochem.* **21**, 199-208.
36. Ross R, Bornstein P (1969) The elastic fiber. I. The separation and partial characterization of its macromolecular components. *J. Cell Biol.* **40**, 366-381.
37. Ruth EB (1936) Metamorphosis of the pubic symphysis. II. The guinea pig. *Anat. Rec.* **67**, 69-79.
38. Ruth EB (1937) Metamorphosis of the pubic symphysis. III. Histological changes in the symphysis of the pregnant guinea pig. *Anat. Rec.* **67,** 409-421.
39. Sherwood OD (1994) Relaxin. In: *The Physiology of Reproduction*. (Knobil E, Neill JD, eds). pp. 861-1009. Raven Press: New York.
40. Shorr E (1941) A new technique for staining vaginal smears. II. Single differential stain. *Science* **5**, 545-546.
41. Steinetz BG, Beach VL, Kroc RL (1957) The influence of progesterone, relaxin and estrogen on some structural and functional changes in the pre-parturient mouse. *Endocrinology* **61,** 271-280.
42. Steinetz BG, Manning JP, Butler MT, Beach V (1965) Relationships of growth hormone, steroids and relaxin in the transformation of pubic joint cartilage to ligament in hypophysectomized mice. *Endocrinology* **76**, 876-882.
43. Storey E (1957) Relaxation in the pubic symphysis of the mouse during pregnancy and after relaxin administration, with special reference to the behavior of collagen. *J. Pathol. Bacteriol.* **74,** 147-162.
44. Talmage RV (1947a) Changes produced in the symphyseal pubis of the guinea pig by the sex steroids and relaxin. *Anat. Rec.* **99,** 91-113.
45. Talmage RV (1947b) A histological study of the effects of relaxin on the symphysis pubis of the guinea pig. *J. Exp. Zool.* **106,** 281-292.
46. Viell B, Struck H (1987) Effects of the hormone relaxin on the metabolism of the glycosaminoglycans in the mouse symphysis pubis. *Horm. Metabol. Res.* **19**, 415-418.
47. Vix VA, Ryu CY (1971) The adult symphysis pubis: normal and abnormal. *Am. J. Roentgenol. Radium Ther. Nucl. Med.* **112**, 517-525.
48. Wahl LM, Blandau RJ, Page RC (1977) Effect of hormones on collagen metabolism and collagenase activity in the pubic symphysis ligament of the guinea pig. *Endocrinology* **100,** 571-579.
49. Weiss M, Nagelschmidt M, Struck H (1979) Relaxin and collagen metabolism. *Horm. Metab. Res.* **11**, 408-410.
50. Wren TAL, Carter DR (1998) A microstructural model for the tensile constitutive and failure behavior of soft skeletal connective tissues. *J. Biomech. Eng.* **120**, 55-61.
51. Zhao L, Samuel CS, Tregear GW, Beck F, Wintour EM (2000) Collagen studies in late pregnant relaxin null mice. *Biol. Reprod.* **63**, 697-703.

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