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CARACTERIZAÇÃO DAS FIBRAS DO SISTEMA ELÁSTICO E DA PLASTICIDADE CELULAR NA SÍNFISE PÚBICA DO CAMUNDONGO DURANTE A PRENHEZ, PARTO E PÓS-PARTO.

ESTUDO PELA MICROSCOPIA DE LUZ E ELETRÔNICA DE TRANSMISSÃO

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Orientador: Prof. Dr. Paulo Pinto Joazeiro

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"Think like a mystery life is!
It emerges from the unknown
and into the unknown it dissolves…
We have learned much, but we are still picking up pebbles on the vast seashore of life."

Paramahansa Yogananda

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RESUMO

A sínfise púbica é um tipo de articulação ligeiramente móvel localizada na confluência dos ossos pubianos, unindo-os através de coxins de cartilagem hialina. A estabilidade apresentada por essa articulação é alterada durante a gestação, período em que tanto a cérvix uterina como toda a cavidade pélvica, inclusive a sínfise, devem se adaptar proporcionando um parto normal. A separação da sínfise púbica fibrocartilaginosa do camundongo na prenhez depende de sua transformação em um ligamento extensível, processo este que envolve o aumento da biossíntese da matriz extracelular (MEC), principalmente colágeno, proteoglicanos e glicosaminoglicanos e a mudança na relação entre síntese e degradação dos mesmos. Na sínfise de camundongo o *turnover* destes componentes foram estimados por estudos bioquímicos e de microscopia de luz; porém pouca atenção tem sido dada aos aspectos ultra-estruturais e histoquímicos, tão pouco encontramos relatos sobre os componentes do sistema elástico e a plasticidade celular. O presente trabalho teve por objetivo conhecer a distribuição das fibras do sistema elástico e caracterizar o imunofenótipo e aspectos ultra-estruturais de células envolvidas na formação e involução do ligamento interpúbico na sínfise púbica de camundongos, durante prenhez, parto e pós-parto. O estudo foi realizado por meio de métodos seletivos de coloração para sistema elástico, imunohistoquímica para identificação das células (utilizando anticorpos específicos anti-α-actina de músculo liso (α-SMA), desmina e vimentina) e análise ultra-estrutural para ambos. Os resultados obtidos mostraram variação de tipos e de distribuição das fibras do sistema elástico, assim como um aumento de diâmetro e de comprimento aparente das mesmas, durante a prenhez. Estas observações indicam que o sistema elástico deve desempenhar importante papel, tanto impedindo o rompimento do ligamento interpúbico durante a evolução do mesmo, quanto recuperando a organização dessa estrutura após o parto. Com relação ao compartimento celular, foi observada uma intensa imunomarcação para anti-α-SMA durante todos os períodos observados, enquanto vimentina e desmina apresentaram uma expressão transitória (entre o 15° dia de gestação e 24 horas pós-parto). A análise ultra-estrutural mostrou ainda que estas células passam por algumas alterações morfológicas durante a formação do ligamento interpúbico, adquirindo características descritas anteriormente para miofibroblastos, como núcleo indentado e presença de fibronexus e geralmente são

vistas próximas às fibras elásticas. Os dados morfológicos e ultra-estruturais sugerem que essas células possuem um aparato de contração formando uma rede entre célula e MEC capaz de transmitir a força gerada pela movimentação dos ossos púbicos durante a prenhez, tanto para células adjacentes como para outros componentes da MEC, a exemplo das fibras do sistema elástico. Portanto, a presença de células e fibras com essas características particulares na sínfise púbica pode contribuir para integridade estrutural e mecânica dessa articulação.

ABSTRACT

Pubic symphysis is a type of slightly mobile joint, formed with a fibrocartilaginous disc lying between the hyaline cartilage-covered medial borders of the pubic bones. The stability presented for this joint is modified during the pregnancy, period where as much cervix uterine as the entire pelvic cavity, included symphysis, must be adapted to providing a normal labor. The spreading apart of mouse pubic symphysis in pregnancy depends on its transformation in an extensible ligament, process this that involves the increase of biosynthesis of the extracellular matrix (ECM), mainly collagen, proteoglycans and glycosaminoglycans and the change in the relation between synthesis and degradation of the same ones. In mouse symphysis the *turnover* of these components had been estimated by biochemical studies and light microscopy, however little attention has been given to the ultrastructural and histochemical aspects, so little we find reports about the elastic system components and the cellular plasticity. The aims of the present work were to know the distribution of the elastic system fibers and to characterize the immunophenotype and ultrastructural aspects of involved cells in the formation and involution of the interpubic ligament in mouse pubic symphysis, during pregnancy, parturition and post-partum. The study applied selective staining methods for elastic system, Immunohistochemistry (using specific antibodies anti-α-actin of smooth muscle (α-SMA), desmin and vimentin) and ultrastructural analysis. Our results showed variation of types and distribution of the elastic system fibers, as well as an increase of diameter and apparent length of the same ones, during the pregnancy. These observations indicate that the elastic system must play important roles, preventing the disruption of interpubic ligament during it evolution, as well as recovering the organization of this structure after the parturition. With concern to the cellular compartment, it was observed an intense immunoreactivity for anti-α-SMA during all the observed periods, while vimentin and desmin presented a transient expression (from 15° day of pregnancy until 24 hours post-partum). The ultrastructural analysis still showed that these cells pass for some morphologic alterations during the interpubic ligament formation, acquiring characteristic described previously for myofibroblasts, as indentated nucleus and presence of fibronexus and generally are seen close to elastic fibers. The morphologic and ultrastructural data suggest that these cells possess a contraction apparatus constituting a network between

cell and ECM capable to transmit the force generated by the movement of the pubic bones during the pregnancy, as much for adjacent cells as for other components of the ECM, such elastic system fibers. Therefore, the presence of cells and fibers with these particular features in pubic symphysis can contribute for structural and mechanical integrity of this joint.

INTRODUÇÃO

1.CARACTERÍSTICAS MORFOLÓGICAS E ESTRUTURAIS DA SÍNFISE PÚBICA

Os ossos articulam-se aos outros para constituir o esqueleto. Tal união não tem apenas a finalidade de colocar os ossos em contato no seu ponto de encontro, mas também possibilitar a mobilidade entre eles. A estrutura responsável por fazer essa união é denominada então articulação ou junta. As articulações podem ser classificadas morfologicamente, dependendo da sua constituição, ou seja, da natureza do elemento que se interpõe às peças que se articulam (Dângelo & Fattini, 1988; Ham, 1972).

A palavra "sínfise", que consiste em uma categoria de articulação, é derivada do termo grego "crescendo junto". Essa descrição se mostra adequada já que a sínfise púbica é um tipo de articulação ligeiramente móvel localizada na confluência dos ossos púbicos. Cada osso púbico consiste num corpo e dois ramos; na puberdade o ramo superior funde-se ao ílio enquanto o ramo inferior, ao ísquio. Em estudos realizados em sínfise humana observou-se que os ossos ilíacos atuam como arcos, transferindo o peso do pilar principal da região sacral para o quadril (Gamble et al., 1986).

A sínfise púbica é responsável então por fazer a conexão entre os corpos dos dois ossos púbicos ou arcos através de coxins de cartilagem hialina (Gamble et al., 1986). Estes coxins se conectam por meio de um tecido fibroso denso, que se funde com a cartilagem resultando numa estreita zona de transição fibrocartilaginosa (Talmage, 1947a-b; Hall, 1947; Crelin, 1954; Storey, 1957; Steinetz et al., 1965; Ham 1972; Gamble et al., 1986). A estabilidade dessa articulação é conferida por um conjunto de ligamentos denominados circunferenciais. Os ligamentos púbicos anterior, posterior e suprapúbico fazem uma pequena contribuição na manutenção da integridade mecânica, enquanto que o espesso ligamento púbico inferior ou ligamento arcuado, é o grande responsável pela estabilidade da articulação. Juntos estes ligamentos neutralizam forças de cisalhamento e tração, possibilitando apenas o mínimo de movimento da articulação durante a maioria das atividades (Gamble et al., 1986).

2. REMODELAÇÃO DA SÍNFISE PÚBICA NA PRENHEZ

A estabilidade apresentada pela sínfise púbica é alterada durante a prenhez, período em que tanto a cérvix uterina como toda a cavidade pélvica, inclusive a sínfise, devem se adaptar proporcionando um parto normal.

O dimorfismo sexual descrito em quase todos os mamíferos, incluindo o camundongo (Gardner, 1936; Crelin, 1960), é um fator que contribui para o aumento do canal de parto. Em camundongos fêmeas por exemplo, durante a puberdade ocorre uma reabsorção parcial das extremidades mediais dos ossos púbicos, portanto estes ossos são menores, menos espessos e se apresentam mais afastados entre si nas fêmeas do que nos machos. Ainda nas fêmeas, o ângulo entre o ísquio e o osso púbico é mais obtuso e a sínfise está situada mais posteriormente (Gardner, 1936; Crelin, 1960; Sherwood, 1994).

Com relação à sínfise púbica, observa-se ainda uma adaptação adicional envolvendo alterações estruturais que variam de acordo com a espécie (Sherwood, 1994). O resultado destas alterações é um aumento de flexibilidade desta articulação, promovendo uma menor ou maior separação dos ossos púbicos de animais prenhes, de forma a facilitar a passagem dos fetos pelo canal de parto (Talmage, 1947a-b; Hall, 1947; Ham 1972; Gamble et al., 1986; Sherwood, 1994).

Durante a prenhez a transformação da articulação cartilaginosa que constitui a sínfise, em um ligamento interpúbico flexível e elástico, pode ser observada em várias espécies, incluindo cobaia (Ruth, 1937; Talmage, 1947a-b; Wahl et al., 1977), camundongos (Gardner, 1936; Hall, 1947; Crelin, 1954; Storey,1957; Kroc et al., 1958; Steinetz et al., 1957), morcegos (Crelin & Newton, 1969; Crelin, 1969a) e humanos (Crelin, 1969c; Vix, 1971; Gamble et al., 1986). Porém tal processo não é encontrado, por exemplo, em ratos ou coelhos (Talmage, 1947; Crelin e Brightman, 1957; Samuel, 1998).

Em camundongos a separação dos ossos púbicos inicia-se no 12^{o} dia de prenhez com o desenvolvimento de um ligamento extensível, podendo este aumentar em média 1mm por dia até o parto, que geralmente ocorre durante a noite do 19^{o} dia de gestação. O afastamento dos ossos púbicos é atribuído também à reabsorção das superfícies mediais dos mesmos e à substituição da cartilagem sinfiseal e de parte do osso reabsorvido, pelo

tecido conjuntivo fibroso que compõe o ligamento interpúbico que se forma neste período. A involução deste ligamento acontece rapidamente nos cinco dias após o parto, porém a estrutura da sínfise não retorna à dimensão e aparência de um animal virgem (Hall, 1947; Crelin, 1954; Storey,1957; Kroc et al., 1958; Steinetz et al., 1957; Horn, 1960).

Vários trabalhos sugerem que uma variedade de hormônios influencia a estrutura e função do trato reprodutivo (Kroc et al., 1958). Extensos estudos sobre a regulação hormonal da transformação do ligamento interpúbico demonstraram que o crescimento dessa estrutura é estimulado principalmente por estrógeno e relaxina. Informações adicionais sobre a atuação hormonal sobre os tecidos conjuntivos da sínfise púbica podem ser encontradas em vários trabalhos (Crelin e Levin, 1955; Crelin e Haines, 1955; Steinetz et al., 1957; para revisão, ver Sherwood, 1994).

Os diferentes fatores hormonais e mecânicos, que atuam sobre o ligamento interpúbico durante a prenhez, produzem alterações na composição, deposição, reabsorção e organização da matriz extracelular.

A transformação desta articulação em um ligamento interpúbico extensível é caracterizada pela deposição de matriz extracelular e pela diferenciação de populações celulares do tecido conjuntivo, durante a prenhez ou experimentalmente sob estímulo de hormônios estrogênicos e da relaxina. Hall (1954) estudou o desenvolvimento deste ligamento em camundongos prenhes e afirmou que a principal fonte de células que contribui para a formação dessa estrutura é provavelmente osteócitos ou células da medula que adquirem características semelhantes a fibroblastos e migram para áreas nas extremidades dos ossos púbicos, que estão sendo reabsorvidas. Em 1955, Crelin & Levin especularam outras possíveis fontes de células envolvidas na formação do ligamento interpúbico, como condrócitos da cartilagem hialina que reveste as extremidades dos ossos púbicos, fibrocondrócitos da transição fibrocartilaginosa presente no centro da articulação e fibroblastos do pericôndrio. Sabe-se que os condrócitos, fibrocondrócitos e fibroblastos da sínfise passam a sintetizar e secretar quantidades crescentes de componentes da matriz extracelular, porém nenhum relato descreve a plasticidade destas células. O desenvolvimento do ligamento extensível, durante os últimos cinco dias que antecedem o parto, e a sua involução, nos cinco primeiros que o sucedem, são marcados por intensa remodelação dos componentes da matriz extracelular (Talmage, 1947a-b; Hall, 1947; Frieden & Hisaw, 1953; Kroc et al., 1958; Whal et al., 1977; Schwabe et al., 1978; Pinheiro, 1998).

Estudos morfológicos e bioquímicos das transformações que ocorrem na sínfise púbica durante a prenhez de roedores ou em ensaios biológicos -sob o estímulo combinado de hormônios estrogênicos e da relaxina, produzida por diferentes espécies de mamíferos -mostraram que na prenhez ocorre um aumento da deposição de complexos macromoleculares na matriz tais como: o colágeno, que no dia do parto atinge aproximadamente 70% do peso seco do ligamento (Whal et al., 1977); os glicosaminoglicanos (Viell & Struck, 1987), bem como síntese e secreção de enzimas envolvidas no processo de remodelação como catepsinas (Mcdonald & Schwabe, 1982) e colagenase (Chihal & Espey, 1973; Whal et al.,1977). Entretanto encontramos somente alguns relatos que descrevem aspectos sobre a modulação de componentes do sistema elástico em apenas uma espécie de mamífero (Crelin & Newton, 1969; Crelin, 1969a).

Portanto, observa-se que são poucos os estudos sobre as características morfológicas, principalmente as ultra-estruturais, histoquímicas e bioquímicas dos componentes da matriz extracelular da sínfise e do ligamento interpúbico, se comparados aos existentes na literatura sobre útero, cérvix e vagina. Em contrapartida, alguns estudos cujo objetivo é a caracterização de polipeptídeos da família da relaxina, utilizam ensaios biológicos que se resumem na quantificação, *in situ*, do grau de distensão do ligamento. Esta quantificação é bastante fidedigna e de emprego rotineiro em experimentos para a descrição de novos membros ou frações de moléculas de relaxina, mesmo em ensaios com emprego de técnicas de biologia molecular (Steinetz et al., 1988; Ferraiolo et al., 1989; Büllesbach & Schwabe, 1995, 1996).

De acordo com Kroc et al. (1958), os órgãos do aparelho reprodutor de fêmeas de roedores respondem de modo semelhante ao mesmo controle hormonal durante os últimos dias da prenhez, parto e pós-parto, possuindo estruturas e funções definidas pelas características do tecido conjuntivo. A cérvix uterina, apesar de apresentar uma grande variação morfológica entre mamíferos, possui o tecido conjuntivo denso como seu principal componente, o que favorece a observação de fenômenos que ocorrem na matriz extracelular durante a prenhez (Golichowski et al. 1980; Junqueira et al., 1980).

Da mesma forma que a cérvix, o ligamento interpúbico é um tecido relativamente homogêneo e a deposição e remodelação da matriz ocorrem rapidamente em resposta aos estímulos hormonais (Wahl et al., 1977), o que também favorece o estudo de fenômenos envolvendo a síntese, deposição e reabsorção de componentes da matriz. Além disso, estudos recentes demonstraram que a prenhez induz complexas alterações

no padrão de expressão de mRNA de proteínas e enzimas da matriz extracelular dos ligamentos do joelho (Hart et al., 1998).

Portanto o modelo experimental em que se constitui o ligamento da sínfise púbica do camundongo, além das vantagens enumeradas por Wahl et al. (1977) para o estudo da síntese e degradação de componentes da MEC em condições fisiológicas, sobretudo do colágeno, apresenta-se como modelo de fácil obtenção e manipulação. Deste modo, acreditamos que estudos enfocando aspectos da remodelação da matriz extracelular da sínfise durante sua rápida transformação podem contribuir para o conhecimento de aspectos da modulação das fibras do sistema elástico e da plasticidade celular.

3. SISTEMA ELÁSTICO

Em determinadas localizações anatômicas o tecido conjuntivo tem função predominantemente mecânica apresentando uma combinação apropriada de dois atributos principais -a capacidade de resistir a grandes forças de tensão e compressão e a propriedade de recuperar a forma e estrutura quando estas forças cessam. Assim sendo, é importante entender como seus constituintes químicos -colágenos, glicosaminoglicanos, elastina, sais minerais e água - conferem ao tecido seus atributos mecânicos, ainda que seja difícil determinar as contribuições individuais destes diversos componentes (Parry & Craig, 1988). Se por um lado as fibras que contêm colágeno conferem resistência ao tecido, por outro as do sistema elástico e os proteoglicanos são essenciais na manutenção das propriedades elásticas da matriz. Os componentes do sistema elástico têm sido descritos na matriz extracelular do tecido conjuntivo de tecidos e órgãos que requerem a habilidade de deformar repetidamente e reversivelmente como bexiga (Koo et al., 1998), útero (Starcher & Percival, 1985; Leppert & Yu, 1991), tendão (Caldini et al., 1990; Carvalho et al., 1994; Carvalho & Vidal, 1995), pele (Cotta-Pereira et al, 1976; Kielty et al., 1993), ligamento periodontal (Takagi et al., 1989), pulmão (Fukuda et al., 1984; Leick-Maldonado et al., 1997), nervos (Ferreira et al., 1987) e outros tecidos.

Porém em determinados momentos no desenvolvimento dos organismos se faz necessário um aumento da elasticidade e ou uma redução da resistência de certas localizações do tecido conjuntivo, a exemplo do que ocorre na sínfise púbica de alguns

roedores, onde as evidências ultra-estruturais mostraram que sob o estímulo da relaxina os fibroblastos liberam enzimas na matriz que promovem a ruptura das fibras de colágeno (Chihal & Espey, 1973; Pinheiro, 1998). Esta ruptura ocorre de modo semelhante ao observado na cérvix uterina do rato onde foi demonstrado que, nos momentos que precedem o trabalho de parto, os polimorfonucleares degranulam no estroma da cérvix a termo e rompem a organização fibrilar do colágeno; havendo uma relação direta entre o número de polimorfonucleares e o grau de colagenólise na cérvix (Junqueira et al., 1980; Luque & Montes, 1989).

Portanto assumindo-se que o tecido conjuntivo é o principal componente da sínfise e que as fibras colágenas representam um importante papel no seu desempenho funcional, é razoável levantar a hipótese que as fibras do sistema elástico podem não apenas funcionar como uma proteção contra a sua ruptura durante a dilatação e o parto, mas também podem estar envolvidas no processo de reestruturação da sínfise no período pós-parto.

Nos tecidos de mamíferos adultos, o sistema elástico é composto por três tipos de fibras: oxitalânicas, elaunínicas e elásticas propriamente ditas ou maduras. As fibras oxitalânicas foram descritas por Fullmer & Lillie em 1958, sendo constituídas exclusivamente por feixes de microfibrilas, que ao MET, em corte transversal exibem um perfil tubular, medindo 10-12nm de diâmetro. As fibras elaunínicas, descritas por Gawlik em 1965, são formadas por feixes de microfibrilas aos quais entremeiam-se grumos irregulares de material amorfo -a elastina. Já as fibras elásticas maduras, devido às suas propriedades tintoriais características são observadas a mais de um século e são compostas por um cilindro sólido de elastina rodeado por microfibrilas (ver contribuições originais em Montes, 1996).

A elastina confere elasticidade às fibras, portanto as fibras elásticas, por apresentarem maior quantidade de elastina, têm suas propriedades elastoméricas mais proeminentes, enquanto que as fibras elaunínicas têm grau intermediário de elasticidade (Souza et al., 1991; Montes, 1996; Leick-Maldonado et al., 1997). A organização molecular presente nas fibras elásticas permite que elas se estirem aproximadamente 1,5 vez o seu comprimento em repouso. As características biomecânicas das fibras elásticas indicam que estas são responsáveis pela distensão reversível do tecido conjuntivo uma vez que exibem deformação elástica e também são capazes de armazenar energia

elástica para restaurar o tecido deformado à sua configuração original (Greenlee et al., 1966; Ross, 1973; Sear et al., 1981; Paniagua et al., 1983).

As variações constitutivas de fibras contendo somente microfibrilas, fibras com pouca elastina, fibras com muita elastina e com poucas microfibrilas, também são observadas durante a ontogênese de uma fibra elástica, e a princípio, acreditou-se que os primeiros dois tipos representavam, sempre, estágios no seu processo de maturação (Gawlik, 1965; Cotta-Pereira et al., 1976). Somente após a descrição das fibras oxitalânicas e elaunínicas em determinadas localizações de tecidos adultos, onde elas nunca iriam originar uma fibra elástica madura, é que estas fibras foram também aceitas como elementos independentes constituintes do tecido conjuntivo (Cotta-Pereira et al., 1977).

Em um organismo adulto, a forma, quantidade e arranjo de componentes do sistema elástico podem variar de acordo com o tipo de tecido ou órgão onde se encontram, dependendo das suas características funcionais (Leppert & Yu, 1991; Koo et al., 1998; Montes, 1996). Um exemplo é a presença de fibras do sistema elástico em determinadas estruturas, como tendões e meniscos, que concomitante transmitem forças de tensão e sofrem compressão, nos quais se tem verificado, em diferentes espécies, que há predominância de um maior número de fibras pré-elásticas; elaunínicas e oxitalânicas, estrategicamente distribuídas pela periferia das fibras de colágeno, a exemplo do que foi observado em meniscos (Ghadially et al., 1983; Cotta-Pereira et al., 1984) e em tendões (Caldini et al., 1990; Carvalho et al., 1994; Carvalho & Vidal, 1995). Estes autores associam a presença das fibras pré-elasticas à manutenção da microarquitetura do tecido e à capacidade dos tendões em suportar forças de tensão e compressão.

Em pulmão, pele e ligamentos os componentes do sistema elástico formam uma pequena rede de fibras, em cartilagens os mesmos se arranjam em uma grande estrutura alveolar tridimensional enquanto que em vasos de grande calibre, aparecem na forma de delgadas lâminas ou lamelas concêntricas, evidenciando assim uma variedade de arranjos necessários para que tecidos distintos desempenhem diferentes funções (Pasquali-Ronchetti, 1997).

Análises bioquímicas e ultra-estruturais demonstraram que as fibras elásticas são estruturas complexas. A formação dessas fibras requer a expressão coordenada de pelo menos quatro proteínas diferentes que não só direcionam a polimerização do monômero de elastina, mas também determinam a arquitetura final da fibra de acordo com as

necessidades funcionais e estresses mecânicos impostos sobre o tecido (Mecham & Davis, 1994). As fibras são constituídas então por dois componentes distintos principais: um componente amorfo formado pela proteína elastina, que compreende aproximadamente 90% da constituição de uma fibra elástica madura e um componente fibrilar, as microfibrilas (Ross & Bornstein, 1969).

3.1. MICROFIBRILAS

3.1.1. CARACTERÍSTICAS GERAIS E ESTRUTURA

Na matriz extracelular de muitos tecidos são encontrados componentes fibrilares denominados microfibrilas. Durante a formação das fibras elásticas, a observação de redes microfibrilares na matriz precede ao aparecimento de elastina, sugerindo assim que elas servem como um arcabouço para a deposição de moléculas de tropoelastina. Através da análise ultra-estrutural as microfibrilas foram caracterizadas como estruturas tubulares, com aproximadamente 10-12nm de diâmetros. Elas também possuem propriedades tintoriais e susceptibilidade à digestão enzimática diferentes da elastina. Em alta resolução, o corte transversal de uma microfibrila aparece como um anel externo elétron-denso que delimita um perfil tubular interno translúcido, enquanto que o corte longitudinal mostra uma cadeia em forma de colar de contas, sugerindo que ela pode ser composta por mais de uma proteína (Rosembloom et al., 1993).

Embora as microfibrilas de tecidos adultos possuam diâmetros aparentemente semelhantes, não se sabe se existem diferenças estruturais ou da composição entre aquelas que não estão associadas com as que se associam a elastina ou ainda entre microfibrilas de diferentes tecidos (Cleary et al. 1981; Rosenbloom et al.1993). Entretanto de acordo com Streeten e Licari (1983) e Gibson e Cleary (1987), estudos imunohistoquímicos mostraram pequenas diferenças entre as microfibrilas.

As microfibrilas são encontradas na matriz extracelular de tecidos ricos em fibras do sistema elástico como pele, pulmão, tendão, vasos sangüíneos, podendo estar ou não associadas a elastina. Em tecidos não elásticos, as microfibrilas desprovidas de elastina apresentam uma função de ancoragem, como por exemplo, ligando a córnea ao corpo

ciliar do olho. Já em tecidos elásticos, além de contribuir como um arcabouço para a deposição de elastina durante a fibrilogênese, as microfibrilas também conectam as fibras elásticas entre si e com outros componentes estruturais e celulares (Ramirez & Pereira, 1999).

Com exceção dos estudos ultra-estruturais, pouco se sabe sobre a síntese, composição e estrutura das microfibrilas. Em parte isto se deve à complexidade e insolubilidade dessas estruturas, dificultando assim a extração tanto das microfibrilas puras, como das proteínas que as constituem. Dessa forma a caracterização bioquímica das microfibrilas tem progredido lentamente. Utilizando ensaios bioquímicos, imunohistoquímicos e mais atualmente técnicas moleculares, alguns componentes microfibrilares puderam ser caracterizados (Rosembloom et al., 1993; Mariencheck et al., 1995; Handford et al., 2000).

As fibrilinas-1 e 2 são os principais componentes estruturais das microfibrilas extracelulares responsáveis pelas propriedades biomecânicas da maioria dos tecidos e órgãos. As fibrilinas são caracterizadas por serem glicoproteínas ricas em cisteína. A fibrilina-1 foi a primeira a ser isolada em 1986 por Sakay et al. Em 1986, a partir de cultura celular de fibroblastos de pele humana utilizando um anticorpo contra microfibrilas. Já a fibrilina-2 foi descoberta cinco anos depois por Lee et al. (1991), durante a clonagem do gene da fibrilina-1. Ambas contém as seqüências RGD (Asp-Gli-Asp), o que sugere um potencial de interação com receptores integrinas na superfície celular (Mecham & Davis, 1994). Além disso, as fibrilinas-1 e 2 apresentam também pequenos segmentos ricos em prolina e glicina respectivamente. A composição e hidrofobicidade dessas seqüências podem facilitar as interações intermoleculares (Ramirez & Pereira, 1999).

Atualmente já se conhecem os genes que codificam as fibrilinas-1 e 2, presentes respectivamente nos cromossomos humanos 15 e 5 (Mecham & Davis, 1994). Grande quantidade de fibrilina-1 é sintetizada no final da morfogênese enquanto que o pico de produção da fibrilina-2 foi observado no início da elastogênese. Além disso, as mutações da fibrilina-1 são responsáveis pelas manifestações pleiotrópicas da síndrome de Marfan, enquanto que defeitos da fibrila-2 resultam em anormalidades esqueléticas na aracnodactilia contractural congênita. Estas diferenças na expressão e patologias têm induzido à idéia de que as fibrilinas têm funções relacionadas porém distintas, que ainda não foram totalmente esclarecidas, porém sugerem que as fibrilinas-1 e 2 estariam

envolvidas na homeostasia do tecido e morfogênese, respectivamente (Ramirez & Pereira, 1999).

3.1.2.OUTRAS PROTEÍNAS ASSOCIADAS AS MICROFIBRILAS

Evidências sugerem que as fibrilinas são codificadas por uma família de genes, sendo descritos até o momento três membros, mas possivelmente existem mais. Uma terceira proteína provisoriamente denominada proteína *fibrilin-like* (FLP) foi isolada e mostrou conter os mesmos domínios repetidos das fibrilinas-1 e 2 (Mechan & Davis, 1994).

Um grande número de outras proteínas descritas como constituintes das microfibrilas parecem não estar relacionadas às fibrilinas. Uma delas é a glicoproteína associada a microfibrila (MAGP) de 31kDa, que foi isolada por Gibson e colaboradores em 1986. MAGP contém duas regiões estruturalmente distintas: um domínio N-terminal rico em glutamina, prolina e aminoácidos ácidos e o domínio C-terminal que possui todos os 13 resíduos de cisteína e a maioria dos aminoácidos básicos. (Gibson et al., 1991. A localização através da imunofluorescência e imunomarcação com ouro mostrou que a distribuição da MAGP corresponde a mesma das microfibrilas associadas ou não à elastina, em uma grande variedade de tecidos (Gibson & Cleary, 1987; Kumaratilake et al., 1989). Assim como as fibrilinas, a exata função e organização da MAGP dentro da estrutura microfibrilar ainda não são conhecidas.

Além da MAGP, outras proteínas também isoladas de extratos de tecidos ricos em fibras elásticas, são candidatas a componentes das microfibrilas como a enzima lisil oxidase (Kagan et al., 1986) e as glicoproteínas de 32kDa (AMP) (Horrigan et al., 1992), de 36kDa (Kobayashi et al., 1989) e de 115kDa (emilina) (Bressan et al., 1993).

3.1.3.BIOSSÍNTESE E DEGRADAÇÃO DAS MICROFIBRILAS

As microfibrilas aparecem, em corte longitudinal ao microscópio eletrônico, como filamentos semelhantes a um fio constituídos de cordões de contas compostos por feixes

de braços lineares unidos por contas globulares. O cordão de contas provavelmente represente o polímero de fibrilina puro que subseqüentemente se arranja junto com outros componentes para formar os filamentos. A natureza bioquímica das contas e as etapas de formação das microfibrilas ainda permanecem desconhecidas (Ramirez & Pereira, 1999).

Embora haja pouco ou nenhum *turnover* das microfibrilas em tecidos adultos, a degradação de tecidos elásticos faz parte de processos normais durante o envelhecimento e é marcante em várias doenças humanas. Parece que microfibrilas ricas em fibrilina podem ser degradadas por proteases séricas e elastases de neutrófilos e macrófagos (Ramirez & Pereira, 1999).

Informações mais detalhadas e atuais sobre as propriedades químicas e físicas das microfibrilas e seus componentes podem ser encontradas em revisões como Rosenbloom et al. (1993) e Mechan & Heuser (1991) e outros artigos Cleary et al. (1981), Sear et al. (1981), Ramirez & Pereira (1999) e Mariencheck et al. (1999).

3.2. ELASTINA

3.2.1. CARACTERÍSTICAS GERAIS E ESTRUTURA

A elastina é a proteína de matriz extracelular encontrada em vertebrados, responsável pela elasticidade de vários tecidos e órgãos que são submetidos à estiramento e subseqüente recuperação da forma como pele, artérias, pulmão, ligamentos e fibrocartilagens.

Muitas técnicas bioquímicas padrões utilizadas na caracterização de proteínas ou determinação de parâmetros cinéticos de reações bioquímicas não são aplicáveis a estudos da biossíntese de elastina devido às propriedades físicas únicas dessa proteína. A recente aplicação de técnicas imunológicas e de biologia molecular, associadas à estudos morfológicos das fibras elásticas em diversos tecidos, têm contribuído com importantes dados para a compreensão do processo de elastogênese (Mechan, 1981).

A elastina é a proteína predominante em fibras elásticas maduras, caracterizada por ser altamente insolúvel e apresentar hidrofobicidade extrema (Partridge, 1962). Essas

propriedades físicas incomuns da elastina fazem dela uma das proteínas mais estáveis do corpo, permanecendo durante toda a vida do organismo (Shapiro et al, 1991).

Devido à extrema insolubilidade da elastina, pesquisas sobre o processo de formação de fibras elásticas só começaram a progredir com a descoberta do seu precursor solúvel, a tropoelastina. A elastina é constituída então por monômeros de tropoelastina que se unem covalentemente através de ligações cruzadas e apresenta uma composição peculiar de aminoácidos, coerente com suas propriedades físicas. Uma análise da elastina presente em várias espécies de vertebrados mostrou que ela é rica em glicina (33%), alanina (24%), valina (15%), prolina (10-13%) e que aminoácidos hidrofóbicos constituem aproximadamente 44% dos resíduos totais. Embora a elastina contenha 4% de lisina, a maior parte desta está incorporada nas ligações cruzadas (Rosembloom et al., 1993; Debelle & Alix, 1995).

Pelo fato de se localizarem entre os domínios de ligações cruzadas altamente rígidos, os segmentos hidrofóbicos exibem uma mobilidade considerável e contribuem grandemente para a entropia do sistema (Debelle & Tamburro, 1999).

Vários modelos estruturais têm sido propostos para explicar a função da elastina e a sua habilidade de retornar à posição relaxada após estiramento. Há uma série de evidências que sugerem que a base da elasticidade desta molécula está na sua origem entrópica, ou seja, o estiramento reduz a entropia do sistema e a volta à condição relaxada é dirigida por um retorno espontâneo para a entropia máxima. O mecanismo pelo qual isto ocorre ainda é objeto de especulação (Weis-Fogh & Andersen, 1970; Dorrington & McCrum, 1977; Gosline, 1978).

3.2.2. BIOSSÍNTESE DE ELASTINA E FORMAÇÃO DAS FIBRAS ELÁSTICAS

Todos os experimentos realizados até o momento, incluindo análise em Southern blot do DNA genômico bovino, ovino e humano, indicam que apenas uma cópia do gene para elastina está presente no genoma de mamíferos (Olliver et al., 1987; Fazio et al., 1991).

Diversos tipos celulares produzem a elastina, como por exemplo, células musculares lisas, células endoteliais, condrócitos e fibroblastos. Estas células podem responder a estímulos biológicos comuns como hormônios ou macromoléculas da matriz extracelular, permitindo assim determinar o início da elastogênese e controlá-la através de fatores externos (Mecham, 1981).

A elastina é sintetizada no retículo endoplasmático granular (REG) na forma de seu precursor solúvel, a tropoelastina, com aproximadamente 70.000 a 75.000 Daltons. Imediatamente após a tradução, a molécula de tropoelastina é associada a uma galactolectina de 67kDa que atua efetivamente como uma chaperona, prevenindo a agregação intracelular prematura. Essa associação persiste no Complexo de Golgi até a secreção da tropoelastina para o espaço extracelular (Mechan et al., 1989; Hinek & Rabinovitch, 1994).

As cadeias nascentes de tropoelastina possuem um peptídeo sinal que serve de vetor de transferência para a proteína em crescimento através da membrana do REG. Embora a via específica do transporte intracelular da tropoelastina não tenha sido completamente descrita, sabe-se que algumas proteínas como a actina estão envolvidas no transporte de vesículas do REG para elementos transitórios, destes para o Complexo de Golgi e em seguida para sítios da membrana celular (Mecham & Heuser, 1991). Ainda no Golgi a tropoelastina é submetida a hidroxilação das prolinas e remoção do peptídeo sinal, sendo então secretada juntamente com a galactolectina para a superfície celular. No espaço extracelular este complexo entra em contato com a fibra elástica nascente e a interação da chaperona com açúcares do tipo galactose presentes nas glicoproteínas microfibrilares reduz drasticamente a afinidade da galactolectina pela tropoelastina, conduzindo à liberação desta no local de formação da fibra (Debelle & Tamburro, 1999; Mecham, 1981).

Enquanto a chaperona é reciclada passando a fazer parte constitutiva do receptor celular para elastina, os domínios de ligação da molécula de tropoelastina são alinhados para interagir com a glicoproteína microfibrilar ou com outros componentes da matriz extracelular e a enzima lisil oxidase catalisa as reações de ligações cruzadas formando então a fibra elástica funcional (Hinek, 1997).

Este processo de fibrilogênese ocorre em sítios próximos à membrana celular, geralmente em invaginações da superfície celular. As microfibrilas são os primeiros componentes visíveis das fibras elásticas e aparecem agrupados em pequenos feixes

perto da membrana plasmática. Com o desenvolvimento da fibra, a elastina aparece como um material amorfo em um discreto locus dentro de cada feixe microfibrilar. Essas áreas amorfas gradualmente se fundem gerando um *core* central de elastina. A maioria das microfibrilas é progressivamente disposta na região periférica da fibra, posição esta que se mantém na fibra madura. A observação de que agregados de microfibrilas tomam a forma e orientação das pressupostas fibras elásticas sugere que elas direcionam a morfogênese das fibras elásticas atuando como um arcabouço para a deposição de elastina (Rosembloom et al., 1993; Mechan & Davis, 1994; Montes, 1996).

3.3.3. DEGRADAÇÃO E REPARO DA ELASTINA

A elastina apresenta um *turnover* muito lento em tecidos normais. Esta visão reflete em parte a alta resistência da elastina à degradação pela maioria das enzimas proteolíticas, com exceção daquelas que têm especificidade por fragmentos de cadeia polipeptídica contendo aminoácidos hidrofóbicos (Mechan, 1981). O grupo de proteases capazes de degradar a elastina é coletivamente conhecido como elastases e geralmente atuam sobre uma grande variedade de substratos além da elastina. As elastases séricas, que incluem a elastase pancreática, elastase produzida por neutrófilos e a catepsina G são as elastases mais abundantes em mamíferos (Stone & Franzblau, 1982).

A degradação da elastina é importante em muitos processos fisiológicos como crescimento, cicatrização, gravidez e remodelação de tecidos. Entretanto, uma elastólise inapropriada ou descontrolada pode contribuir para doenças como enfisema pulmonar e ateroesclerose em artérias. A degradação da elastina também é uma característica do envelhecimento normal (Braverman & Fonferko, 1982).

Ao contrário da elastina, o seu precursor tropoelastina é extremamente susceptível à ação de proteases (Mecham & Foster, 1978) e mesmo estando covalentemente ligada na fibra elástica, ela ainda contém resíduos de lisina que podem servir como sítios de clivagem para algumas enzimas (Mecham, 1981).

Alguns estudos têm mostrado que os peptídeos solúveis derivados da degradação de elastina e de tropoelastina desempenham vários papéis biológicos. Peptídeos originados pela ação de elastase de leucócito humano sobre elastina madura, por exemplo, são quimiotáticos para células inflamatórias humanas e podem ser sinais necessários para respostas inflamatórias em tecidos que contém elastina. Fragmentos de elastina também são capazes de gerar respostas imunes que podem ser importantes na etiologia de algumas doenças de tecido conjuntivo, assim como podem penetrar no fluxo sangüíneo e causar vasodilatação (Mecham, 1981).

Ainda resta investigar se estes fragmentos podem ser capazes de regular a biossíntese da elastina. Interações entre peptídeos de elastina com a superfície celular podem representar uma importante via ou mecanismo de comunicação das condições celulares na matriz extracelular. Sendo assim, fragmentos de elastina e de tropoelastina podem fornecer sinais distintos pela natureza de suas estruturas químicas e susceptibilidade à proteases diferentes (Mecham, 1981).

O reparo de elastina danificada por protease pode ocorrer, mas não parece produzir uma elastina com a mesma qualidade daquela originalmente sintetizada durante o crescimento. No reparo de tecido pulmonar após um enfisema induzido experimentalmente, os níveis de elastina podem voltar ao normal, mas as novas fibras elásticas são altamente desorganizadas e não são completamente funcionais (Vrhovski & Weiss, 1998).

Atualmente, com todas essas novas informações sobre a elastina, fica claro que de modo algum ela pode ser considerada uma molécula estática cuja função se restringe à elasticidade. Novas pesquisas devem ser desenvolvidas com o objetivo de investigar as interações entre elastina e o sistema biológico completo, de forma a fazer novas revelações sobre a biologia do tecido conjuntivo.

Detalhes da biossíntese e composição bioquímica das fibras do sistema elástico, da sua distribuição, organização ultra-estrutural, e função em diferentes órgãos e tecidos podem ser encontradas em algumas revisões clássicas (Ross, 1973; Fullmer et al., 1974; Cotta-Pereira et al., 1977; Sandberg et al., 1981; Ghadially, 1982; Cleary & Gibson, 1983; Mecham & Heuser, 1991; Mecham et al., 1991; Montes, 1992; Rosenbloom et al., 1993; Montes, 1996; Vrhovski & Weiss, 1998; Debelle & Tamburro, 1999). Nestas revisões estão as referências e as contribuições originais aos diferentes tópicos.

4. MÉTODOS DE ESTUDO

Os principais componentes que conferem a característica elástica aos tecidos e órgãos foram inicialmente descritos, ao nível da microscopia de luz, pelo emprego de colorações multicompetentes que datam do final do século passado e início deste: a resorcina-fucsina (Weigert, 1898) e a hematoxilina férrica (Verhoeff, 1908). Posteriormente a estas colorações foram adaptadas técnicas como a forte oxidação do tecido, com agentes como o ácido peracético ou monopersulfato de potássio (oxona), seguida de coloração por corantes como a orceína ou a resorcina-fucsina (Fullmer & Lille, 1958; Fullmer, 1960); estas adaptações conferiram caráter seletivo às colorações.

Apesar do grande interesse no estudo do papel biomecânico das fibras do sistema elástico são relativamente poucos as facilidades e reagentes disponíveis para o estudo deste componente dos tecidos conjuntivos. Recentemente o emprego de novos recursos técnicos, no que diz respeito à microcopia de luz, a exemplo do microscópio confocal que, permite explorar espectros de emissão luminosa flourescente resultante da interação de corantes empregados rotineiramente, como por exemplo hematoxilina-eosina-floxina, tem aberto novas possibilidades para estudos retrospectivos da distribuição deste componente na matriz (Carvalho & Taboga, 1996 a, b).

5. CONSIDERAÇÕES

Levando-se em consideração que:

1) existe uma distribuição diferencial das fibras do sistema elástico em compartimentos de diversos órgãos, a saber: pele (Cotta-Pereira et al., 1976), nervos (Ferreira et al., 1987), pleura e vias respiratórias (Lemos et al., 1997; Leick-Maldonado et al., 1997), nas cartilagens hialinas e fibrosas (Cotta-Pereira et al., 1984) e tendões (Caldini et al., 1990; Carvalho et al., 1994, Carvalho & Vidal, 1995; Battlehner et al., 1996);

2) no útero de ratos foi observado o aumento de fibras elásticas com um menor número de *crosslinks* na elastina durante a prenhez e sua redução após o parto (Starcher & Percival, 1985: Gunja-Smith et al., 1989);

3) grande variação hormonal desempenha papel significativo na manutenção da prenhez e contribui para acomodação fetal e na parturiação, através da expansão do canal de parto (Vasilenko & Mead, 1987);

4) ficou demonstrado que células, tanto do útero quanto da sínfise do camundongo têm as maiores concentrações de receptores para relaxina quando comparado com aquelas de outros órgãos (Yang et al. 1992), e que este hormônio estimula o aumento do volume do útero, da cérvix e da vagina através da hidratação da matriz extracelular, diminuindo a concentração do colágeno e aumentando a de glicosaminoglicanos (Downing & Sherwood; 1986; Vasilenko et al. 1986; Vasilenko & Mead, 1987) .

Constata-se então que é relevante realizar estudos sobre a distribuição diferencial das fibras do sistema elástico. Este estudo foi realizado em camundongos adultos fêmeas virgens; na última semana da prenhez; durante o parto e até o quinto dia pós-parto, com o objetivo de verificar a influência da prenhez nas possíveis transformações das fibras do sistema elástico na sínfise.

Além disso, caso estes efeitos sobre o sistema elástico se confirmem, a sínfise púbica do camundongo fêmea poderá constituir-se em um excelente modelo para o estudo da remodelação das proteínas do sistema elástico em condições fisiológicas.

OBJETIVOS

Considerando as características biológicas do processo de transformação gradual da sínfise púbica em um ligamento extensível, não somente às custas da proliferação de condrócitos, fibrocondrócitos e fibroblastos mais principalmente de modificações no metabolismo de componentes da matriz extracelular, é razoável pressupor que o *turnover* das fibras do sistema elástico deva representar um papel importante neste processo, assim como a modulação fenotípica das células envolvidas no mesmo.

Neste sentido, constituem-se objetivos do presente trabalho:

1. Identificar os tipos de fibras do sistema elástico presentes na sínfise e ligamento do camundongo, durante a prenhez, parto e pós-parto.
2. Conhecer a distribuição diferencial das fibras do sistema elástico na sínfise púbica do camundongo, submetida às condições fisiológicas resultantes da prenhez, por meio de métodos seletivos de coloração associados ao estudo ultra-estrutural.
3. Avaliar quantitativamente o conteúdo de fibras do sistema elástico através de técnicas morfométricas.
4. Avaliar a expressão de proteínas do citoesqueleto, vimentina , desmina e α-actina de músculo liso, em células da sínfise púbica durante a formação e involução do ligamento interpúbico.
5. Explorar aspectos ultra-estruturais das células que podem ser relevantes para a organização das fibras do sistema elástico na matriz extracelular da sínfise púbica.

De acordo com os resultados obtidos, este trabalho pretende ainda: a) fornecer subsídios para a compreensão do processo de remodelação do tecido conjuntivo da sínfise púbica. b) contribuir tanto para trabalhos subseqüentes que visam caracterizar outros componentes da matriz extracelular e suas interações, quanto para outros que visam promover o desenvolvimento de métodos hormonais que permitam um aporte à indução ou inibição do parto e diminuam os riscos de traumatismo fetal por distocia obstétrica.

ARTIGOS

Durante a realização deste trabalho foram elaborados os seguintes artigos para serem submetidos à publicação:

1. **Differential Distribution and Ultrastructural Study of Elastic System Fibers in the Mice Pubic Symphysis During Pregnancy, Partum and Post-Partum**
Suzana Guimarães Moraes, Mônica de Campos Pinheiro, Áureo Tatsumi Yamada, Paulo Pinto Joazeiro.

2. **Phenotypic Modulation of Fibroblastic Cells in the Mice Pubic Symphysis During Pregnancy, Partum and Post-partum**
Suzana Guimarães Moraes, Olga Maria S. Toledo, Paulo Pinto Joazeiro.

Differential Distribution and Ultrastructural Study of Elastic System Fibers in the Mice Pubic Symphysis During Pregnancy, Partum and Post-Partum

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Running Title: Elastic system fibers in pregnant mice pubic symphysis

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ABSTRACT

The spreading apart of the pubic joint depends on the growth of the ligament formed in some pregnant rodents. The development of the interpubic ligament involves the increase of biosynthesis of the extracellular matrix (ECM). Early studies with the female reproductive tract indicate that significant metabolism of elastin is also occurring during pregnancy, but little attention has, so far, been paid to the architecture and ultrastructural aspects of the elastic system fibers in the mouse pubic symphysis. In order to evaluate the main characteristics of the elastic system, mice pubic symphysis during pregnancy, partum and post-partum were used for light microscopical and ultrastructural studies. A distinct pattern of arrangements of the microfibrils and an elastin deposition process were observed by ultrastructural analysis during pregnancy and post-partum. Quantitative evaluation of elastic system in histological preparations was computed on the basis of determining the number of intersections between a test system with fibers deeply stained by Weigert's resorcin-fucsin (after oxidation of the tissue sections). The data obtained on the virgin mice pubic symphysis were similar to that seen on the 12th day of pregnancy, where the fibers consisted of small bundles of 12nm microfilaments. At 12-18 days of pregnancy there were twice as many intersections between the test system and pre-elastic fibers. This may be an indication of the increase of elastic system components.

Key words: elastic system fibers; pubic symphysis; ligament; histochemistry, ultrastructure

INTRODUCTION

The pubic symphysis is a joint formed with a fibrocartilaginous disc lying between the hyaline cartilage-covered medial borders of the pubic bones. Four ligaments reinforce this articulation. The anterior and inferior or arcuate ligaments contribute to the strength of the joint (Gamble et al., 1986). Attention has been paid to pubic symphysis changes during pregnancy. The mechanism of passage of young through the birth canal at delivery has been explained by means of hormonally regulated adaptations of the pelvis. Transformation of the pubic joint cartilage to a flexible and elastic interpubic ligament occurs during the pregnancy, increasing the birth canal. According to the historical briefing by Thoms (1936), this was first documented by LeGallois in 1812 when he noted that the head of the fetus of the guinea-pig was approximately twice the size of the normal pelvic channel and that a sizable ligament formed between the two pubic bones enabling the birth canal to accommodate the passage of the fetuses.

Extensive studies about hormonal regulation of the interpubic ligament development demonstrated that growth of this structure is stimulated primarily by estrogen and relaxin (Sherwood, 1994). This first morpho-physiological change, in the mouse, involves the increasing of biosynthesis activity of the extracellular matrix components in the center of pubic joint (Crelin, 1954). During the last week of pregnancy, the pubic bones separate. This event is attributable to resorption of the medial ends of the pubic bones, swelling of the cartilaginous matrix, and transformation of the cartilage caps into a fibrous interpubic ligament that attains a maximum length of 3 to 6mm at parturition. Following parturition, (which occurred between the 19 th and 22 nd day), a rapid regression of the interpubic ligament and cervix, that approaches cycling virgin dimensions about 5 days post-partum were observed (Hall, 1947; Crelin, 1954; Storey, 1957; Kroc et al., 1958; Steinetz et al., 1957; Horn, 1960).

The physiologic function of many tissues requires that they possess elastic properties, which are due to the presence of elastic fibers in the extracellular space (Rosembloom et al., 1993). Tissues are not homogeneous structures from the point of view of the distribution of elastic system fibers (Montes, 1992).

Three types of elastic fibers have been described: mature elastic fibers, elaunin fibers and oxytalan fibers. These fibers are identified by different histological, biochemical

and immunological methods (Cotta-Pereira et al.1976; Cotta-Pereira et al.1984; Fukuda et al., 1984). Ultrastructural analysis is also necessary to distinguish the different types of fibers with accuracy (Ross, 1973). At the ultrastructural level, mature elastic fibers have been show to be composed of two components – a central electron-lucent, abundant and homogeneous amorphous core of elastin, and peripherally located microfibrils that measure from 10 to 12nm in diameter and in cross-sections, exhibit an electron-dense tubular profile (Ross and Bornstein, 1969). Elaunin fibers consist of bundles of microfibrils that cross discontinuous aggregates of elastin while oxytalan fibers are composed exclusively of microfibrils identical to those presented by mature elastic fibers, but without elastin (Montes, 1996).

These three different types of fibers possess distinct mechanical properties. Elastic fibers, because of their large proportion of elastin, are able to distend, whereas oxytalan fibers (which are devoid of elastin) are practically inextensible. Elaunin fibers present an intermediate behavior between these two extremes (Montes, 1996).

Because organ systems possess different functional characteristics, it is not surprising that connective tissue components found in such distinct compartments differ both qualitatively and quantitatively (Montes, 1992). The distribution of oxytalan fiber, for example, appears to be restricted to sites where connective tissue is subjected to mechanical stress (periodontal membranes, skin and adventtia), thus suggesting that this fiber type acts to prevent overstretching of the structures present in these areas (Montes, 1992; Fullmer, 1960).

Pregnancy induce reversible changes in mRNA for matrix molecules in ligaments, but their complexity indicates that there is probably no simple cause and effect relationship between laxity changes and the molecular alterations, moreover the difference in responsiveness exist between different ligaments (Hart et al, 1998).

Regarding the elastic system early studies with the female reproductive tract indicate that significant metabolism of elastin is also occurring during pregnancy (Starcher and Percival, 1985), but little attention has so far been paid to the architecture and ultrastructural aspects of the elastic system fibers in the mouse pubic symphysis. The pubic symphysis ligament has several features which make it an usual system for studying collagen and also further investigation into the metabolism of ligament connective tissue, as it was observed in the study of collagen metabolism under hormonal control in the guinea-pig (Wahl et al. 1977). Among the constitutive features, the relative tissue

homogeneity, in which fibroblast are the main cellular component, the rapid increase in the ligament size prior to delivery and post-partum decrease in size, as consequence of a short period of time for both formation and degradation of extracellular matrix molecules, makes it useful when compared with another frequently used system, like uterus, where a tissue heterogeneity is present and a gradual extracellular matrix formation is observed (Wahl et al. 1977). Starting from the observation of numerous elastic system fibers in pubic symphysis of pregnant free-tailed bat in previous morphological investigations (Crelin, 1969a), we designed the present experiments to study the distribution and ultrastructural characteristics of elastic system fibers in the mice pubic symphysis during pregnancy, partum and post-partum.

EXPERIMENTAL PROCEDURES

Animals

Virgin female Swiss mice, 3 months old and over 25-30g of body weight (Center for Animal Care of State University of Campinas, SP, Brazil) were used. In this study animals were maintained under controlled environment (25 ± 2 °C; exposed to light daily cycle of 12 hours) and had free access to pellet laboratory chow and tap water. Animal's studies were conducted in accordance with the Guide for the Care and Use of Laboratory Animals (National Academy of Science, 1996).

To obtain pregnant specimen, virgin females were caged overnight with males (in proportion of 2:1). A "plug" in the vagina the following morning indicated successful mating and this day was designated the first day of pregnancy (D1). The delivery could be expected on day 19.

Pubic symphysis samples were achieved from animals during pregnancy (D12, D15, D17 and D18), immediately after delivery (D19), and during post-partum involution (D20, D21, D22, D23 and D24). Cycling virgin mice in estrus were also used as control. Estrus was determined by vaginal smears according to Shorr (1941).

Tissue Sample Collection

For light microscopy and morphometry analysis five animals were used for each experimental point and two animals per group were studied in electron microscopy. The animals were sacrificed by cervical dislocation. The pubic symphysis were dissected clean and fixed *in situ* with appropriate fixative for 10 minutes. Then the specimens were removed and immediately immersed in the same fixative.

Light Microscopy

Specimens were fixed in 4% paraformaldehyde in 0.1M phosphate buffer pH 7.4 for 24 hours at 4 °C, decalcified in 7% EDTA with 2% paraformaldehyde in 0.1M phosphate buffer pH 7.4 for 5 days at 4 °C, dehydrated and embedded in paraffin. Serial sections (7 μm in thickness) were cut in frontal plane throught the symphyseal region and mounted on poly-L-lysine solution (Sigma Chemical, St. Louis, MO, U.S.A.) 0.1% w/v in water coated slides and dried for 24h at 37 °C.

Sections were stained by the following methods: hematoxylin and eosin, Verhoeff's iron-hematoxylin (Verhoeff, 1908), Weigert's resorcin-fuchsin (Weigert, 1898) with and without previous oxidation according to Caldini et al (1990). Oxidation was performed using oxone as previously described (Fullmer et al, 1974).

Samples of human skin (obtained from autopsies) were used as controls of staining techniques (Cotta-Pereira et al., 1976).

Slides were mounted with permanent mounting medium.

Transmission Electron Microscopy

Small segments (approximately $1mm^3$ in size) of the pubic symphysis or interpubic ligament were fixed in a solution containing 0.1% tannic acid in 2.5% glutaraldehyde dissolved in 0.1M sodium cacodylate buffer (pH 7.2) for 2 hours at room temperature, followed by post fixation in 1% osmium tetroxide for 1h at 4°C.

Fixed materials were stained en bloc in 1% aqueous uranyl acetate overnight. They were then dehydrated through graded ethanols. The samples were immersed in propylene oxide and then placed in a 1:1 dilution of Epon 812 (Electron Microscopy Science) in propylene oxide overnight. Next they were placed in pure resin and were put into molds containing fresh resin to polymerize for 72 hours at 60°C.

Semithin sections (1μm) were cut in LEICA ultratome and stained with toluidine blue. Representative areas of the pubic symphysis were selected. Ultrathin sections were obtained with the same ultratome and were double-stained by 2.0% uranyl acetate and 0.5% lead citrate. Ultrathin sections were studied and micrographed in a LEO 906 transmission electron microscope operating at.

Morphometry

The length and diameters profiles of the elastic system to be quantified were chosen based on either its known role in giving resiliency to the extracellular matrix or its ability to influence the special arrangement of matrix fibrous elements.

For light-microscopic analysis, the joinst were cut in semi-serial sections of 7 μm in coronal plane, and stained by Weigert's resorcin-fuchsin with previous oxidation. Only the medial ones where both fibrocartilaginous disc (cycling virgin and D12) and dense regular connective tissue ligament (D15, D17, D18, D19, D22 and D24) appeared anchored into

outer hyaline cartilage endplates or pubic bones were photographed and enlarged at x320 magnification. The medial sections were used because they included both the fibrocartilaginous discs where the first changes were related and it will ultimately become the ligaments; thus they constitute the most representative support to compressive/tensile loading.

Three randomly selected fields for each semi-serial sections were photomicrographed and three different sections were used for each joint specimen. In order to estimate the mean apparent profile length in a vertical projection of elastic system fiber population (oxytalan, elaunin and elastic fibers), the point counting methodology for structures length measurement was chosen by reference to standard Buffon's morphometric principles (Arherne and Dunnill, 1982). The photomicrography was overlaid in grid, with an area frame corresponding to 0,12mm^2, where parallel lines were 28,12μm apart (d). So one, "d" chosen were larger than mean profile length of 30 elastic system fibers (l = 21,46 ± 6,45 μm), directly measured over the photomicrograph taken on cycling (virgin) medial fibrocartilaginous tissue. The apparent length profile was calculated by the equation to the estimated length of a "needle" (***l* = (π/2).(n/N).d**) according to (Arherne and Dunnill, 1982).

Because of the small volume occupied by elastic system fibers when compared with other components of connective tissues (Cullen et al 1981; Wright and Churg, 1995), large number of intersections is required to achieve reasonable counts.

Since within section-slab the apparent profile length of a fiber is the sum of projected fiber profile (vertical projection) and the transected profile at the surface of the section, increase in the apparent profile length of a fiber results in the proportional increase in both projected fiber profile and transected profile. According to Parfitt (1983) for structures distributed between 0 and π/2 angle, the ratio between mean projected profile and mean transected profile is expressed by the formula 2/π . (D/T), where D = 7μm section thickness; T = elastic system diameter measured in micrometers on electron micrographs. Once the mean apparent profile length in a vertical projection was measured by reference to standard Buffon's morphometric principles and the ratio between profiles is a function of both section thickness and diameter of elastic system fibers, the contribution of either projected fiber profile or transected profile at the surface of the section to the mean apparent profile length may be readily calculated. Although the 2/π . (D/T) formula is

valid only for isotropic structures, the range of errors predicted from this formula usually not exceed 2,5 to 10% and only rarely exceed 4 to 16% for degrees of anisotropy found in trabecular bone (Parfitt, 1983).

For transmission electron microscopy analysis, the small diameter of cross-sectioned round or slightly elliptical elastic system fibers was measured directly over electron micrographs (some magnification of those micrographs illustrate the foregoing results), with a Bausch and Lomb measuring magnifier. At least of 30 measurements were made in each experimental specimen. The magnification of the electron microscope was calibrated with a diffraction grating.

Statistical Analysis

The values of each parameter were pooled together and analyzed using the ANOVA one way test to compare data in both symphyseal fibrocartilage of cycling and interpubic ligament of pregnant and post-partum mice. All data are presented as mean ± SEM. In all case differences having statistical probability P less than 0.05 was reported as significant. A personal computer was used to perform statistical analysis.

RESULTS

Light Microscopy

Light microscopical analysis of coronal section stained by hematoxylin and eosin showed that the structure of mature virgin and early pregnant (D12) mice pubic symphysis is composed by a fibrocartilaginous disc lying between the hyaline cartilage-covered articular surfaces of the pubic bones. A typical layer of dense connective tissue, containing numerous fibroblasts and vascular elements, covers the hyaline and fibrocartilage ventrally and dorsally. Sometimes in a few cycling virgin mice, a cleft between the cartilaginous caps, i.e., the symphyseal space, was seen. The cartilage adjoining this space contains long thin lacunae running parallel to it. The chondrocytes are larger and rounded where the cartilage merges into the surrounding connective tissue or the growing cartilage (Fig.1).

The morphology of mouse pubic symphysis on the D12 is quite similar to cycling, but a rapid proliferation of connective tissue cells and resorption of the symphyseal faces of the pubic bones occur during the last week of pregnancy. So, the cartilaginous pubic symphysis of the pregnant female is gradually replaced by fibrous connective tissue that forms a ligament between the pubic bones (data not show).

Pubic symphyses of animals in all physiological stages analyzed have elastic system fibers. According to Cotta-Pereira et al. (1984) except for the fibrous layer of the pericondrium, no elastic fibers could be detected when the cycling and pregnant mice tissue sections were stained with Verhoeff's iron hematoxylin. By this method some mature elastic fibers were observed in the interpubic ligament from intra-partum, mainly at the D24 (data not show). Using Weigert's resorcin-fuchsin, a little amount of elaunin fibers in the fibrocartilaginous tissue was also observed amongst the bundles of collagen fibers running along the fibrocartilage periphery. Using Weigert's method preceded by oxidation the presence of all elastic system components (i.e., oxytalan, elaunin and mature elastic fibers) was demonstrated forming a meshwork in the virgin fibrocartilage and interpubic ligament of the pregnant mice, where oxytalan and elaunin fibers predominated.

At cycling pubic symphysis a few thin fibers of the elastic system were localized mainly in the fibrocartilage and hyaline caps, and were disposed obliquely in relation of pubic bones separation (Fig.2). At early pregnancy (Fig.3), the elastic system fibers do not

display a preferential disposition, but at the late pregnancy they change progressively to a polarized direction clearly observed with thicker fibers at term (Fig.4). After delivery, the elastic system fibers were seen disposed in a similar organization of that pregnant mouse (Fig.5).

Electron Microscopy

The ultrastructural picture of the distribution of the elastic system fibers was equivalent to the description made by means of light microscopy as much as with the virgin as with the pregnant and post-partum animals.

Ultrathin sections of virgin (Fig.6) and D12 (Fig.7) mice showed thinner bundles of microfibrils, about 10-12nm in diameter, always interspersed with fibrils of collagenous system. These elastic system fibers were devoid of elastin, exhibiting a pattern characteristic of oxytalan fibers.

From D15, the start of the elastin deposition process was observed, so when fibers have been sectioned transversely it comprises a bundle of microfibrils interspersed with patches of amorphous and homogenous material that correspond to elaunin fiber (Fig.9). The elastic system fibers which lie more frequently in the vicinity of fibroblast-like cells (Figs.9, 10, 11) and in a longitudinal section (Fig.10) appear as linear aggregates of microfibrils.

In the partum, the pre-elastic fibers present in the interpubic ligament showed two distinct ultrastructural patterns. In some areas it can be seen that typical elaunin fibers composed by preserved microfibrils associated with variable amounts of amorphous materials (Fig.12). However, in other regions the pre-elastic fibers were constituted by scattered microfibrils, which present irregular outline and were involved with disorganized extracellular matrix (Fig13).

On D22 pre-elastic fibers in close contact with fibroblast-like cells were also seen, most of them showing a convoluted arrangement (Fig15). The microfibrilar component at D24 presented the same convoluted pattern as in D22 but the observation of microfibrils was difficult due to great amorphous material that was laid down among the microfibrils (Figs.16, 17).

Morphometric Analyses

The media values for estimated mean apparent profile length and diameter of elastic system fibers determined over histologic tissue sections, during the evolution of pregnancy, are presented in Table 1. The P values reflect the statistical significance derived from ANOVA test when successive morphometrical data was compared. Figures 18 and 19 show the histogram and box-plot for the apparent profile length and diameter increments respectively of elastic system fibers in pubic symphysis and ligament of cycling, pregnant, and post-partum mice. The extension to which transected profile at the histological section surface contribute to the mean apparent profile length was calculated and it was comprised between 4% (Virgin) to 10,2% (D17), even when the largest mean diameter (D19) was observed.

At the early pregnancy for 12 days, there was a progressive and significant ($P < 0.001$) increase in the diameter of elastic system fibers (oxytalan and elaunin) with time, disclosing a progressive organization of the elastic system components in the extracellular matrix during the early pregnancy compared with virgin cycling mice, however it was found with an approximately similar mean apparent profile length in photomicrographs of fibrocartilage at D12 pregnant mice and cycling mice.

As shown in the morphometrical data, during the late pregnancy (D15 to D17) the elastic system fibers now in ligament extracellular matrix showed larger diameters in relation to virgin and D12. At this time the mean apparent profile length of the elastic system fibers also progress significantly ($P < 0,001$). However, it must be emphasize, that contribution of the fiber diameters to mean apparent profile is negligible, less than 10 % of the profile length. Therefore this result does not indicate a positive association between the progressive thickening of the elastic system fiber diameters (34% and 28% from D12 to D15 and D15 to D17 respectively), rather than a change in the obliquity of elastic system fibers.

By D18 a significant decrease in the mean apparent profile length ($P < 0,001$) began, however the mean diameter of elaunin fibers remained similar to D17. Values of both mean apparent profile length and mean diameter of elaunin fibers do not change at the delivery (D19). At D22 and D24 the mean apparent profile length showed an opposite behavior to that observed for diameters. The diameter proportions of elastic system fibers remained similar at the end of late pregnancy (D18), partum (D19) and post-partum (D22 though D24). At the same times it was observed that the mean apparent profile length of

elastic system fibers tend to decrease significantly at the post-partum (D24) and it was near to the values observed at D15.

DISCUSSION

The dynamic modification of the birth canal at delivery, which includes an adaptation in the pubic bones, is a basic problem in the physiology of mammalian pregnancy and parturition. There is an extensive literature concerned with the changes taking place in the pubic symphysis during pregnancy or hormone treatment (Sherwood, 1994).

The separation of the pubic bones is attributable to resorption of their articular surfaces, to swelling of the cartilaginous matrix, and transformation of the cartilage into an interpubic ligament. After parturition, the ligament is reduced in length and the pubic bones move together. Cartilage is remodeled through the pressure of surrounding tissues and new bone formation restores the architecture of the bony symphysis. These changes were described in the mouse (Hall, 1947; Crelin, 1954; Crelin and Haines, 1955; Storey, 1957; Steinetz et al., 1957, 1965), guinea pig (Ruth 1936,1937; Talmage 1947a-b; Wahl et al., 1977), bats (Crelin and Newton, 1969; Crelin, 1969a) and human (Crelin, 1969b; Vix, 1971; Gamble et al., 1986). Storey (1957) showed that relaxin given to the estrogen-sensitized mouse reproduces these changes of pregnancy in the pubic symphysis. Altered interpubic histoarchitecture in the mice by castration, pregnancy or injections of estradiol benzoate and relaxin have been frequently reported in the literature, but this process was not observed in rat neither during pregnancy nor in other experimental assay (Crelin and Brightman, 1957; Ortega et al., in press).

Recently it was histologically demonstrated that relaxin null mutant female mice do not enlarge the interpubic ligament significantly at the end of pregnancy, due a more compact collagen arrangement, without significant biochemical differences or water increase, but they were fertile, produce normal litters and delivery normally (Zhao et al., 1999, 2000). Those studies do not give detail about the elastic system fibers neither in mutant nor in wild type mice.

Despite the morphological and biochemical studies about the composition and metabolism of the extracellular matrix components in the mouse pubic symphysis and interpubic ligament (Linck et al, 1976; Weiss et al, 1979; Viell and Struck, 1987; Zhao et al., 2000), until the present moment no elastic system study was realized in this model.

The aim of this study was to analyze the distribution and ultrastructural features of the elastic system fibers present in the mouse pubic symphysis during pregnancy, partum and post-partum.

Elastic fibers constituted of two components, elastin and microfibrils interconnected during elastogenesis, being among the most complex structures of the extracellular matrix. Their assembly requires the coordinate expression of at least four different proteins that not only organize the polymerization of the elastin monomer but also determine the final architecture of the fiber according to the functional requirements and mechanical stresses imposed on the tissue (Mecham and Davis, 1994).

Mature elastic fibers, rich in elastin, do provide the elastomeric properties required in several tissues such as blood vessels. These fibers are characterized by a strong staining with resorcin-fuchsin and by a high elastin/microfibrils ratio (Montes, 1996). Oxytalan fibers (Fullmer and Lillie, 1958), devoid of elastin, probably do not elongate much under mechanical stress (Cotta-Pereira and Iruela, 1989). They are more abundant in locations where resistance to mechanical stress is required, such as dermo-epidermal junction (Cotta-Pereira and Iruela, 1989), periodontium (Fullmer and Lillie, 1958), ciliary zonule (Cotta-Pereira and Iruela, 1989) and tendon (Caldini et al., 1990; Carvalho et al., 1994; Carvalho and Vidal, 1995). In 1965, Gawlik described the elaunin fibers as being composed of microfibrils and a small amount of elastin, which having mechanical properties in between mature and oxytalan fibers (Cotta-Pereira and Iruela, 1989). A large number of studies has focused on the presence and function of the fibers of the elastic system in different organs and tissues (see some classical reviews, Ross, 1973; Fullmer et al., 1974; Sandberg et al., 1981; Cleary and Gibson, 1983; Ghadially, 1988; Mecham and Heuser, 1991; Rosenbloom et al., 1993; Montes, 1996).

The chemical and mechanical characteristics of elastic fibers indicate that these fibers are responsible for the reversible distensibility of the connective tissue (Greenlee *et al.,* 1966; Ross, 1973; Sear *et al.,* 1981; Paniagua *et al.,* 1983; Debelle and Alix, 1999; Montes, 1996;), since they exhibit elastic deformation and are also able to store elastic energy to restore the deformed tissue to its original configuration.

The capability of storing elastic tension would be of interest in a structure such as the mice pubic joint, which are able to increase and decrease their size during hormone induced changes or pregnancy (Storey, 1957). Elastic system fibers could participate in pubic joint opening and closing, by storing and transferring energy quanta to both pull or

push back pelvic components when the hormonal and mechanic stimulations of the connective tissue cells were up or down regulated.

As a matter of fact, the presence of elastic fibers in the pubic joint were classically described in free-tailed bat (Crelin, 1969a; Crelin and Newton 1969), but the mechanisms about their arrangement and connection with cells and, on the other hand, with surrounding macromolecular system of extracellular matrix were not explained.

The results of this study show that the distribution and ultrastructural characteristics of elastic system fibers in mouse pubic symphysis change during the course of pregnancy and post-partum. By light and electron microscopy analysis a predominant presence of oxytalan and elaunin fibers was observed in pregnancy stages, whereas elaunin and elastic fibers predominated at post-partum.

In cycling virgin and early pregnancy we demonstrated, in the fibrocartilaginous tissue in the medial region, that slender fibers of the elastic system are observed frequently interspersed with fibrils of collagenous system (Fig, 6 and 7), possibly acting as the effectors of the interdependence between both systems. Thus oxytalan and elaunin fibers in collaboration with the collagen fiber bundles may provide the pubic joint with special elasticity to support the joint spreading apart. This interaction could trigger a positive biochemical synergism (Oakes and Bialkower, 1977), which would strengthen the whole structure (Greenlee et al., 1966; Oakes and Bialkower, 1977; Höpker et al., 1986). The collagen fibers can be acting as sources of tensile strength, while oxytalan and elaunin fibers contribute to matrix resiliency, restricting the stretching of interpubic ligament. In fact, the presence of elastic system fibers in the tissues under compression/tension as well as cartilaginous, fibrocartilaginous and compressional zones of the tendons is classically known (Cotta-Pereira et al, 1984; Hess, 1987; Carvalho et al., 1994; Carvalho and Vidal, 1995).

Elaunin and oxytalan fibers have an adequate mechanical profile to transmit tension without significant viscous or plastic losses (Fullmer and Lillie, 1958; Manning, 1974; Cotta-Pereira *et al.,* 1976; Marsch *et al.,* 1979; Goldfischer *et al.,* 1983). Viscous losses are those related to friction of moving structures (Mijailovich et al 1993), and plastic forces (elastic moduli) are those related to changes in shape or deformation of connective tissue components when submitted to loading (Rapoff et al., 1999). It is noteworthy that Mijailovich et al (1994), refer that elastin rich ligament under uniaxial cycling loading, as pigeon propatigiale ligament, behaves virtually as a Hookean spring, i.e. capable of

sustaining the distension changes to which the tissue is submitted periodically, with little change in the elastance and hysterisivity.

During the time course of pregnancy and post-partum we have observed a polarized arrangement and a significant increase in mean of the estimated length profile of elastic system fibers in the interpubic medial region. This progressive polarization of the elastic fibers is similar to the mechanical behavior described by Wren and Carter (1998) for a tissue fiber, which rotates and elongates instead of simply elongating, to become more aligned in the direction of loading at the same time that the reorientation resistance parameter of soft skeletal connective tissue changes.

The notice of an increase of fibers diameters and an elastin deposition process in the same period by electron microscopy analysis may indicate relatively rapid elastogenesis in the pregnant mouse pubic symphysis, when the entire reproductive tract is adapting to different degrees of stress. Coincidently studies of elastogenesis in the uterus, during rat pregnancy, showed that elastin synthesis increases and reaches the most elevated values at term, and progressively declines after parturiarion (Gunja-Smith et al., 1989; Starcher and Percival, 1985). In the human uterine cervix, the fact that elastic fibers to distend to twice their length allowing the cervix to dilate for parturition, suggests that elastin has an essential role in maintaining the shape of the cervix and helping to keep it close to retain the fetus (Leppert, 1995; Ludmir and Sehdev, 2000). However, in the mouse pubic symphysis, the elastic fibers rich in elastin were observed only at the 5 day post-partum (D24), so the microfibrils appear to serve as sites in which a aggregation of elastin occurs and also the presence of oxytalan and elaunin fibers in the adult mouse pubic symphysis can be an evidence that the microfibrils carry out a structural role independent of its role in the elastogenesis.

Previous study reporting quantitative analysis of elastic system fibers to quantificate modifications in relations to physiological variation, in tissue sections, showed the existence of a significant positive relation between the diameter and volume of fibers, sugesting that volume changes are mainly due to size variations (Vitellaro-Zuccarello et al.; 1994). It was demonstrated the mean project fiber length within a tissue section were much longer than 100 μm, consequently the real lengths estimated for elastic fibers is longer than the mean length (Cullen et al. 1981).

Electron microscopy study still showed that the close topographic relationship between elastic fibers and the fibroblast-like cells of the fibrocartilage and interpubic

ligament, coupled with their absence near cells with a chondral or osteoblastic phenotype, seem to indicate that these fibers are synthesized exclusively by these fibroblast-like cells. Usually, the interaction between connective tissue cell and the fibers of the elastic system is made through microfibrils (Böck and Stockinger, 1984; Caldini et al., 1990; Montes, 1996).

With respect to drastic compositional changes observed in the wild-type mouse pubic joint at late pregnancy, it was observed that relaxin induces both collagen degradation and larger change in the osmolarity, by increasing water content (20%) in the ligament extracelullar matrix (Zhao et al., 2000). Recently it was demonstrated, in vitro, that the binding of fibrilins 1 and 2 to tropoelastin was sensitive to ionic concentration, the raising of sodium chloride concentration results in a dramatic decrease in this molecular event (Trask et al., 2000). Additionally, considering the plasticizer role of water with respect to the elasticity of elastin polymers, the relaxed and extend states were described as more hydrated, essentially unstructured and less hydrated, more structured, respectively (Debelle and Tamburro, 1999).

At late pregnancy, when water content is high, we found morphologic evidence that fibroblasts-like cells retain an elastogenic potential, incorporating progressively more clumps of amorphous and homogeneous material (elastin) in the microfibrilar scaffold of ligament extracellular matrix, producing change at the medium diameter of elastic system fibers and modifications in the estimated profile length of the elauninic fibers.

It is well recognized in uterine cervix, when the pregnancy advances closer to term, that there is a further increase in the overall dispersion of collagen (Junqueira et al., 1980; Ludmir and Sehdev, 2000). As the fibers disperse, both water and hyaluronic acid concentration increases (Ludmir and Sehdev, 2000). Recently it was showed that hyaluronic acid in the pulmonary matrix has a protective effect over the elastic system fibers, since an increase in the hyaluronic acid content decrease the susceptibility of elastin to degradation by elastase (Cantor et al., 1999; Cantor et al., 2000).

Coincidentally, we observed well-preserved elastic system fibers during all the course of pregnancy when the pubic symphysis slowly weakens to allow delivery. By this time the increase of the water and hyaluronic acid contents in the mouse pubic symphysis were reported (Zhao et al., 2000). The same was observed after relaxin treatment (Weiss et al., 1979; Viell and Struck, 1987).

There are several characteristics of this joint scaffold that are particularly interesting as putative factors of importance in interpubic ligament morphogenesis. The pattern of arrangements of elastic system fibers follows closely the sequence of elastogenesis of the skeletal elements. This feature is reinforced by the ability of the fibroblast-like cells to reorientate and aggregate elastic fibers adding versatility to the elastic system fibers (Montes 1996).

As it was proposed in the chick limb development (Hurle et al., 1994), the elastin scaffold formation may be linked to the occurrence of mechanical stress associated with the anisotropic growth of the structures with tissues of different physical characters. According to Hurle et al (1994), if this assumption is true, the elastin scaffold provides molecular evidence for the hypothesis involving mechanical tension in the morphogenesis, maintenance, repair and regeneration of both the ligament and adjacent hard tissue.

The different configuration adopted by the elastic system is compatible with the idea that the tension stored in the post-partum elastic fibers acts in the sense of pulling back the pubic bones in direction of medium line when hormonal and mechanical stimulation to spreading apart ends. The distribution of the different components of the elastic system described in this study is coherent with the hypothesis that these fibers contribute to joint stiffness and thus interfere with the magnitude of time course regulation of pubic spreading apart during mouse pregnancy, partum and post-partum.

Considering the potential role of fibers of the elastic system in limiting the separation of pubic bones, further studies of pubic symphysis relaxation should be performed to clarify their possible role in this process.

FIGURE LEGENDS

FIG.1. Coronal section through the mouse interpubic joint of a D12 mouse stained by hematoxylin and eosin. Observe the symphyseal medium ends of the pubic bones (pb) with hyaline capsule (hc), a fibrocartilage portion (fc), symphyseal space (arrowhead) and sheet of connective tissue (CT). Bar, 65μm.

FIG.2. Coronal section of a virgin female mouse pubic symphysis stained by Weigert's resorcin-fuchsin with previous oxidation. Among the abundant collagen fibers, a small amount of thin fibers of the elastic system stained in black (arrowheads) can be observed. These fibers are organized in the direction of pubic bone separation. Bar, 25μm.

FIG.3. Coronal section of a mouse interpubic ligament at the D15 stained by Weigert's resorcin-fuchsin with previous oxidation. Note the numerous fibers of elastic system (arrowheads) orientated in several directions forming a three-dimensional meshwork. These fibers show a considerable variation in thickness. Bar, 20μm.

FIG.4. Coronal section of a mouse interpubic ligament at the parturition (D19) stained by Weigert's resorcin-fuchsin with previous oxidation. This picture illustrates the presence of the thick fibers (arrowheads) of the elastic system near the bone insertion. Bar, 25μm.

FIG.5. Coronal section of a mouse interpubic ligament at the D24 stained by Weigert's resorcin-fuchsin with previous oxidation. The fibers of the elastic system are interwoven with collagen (C). Thin elastic fibers (arrowhead) are stained slightly. Bar, 10μm.

FIG.6A, B. Transmission electron micrograph of a thin cross section of pubic symphysis of virgin female mouse. A, Ultrastructure of an oxytalan fiber showing that it is formed solely of a bundle of 10 to 12nm thick microfibrils, which exhibit an electron-dense tubular profile when cross-sectioned (arrowheads). B, higher magnification. Bar, 200nm (A) and 100nm (B).

FIG.7A, B; 8. Transmission electron micrographs of a thin cross section of pubic symphysis at the D12. 7A, this picture illustrates fibers of the elastic system showing the same pattern observed in the virgin mouse, which are exclusively composed by microfibrils (arrows). 7B, higher magnification. In figure 8 notes in the fibrocartilage a longitudinal section of the oxytalan fiber (asterisk), formed by microfibrils arranged in straight way to the long axis of the fiber in close contact with collagen fibrils (c). Bar, 300nm (7A), 200nm (7B) and 400nm (8).

FIG.9, 10. Transmission electron micrographs of a thin cross section of pubic symphysis at the D15. 9, a transverse section of an elaunin fiber (arrow) which consists of a bundle of microfibrils (arrowhead) in parallel array interspersed with patches of amorphous and homogeneous material (elastin – E). 10, elastic system fibers with pattern of elaunin fiber, in longitudinal (arrowheads) and oblique (arrow) sections. Bar, 100nm (9) and 400nm (10).

FIG.11. Transmission electron micrograph of a thin cross section of pubic symphysis on the D18, showing an elastic system fiber in close contact with the cell membrane. Bar, 500nm.

FIG.12, 13. Transmission electron micrographs of a thin cross section of pubic symphysis mouse during the parturition (D19). 12, note the long cellular processes (arrowheads) involving the elaunin fiber (arrow), rich in elastin (E). 13, degradation pattern of the elastic system fibers (arrow). Bar, 300nm (12) and 450nm (13).

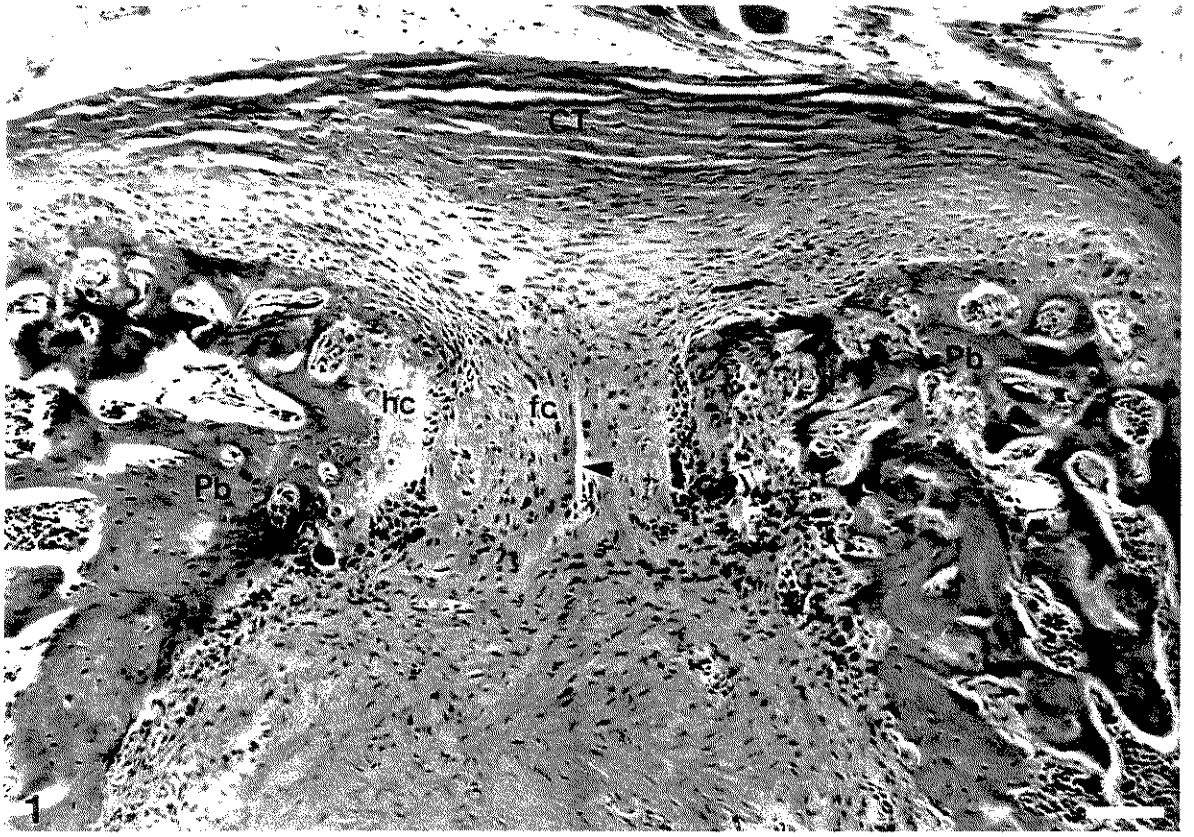
FIG.14, 15. Transmission electron micrographs of a thin cross section of pubic symphysis mouse on the D22. 14, longitudinal section illustrating the great proximity of an elastic system fiber (arrow) with elastin patches (asterisk) to the fibroblast-like cell. 15, the microfibrilar component is disposed in convoluted state (arrowheads). Bar, 350nm.

FIG.16, 17. Transmission electron micrographs of a thin cross section of pubic symphysis mouse on the D24. Longitudinal sections of mature elastic fibers (arrow). These fibers are composed of a central dense core of homogenous materials (elastin – E)

surrounded by a peripheral mantle of microfibrils in straight way. The inset contains a medium magnification of a cross-section of a mature elastic fiber. Bar, 750nm (16), 700nm (17) and 600nm for inset.

Fig.18. Estimated length profile of elastic system fibers in pubic symphysis and interpubic ligament of cycling, pregnant and post-partum mice.

Fig.19. Box-plots showing the changes in the diameter of elastic system fibers in pubic symphysis and interpubic ligament mice cycling, during pregnancy and post-partum. The boxes indicate 25th-75th percentiles, the whiskers indicate the range of values excluding outliers (O), and the line across each box represents the median value.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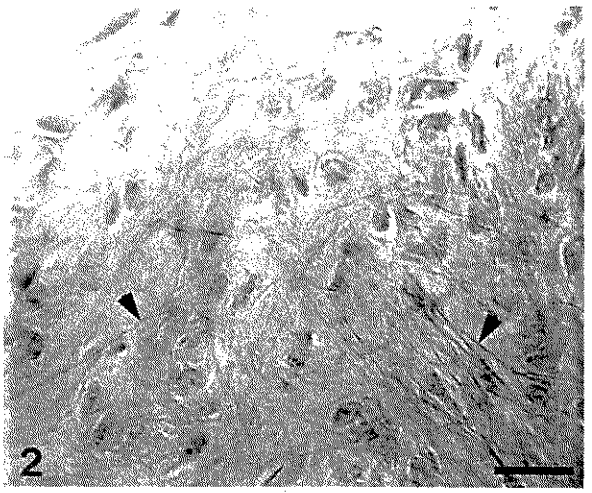
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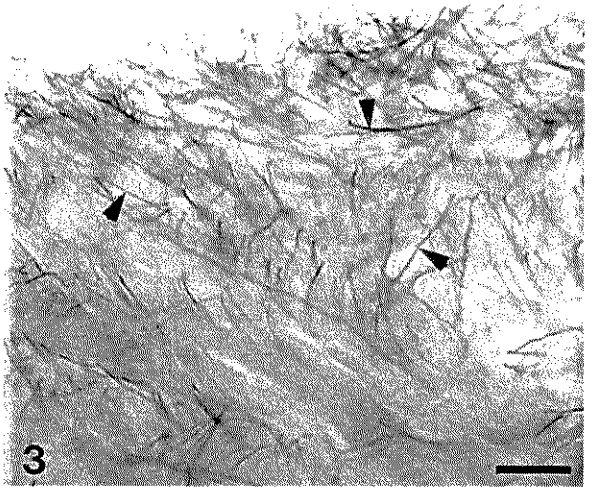
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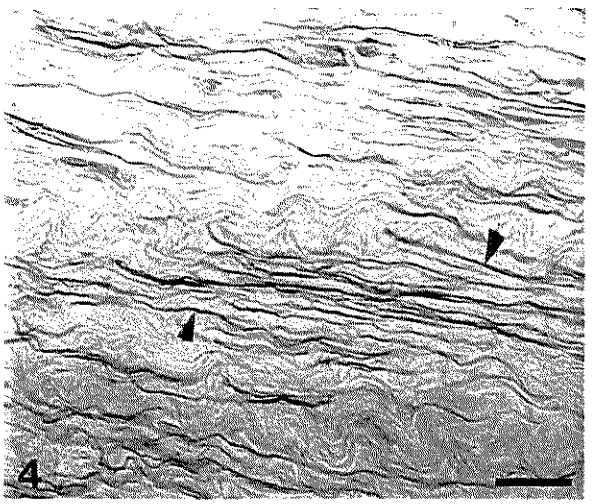
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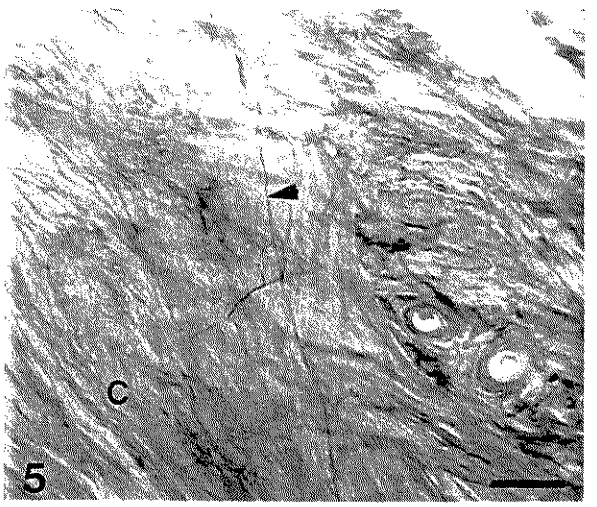
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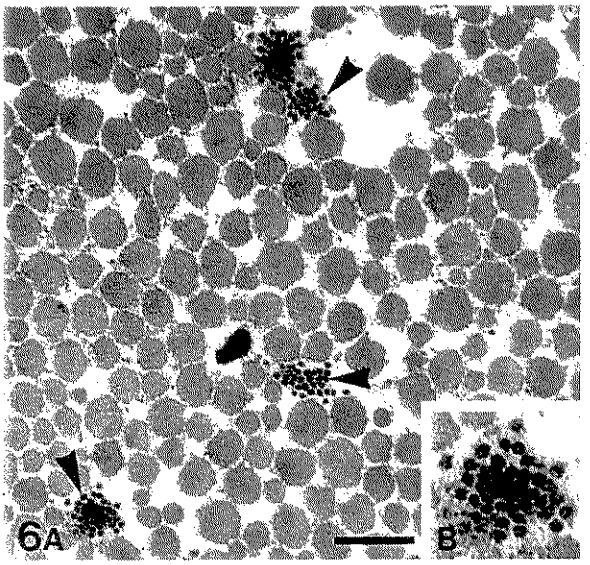
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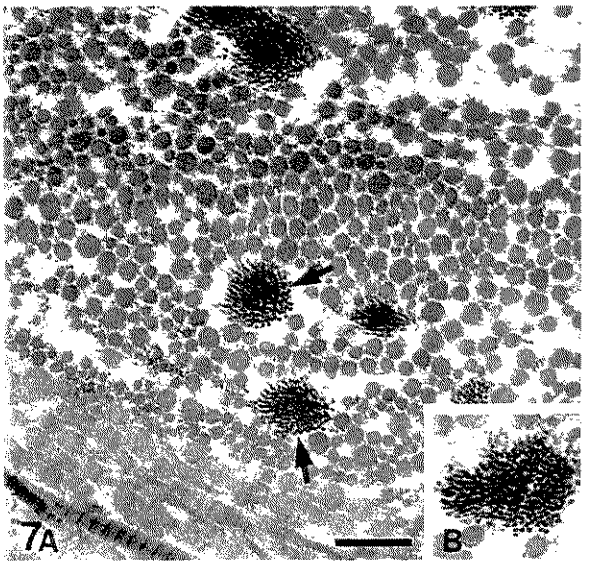
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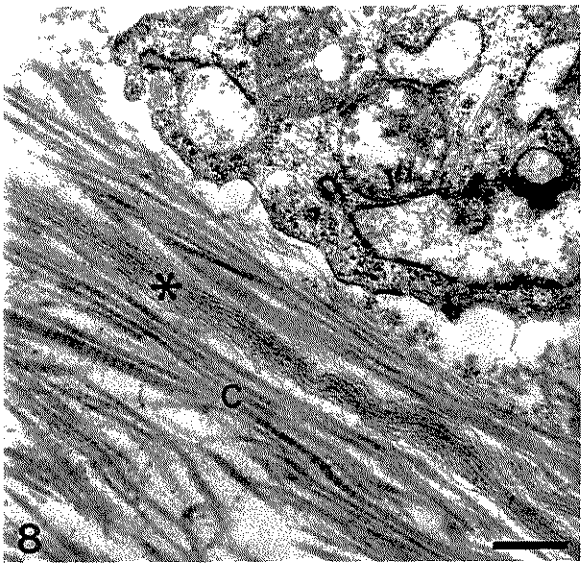
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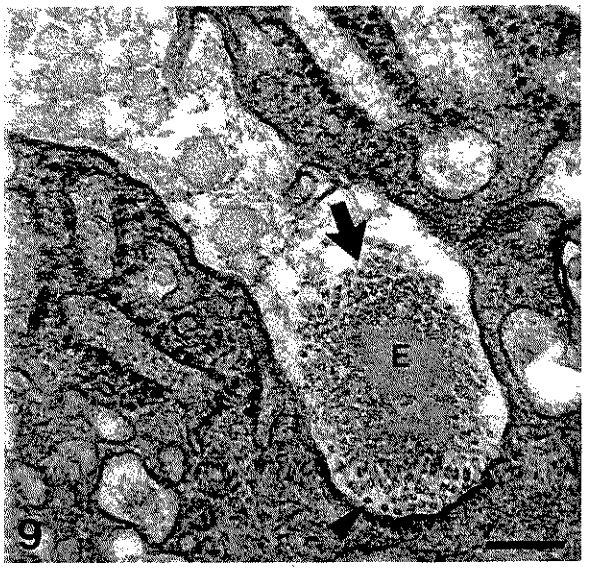
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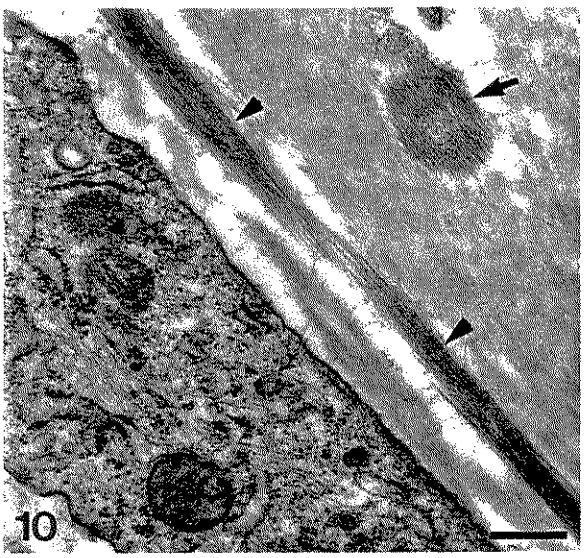
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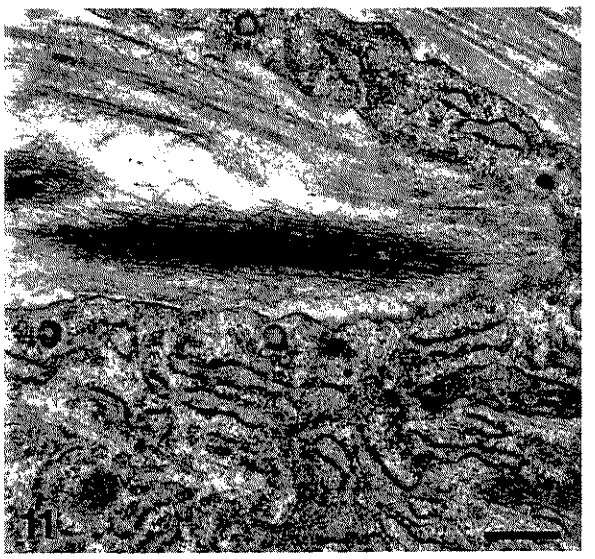
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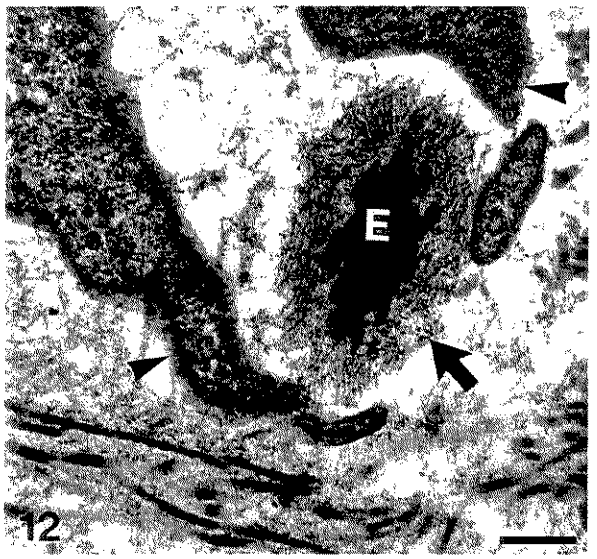
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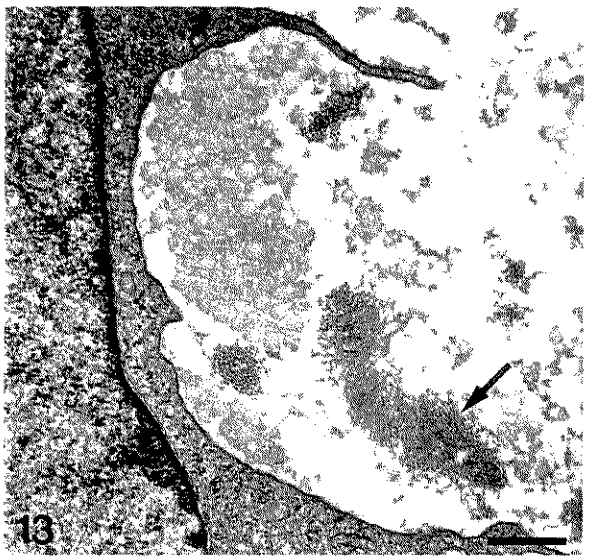
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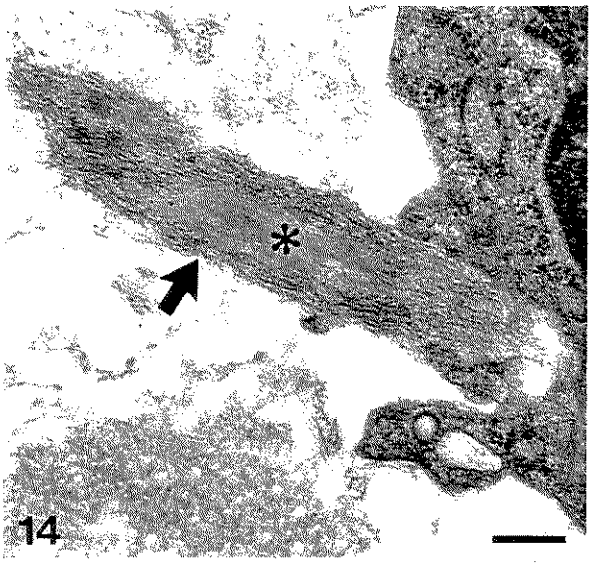
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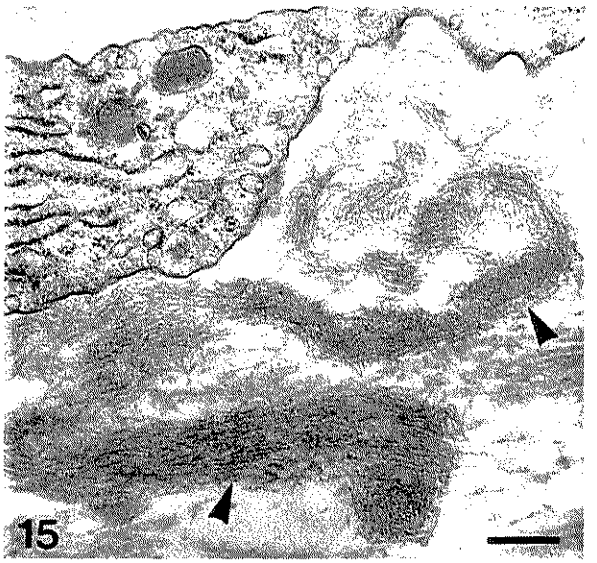
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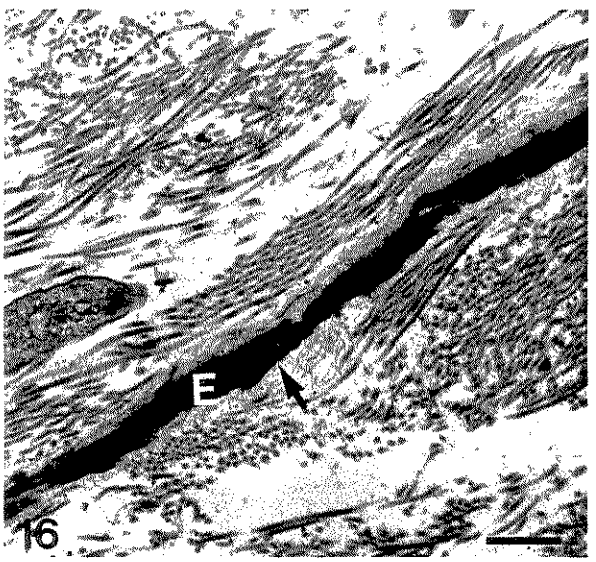
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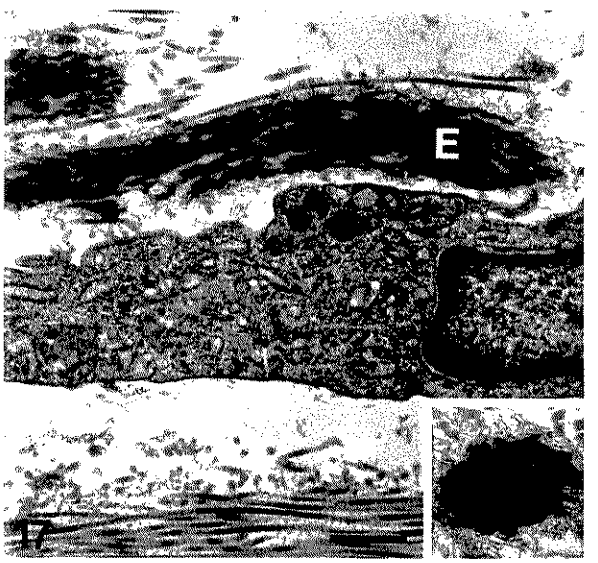
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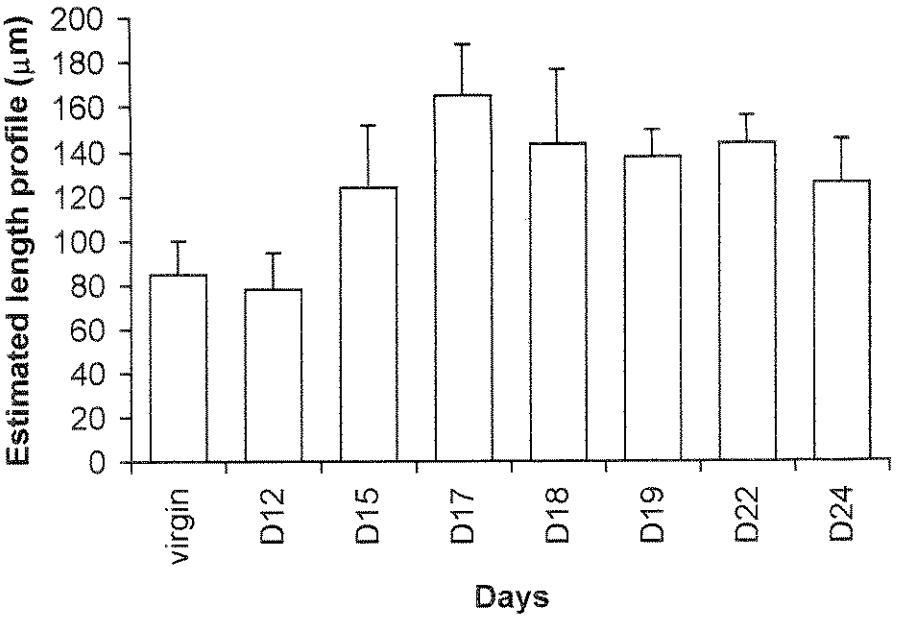
Estimated length profile (µm)
200
180
160
140
120
100
80
60
40
20
0
virgin
D12
D15
D17
D18
D19
D22
D24
Days

Fig.18

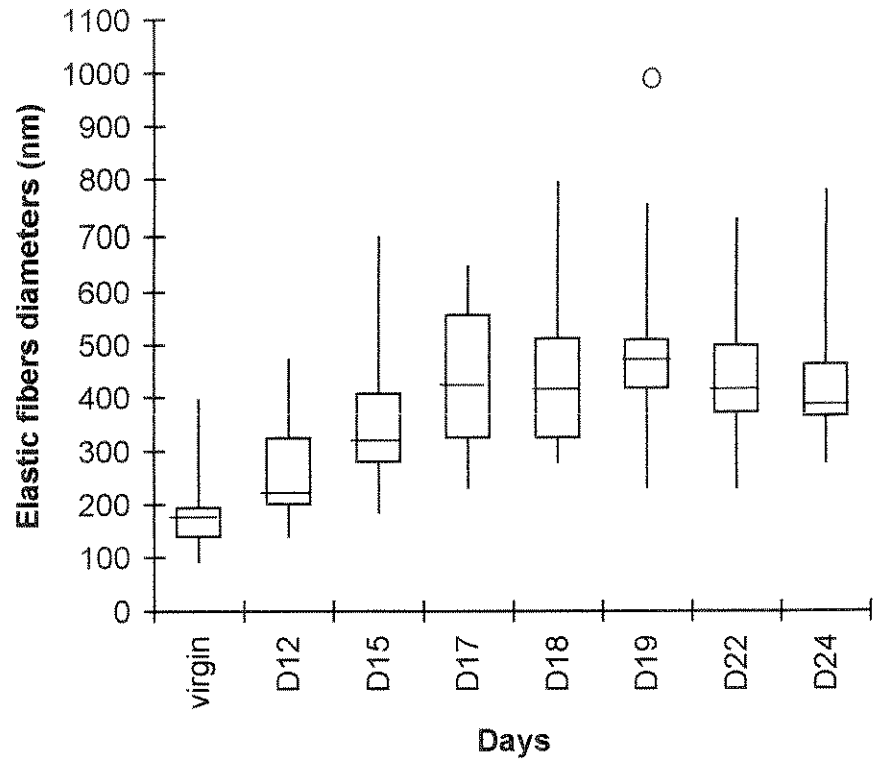
Elastic fibers diameters (nm)
1100
1000
900
800
700
600
500
400
300
200
100
0
virgin
D12
D15
D17
D18
D19
D22
D24
Days

Fig.19

Table 1. Estimated length and small diameter profile of elastic system fibers in pubic symphysis and interpubic ligament of cycling, pregnant and post-partum mice

	Light Microscopy				Electron Microscopy		
Animals	Mean number of intersections per throw ± SD (N=45)	Mean estimated profile length (*l*)	Δ% Values	P values	Mean diameter of elastic system fibers (nm)	Δ%Values	P Values
Cycling (virgin)	86,51±15,80	84,78±15,48	0	-	180±60	0	-
D12	79,66±16,73	78,07±16,39	-7	NS	260±80	44	P<0,001
D15	127,13±27,81	124,46±27,22	59	P<0,001	350±100	34	P<0,001
D17	168,4±23,65	164,64±23,12	32	P<0,001	450±140	28	P<0,001
D18	146,73±33,31	143,77±32,63	-12	P<0,001	460±180	2	P<0,001
D19	141,22±11,40	138,38±11,17	-3	NS	470±140	2	NS
D22	146,55±12,16	143,94±11,94	-4	NS	430±100	-8	NS
D24	129,68±18,80	127,09±18,52	-11	P<0,001	440±120	2	NS

l mean estimated profile length, $l = (\pi/2).(n/N).d$.

n/N mean number of intersections through elastic fiber profiles per throw (N=45).

d = 28,12μm.

The Δ% Values were calculated from estimated profile length and diameter as follow: {[D(a)-D(b)]÷D(b)}.100, where (a) and (b) are successive values.

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REFERENCES

AHERNE, A.W., DUNNILL, M.S. 1982. Morphometry. Edward Arnold, London.

BASSET, E.G., PHILLIPPS, D.S.M. 1955. Changes in the pelvic region of the ewe during pregnancy and parturition. *N. Z. Vet. J.* ***3***, 20-25.

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.

CALDINI, E. G., CALDINI, N. De-PASQUALE, V., STROCCHI, R., GUIZZARDI, S., RUGGERI, A., MONTES, G.S. 1990. Distribution of elastic system fibers in the rat tail tendon and its associated sheaths. *Acta Anat.* ***139***, 341-8.

CANTOR, J.O., CERRETA, J.M., ARMAND, G., OSMAN, M., TURINO, G.M. 1999. The pulmonary matrix, glycosaminoglycans and pulmonary emphysema. *Connect. Tiss. Res.* ***40***, 97-104.

CANTOR, J.O., SHTEYNGART, B., CERRETA, J.M., LIU, M., ARMAND, G., TURINO, G.M. 2000. The effect of hyaluronan on elastic fiber injury in vitro and elastase-induced airspace enlargement in vivo. *Proc. Soc. Exp. Biol. Med.* ***225***, 65-71.

CARVALHO, H.F., NETO, J.L., TABOGA, S.R. 1994. Microfibrils, neglected components of pressure-bearing tendons. *Ann. Anat.* ***176***, 155-159.

CARVALHO, H.F., VIDAL, B.C. 1995. The elastic system of a pressure-bearing tendon of the bullfrog *Rana catesbeiana. Ann. Anat.* ***177***, 397-404.

CHIHAL, H.J., ESPEY, L.L. 1973. Utilization of the relaxed symphysis pubis of guinea pigs for clues to mechanism of ovulation. *Endocrinology* ***93***, 1441-5.

CLEARY, E.G. & GIBSON, M.A. 1983. Elastin-associated microfibrils and microfibrilar proteins. *Int. Rev. Connect. Tiss. Res.* ***10***, 97-109.

COTTA-PEREIRA, G., RODRIGO, F.G., BITTENCOUT-SAMPAIO, S. 1976. Oxytalan, elaunin and elastic fibers in the human skin. *J. Invest. Dermatol.* ***66***, 143-8.

COTTA-PEREIRA, G., DEL-CARO, L. M., MONTES, G.S. 1984. Distribution of elastic system fibres in hyaline and fibrous cartilages of the rat. *Acta Anat.* ***119***, 80-5.

COTTA-PEREIRA, G., IRUELA-ARISPE, M.L. 1989. Extracellular matrix, functional significance of oxytalan, elaunin and elastic fibers. *Prog. Clin. Biol. Res.* ***295***, 101-107.

CRELIN, E.S. 1954. The effect of estrogen and relaxin on intact and transplanted pubic symphyses in mice. *Anat. Rec.* ***118***, 380-381.

CRELIN, E.S., HAINES, A.L. 1955. The effects of locally applied estrogen on the pubic symphysis and knee joint in castrated mice. *Endocrinology* ***56***, 461-470.

CRELIN, E.S., BRIGHTMAN, M.W. 1957. The pelvis of the rat, its response of estrogen and relaxin. *Anat. Rec.* ***128***, 467-484.

CRELIN, E.S., NEWTON, E.V. 1969. The pelvis of the free-tailed bat, Sexual dimorphism and pregnancy changes. *Anat. Rec.* ***164,*** 349-358.

CRELIN, E.S. 1969. Interpubic ligament, elasticity in pregnant free-tailed bat. *Science.* ***164***, 81-2. a.

CRELIN, E.S. 1969. The development of bony pelvis and its changes during pregnancy and parturition. *Trans. NY Acad. Sci.* ***31***, 1049-1058, b.

CULLEN, W.C., FLETCHER, T.F., BRADLEY, W.E. 1981. Histology of the canine urethra. I Morphometry of the female urethra. *Anat. Rec.* ***199***, 177-189.

DEBELLE, L., ALIX, A.J.P. 1999. The structures of elastins and their function. *Biochimie* ***81***, 981-994.

DEBELLE, L., TAMBURRO, A.M. 1999. Elastin, molecular description and function. *Int. J. Biochem. Cell Biol.* ***31***, 261-272.

FUKUDA, Y, FERRANS, V.J., CRYSTAL, R.G. 1984. Development of elastic fibers of nuchal ligament, aorta, and lung of fetal and postnatal sheep,an ultrastrucutral and electron microscopic immunohistochemical study. *Am. J. Anat.* ***170***, 597-629.

FULLMER, H.M., LILLE, R.D. 1958. The oxytalan fiber, a previously underscribed connective tissue fibers. . *J. Histochem. Cytochem.* ***6***, 425-30.

FULLMER, H.M. 1960. A comparative histochemical study of elastic, pre-elastic and oxytalan connective tissue fibers. *J. Histochem. Cytochem.* ***8***, 290-5.

FULLMER, H.M., SHEETZ, J.H., NARKATES, A.J. 1974. Oxytalan connective tissue fibres, a review. *J. Oral. Pathol.* ***3***, 291-316.

GAMBLE, J.G, SIMMONS, S.C., FREEDMAN, M. 1986. The symphysis pubis, anatomic and pathologic considerations. *Clinical Orthopaedics and Related Research* ***203,*** 261-72.

GAWLIK, Z. 1965. Morphological and morphochemical properties of the elastic system in the motor organ of the man. *Folia Histochem. Cytochem.* **3**,233-251.

GHADIALLY, F.N. 1988. Ultrastructural pathology of the cell and matrix. London, Butterworths. pp, 1252-1261.

GOLDFISCHER, S., COLTOFF-SCHILLER, B., SCWARTZ, E., BLUMENFELD, O.O. 1983. Ultrastructure and staining properties of aortic microfibrils (oxytalan). *J. Histochem. Cytochem.* ***31***, 382-390.

GREENLEE, T.K., ROSS, R., HARTMAN, J.C. 1966. The fine structure of elastic fibers. *J.Cell Biol.* ***30,*** 59-71.

Guide for the Care and Use of Laboratory Animals (National Academy of Science, 1996)

GUNJA-SMITH, Z., LIN, J., WOESSNER JR., J.F. 1989. Changes in desmosine and Pyridinoline crosslinks during rapid synthesis and degradation of elastin and collagen in the rat uterus. *Matrix.* ***9***, 21-27.

HALL, K. 1947. The effects of pregnancy and relaxin on the histology of the pubic symphysis in the mouse. *J. Endocrinol.* ***5,*** 174-82.

HART, D. A; SCIORE, P.; BOYKIW, R.; RENO, C. 1998.Pregnancy induces changes in the pattern of mRNA expression in knee ligaments of the adolescent rabbit. *Matrix Biology,* ***17,*** 21-34.

HESSE, I. 1987. The occurrence of elastic fibres in the matrix of normal articular cartilage. *Cell Tissue Res.* ***248***, 589-593.

HÖPKER, W-W, ANGRES, G., KLINGEL, K., KOMITOWSKI, D., SCHUCHARDT, E. 1986. Changes of the elastin compartment in the human meniscus. *Virchows Arch. A. Pathol. Anat. Histopathol.* ***408***, 575-592.

HORN, E.H. 1960. Interpubic ligament regression in relaxin-treated virgin and primiparous, post-partum mice. *Endocrinology* ***67***, 668-673.

HURLE, J.M., CORSON, G., DANIELS, K., REITER, R.S., SAKAI, L.Y., 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.

JUNQUEIRA, L.C.U., ZUGAIB, M., MONTES, G.S., TOLEDO, O.M.S., KRISZTAN, R.M., SHIGIHARA, K.M. 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***, 273-81.

KROC, R.L., STEINETZ, B.G., BEACH, V.L. 1958. The effects of estrogens, progestagens, and relaxin in pregnant and nonpregnant laboratorory rodents. *Ann. N. Y. Acad. Sci.* ***75***, 942-80.

LEPPERT, P.C. 1995. Anatomy and physiology of cervical ripening. *Clin. Obstet. Gynecol.* ***38***, 267-279.

LINCK, G., PETROVIC, A., STOECKLE, M.E., PORTE, A. 1976. Fine structure of the public symphysis in the mouse. *Bull Assoc. Anat. (Nancy)* ***60***, 201-9.

LUDMIR, J., SEHDEV, H.M. 2000. Anatomy and Physiology of the uterine cervix. *Clin. Obstet. Gynecol.* ***43***, 433-439.

MANNING, P.J. 1974. The staining of elastic tissue and related fibers in uterine blood vessels. *Med. Lab. Technol.* ***31***, 115-125.

MECHAM, R.P., HEUSER, J.E. 1991. The elastic fiber In, HAY, E.D. Cell Biology of Extracelular Matrix. New York. Plenum Press. pp.79-101.

MECHAM, R.P., DAVIS, E.C. 1994. Elastic fiber structure and assembly. In Yurchenco, P.D., Birk, D.E., Mecham, R.P. (Eds), Extracellular Matrix Assembly and Structure, Academic Press, San Diego. pp. 281-314.

MIJAILOVICH, S.M., STAMENOVIC, D., FREDBERG, J.J. 1993.Toward a kinetic theory of connective tissue micromechanics. *J. Appl. Physiol.* ***74***, 665-81.

MIJAILOVICH, S.M., STAMENOVIC, D., BROWN, R., LEITH, D.E., FREDBERG, J.J. 1994. Dynamic moduli of rabbit lung tissue and pigeon ligamentum propatagiale undergoing uniaxial cycling loadind. *J. Appl. Physiol.* ***76***, 773-782.

MONTES, G.S. 1992. Distribution of oxytalan, elaunin and elastic fibres in tissues. *Cienc. Cult* ***44***, 224-33.

MONTES, G.S. 1996. Structural biology of the fibres of the collagemous and elastic systems. *Cell Biology Int.* ***20***,15-27.

OAKES, B.W., BIALKOWER, B. 1977. Biomechanical and ultrastructural studies on the elastic tendon from the domestic fowl. *J. Anat.* ***123***, 369-387.

ORTEGA, H.H., JOAZEIRO, P.P., MUÑHOZ-DE-TORO, M.M., LUQUE, E.H., MONTES, G.S. Differential distribution of the collagenous and elastic systems and of glycosaminoglycans In the rat pubic joint. Submitted to *J. Submicrosc. Cyt. Pathol.*

PANIAGUA, R., REGADEIRA, J., NISTAL, M., SANTAMARIA, L. 1983. Elastic fibres of the human ductus deferens. *J. Anat.* ***137,*** 467-476.

PARFITT, A.M. 1983. Stereologic basis of bone histomorphometry, theory of quantitative microscopy and reconstruction of the third dimension. In Recker, R.R., Bone histomorphometry, CRC Press Inc. Boca Raton, Florida, U.S.A. pp. 53-87.

RAPOFF, A.J., HEISEY, D.M., VANDERBY Jr., R. 1999. A probabilistic rule of mixtures for elastic moduli. *J. Biomechanics* ***32***, 189-193.

ROSENBLOOM, J., ABRAMS, W.R., MECHAM. R. 1993. Extracellular matrix, the elastic fiber. *FASEB J.* ***7***, 1208-18.

ROSS, R., BORNSTEIN, P. 1969. The elastic fiber. I. The separation and partial characterization of its macromolecular components. *J. Cell Biol.* ***40***, 366-381.

ROSS, R. 1973. The elastic fiber. A review. *J. Histochem. Cytochem.* **21**, 199-208.

RUTH, E.B. 1936. Metamorphosis of the pubic symphysis. II. The guinea pig. *Anat. Rec.* ***67***, 69-79.

RUTH, E.B. 1937. Metamorphosis of the pubic symphysis. III. Histological changes in the symphysis of the pregnant guinea pig. *Anat. Rec.* ***67,*** 409-421.

SANDBERG, L.B., SOSKEL, N., LESLIE, J.G. 1981. Elastin structure, byosinthesis and relation to disease state. *New Engl. J. Med.* ***304***, 566-79.

SEAR, C.H.J., GRANT, M.E., JACKSON, D.S. 1981. The nature of microfibrillar glycoproteins of elastic fibers. A biosynthetic study. *Biomech. J.* ***194,*** 587-598.

SHERWOOD, O. D. 1994. Relaxin. En, Knobil E, Neill j. d. (eds). The physiology of reproduction. Raven Press, New York. Chapter 16, pp.861-1009.

SHORR, E. 1941. A new technique for staining vaginal smears. II. Single differencial stain. Science **5**,545-546.

STARCHER, B., PERCIVAL, S. 1985. Elastin turnover in the rat uterus. *Connect. Tiss. Res.* ***13***, 207-215.

STEINETZ, B.G., BEACH, V.L., KROC, R.L. 1957. The influence of progesterone, relaxin and estrogen on some structural and functional changes in the pre-parturient mouse. *Endocrinology* ***61,*** 271-280.

STEINETZ, B.G., MANNING, J.P., BUTLER, M.T., 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.

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.

TALMAGE, R.V. 1947. Changes produced in the symphyseal pubis of the guinea pig by the sex steroids and relaxin. *Anat. Record* ***99,*** 91-113, $_{a}$.

TALMAGE, R.V. 1947. A histological study of the effects of relaxin on the symphysis pubis of the guinea pig. *J. Exp. Zool.* ***106,*** 281-92, $_{b}$.

THOMS, H. 1936. Relaxation of the symphysis pubis in pregnancy. *Journal of the American Medical Association* **106,** 1364-1366.

TRASK, T.M., TRASK, B.C., RITTY, T.M., ABRAMS, W.R., ROSEMBLOOM, J., MECHAM, R.P. 2000. Interaction of tropoelastin with the amino-terminal domains of fibrillin-1 and fibrillin-2 suggests a role for the fibrillins in elastic fiber assembly. *J Biol Chem.* ***275***, 24400-6.

VERHOEFF, F.H. 1908. Some new staining methods of wide applicability. Including a rapid and differential stain for elastic tissue. *J. Am. Med. Ass.* ***50***, 876-7.

VIELL, B., STRUCK, H. 1987. Effects of the hormone relaxin on teh matabolism of glycosaminoglycans in the mouse symphysis pubis. *Horm. Metabol. Res.* ***19***, 415-18.

VITELLARO-ZUCCARELLO, L., CAPPELLETTI, S., ROSSI, V.D.P, SARI-GORLA, M. 1994. Stereological analysis of collagen and elastic fibers in the normal human dermis, variability with age, sex, and body region. *Anat. Rec.* ***238***, 153, 162.

VIX, V.A., RYU, C.Y. 1971. The adult symphysis pubis, normal and abnormal. *Am J Roentgenol Radium Ther Nucl Med.* ***112***, 517-25.

WAHL L.M., BLANDAU, R.J., PAGE, R.C. 1977. Effects of hormones on collagen metabolism and collagenase activity in the pubic sysmphysis ligament of the guinea pig. *Endocrinology* ***100,*** 571-9.

WEIGERT, C. 1898. Über eine Method zur Färbung elasticher Fasern. *Zentbl.Allg. Pathol. Pathol. Anat.* ***9***, 289-92.

WEISS, M., NAGELSCHMIDT, M., STRUCK, H. 1979. Relaxin and collagen metabolism. *Hormone and Metabolic Res.* ***11***, 408-410.

WREN, T.A.L., CARTER, D.R. 1998. A microstructural model for the tensile constitutive and failure behavior of soft skeletal connective tissues. *J. Biomech. Eng.* ***120***, 55-61.

WRIGHT, J.L., CRURG, A. 1995. Smoke-induced emphysema in guinea pigs is associated with morphometric evidence of collagen breakdown and repair. *Am. J. Physiol.* ***268,*** 17-20.

ZHAO, L., ROCHE, P.J., GUNNERSEN, J.M., HAMMOND, V.E., TREGEAR, G.W., WINTOUR, E.M., BECK, F. 1999. Mice without a functional relaxin gene are unable to delivery milk to their pups. *Endocrinology* ***140***, 445-453.

ZHAO, L., SAMUEL, C.S., TREGEAR, G.W., BECK, F., WINTOUR, E.M. 2000. Collagen studies in late pregnant relaxin null mice. *Biol. Reprod.* ***63***,697-703.

Phenotypic Modulation of Fibroblastic Cells in the Mice Pubic Symphysis During Pregnancy, Partum and Post-partum

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ABSTRACT

In many species, the cartilaginous pubic symphysis of the pregnancy female is gradually replaced by fibrous connective tissue, that forms a flexible and elastic interpubic ligament, separating the pubic bones to enable safe delivery of the young. After labor the interpubic ligament undergoes rapid involution. The phenotypic modulation of the cellular compartment during the pubic symphysis relaxation and closing has not hitherto been reported. The purpose of this study was to investigate the ultrastructure features and immunophenotype of fibroblast-like cells of pubic symphysis in virgin, pregnant and post-partum mice. Expression of the cytoskeletal proteins desmin, vimentin and α-smooth muscle actin (α-SMA) was studied by immunohistochemistry. In this model, the fibroblast-like cells always expressed α-SMA, but the vimentin and desmin expressions were transient, from the 15th day of pregnancy to the first day post-partum. The expression patterns of the three cytoskeletal proteins also were distinct. Cells present in virgin and at the 12th day of mouse pregnancy displayed ultrastructural features characteristic of typical fibroblast. In contrast, at the last week of pregnancy and in post-partum fibroblast-like cells ultrastructural features of a myofibroblast were found, as the presence of fibronexus and contractile apparatus, always lying in close contact with elastic system fibers. Taken together, these results strongly suggest a contractile function for these cells and indicate that this cellular network with extracellular matrix interaction may contribute to support varying mechanical stress during of the pubic bones movement.

Key words: mouse, pubic symphysis, pregnancy, immunohistochemistry, desmin, vimentin, α-SMA.

INTRODUCTION

During the last week of mouse pregnancy, the pubic bones separate to enable safe delivery of the young. This separation is attributable to resorption of the symphyseal surfaces of the pubic bones, swelling of the cartilaginous matrix, and transformation of the cartilage caps into a fibrous interpubic ligament that attains a maximum length of 3 to 6mm at parturition (Hall, 1947; Crelin, 1954; Storey, 1957; Kroc et al., 1958; Steinetz et al., 1957; Horn, 1960). This process, which is primarily hormonal regulated by estrogen and relaxin (Sherwood, 1994), occurs in several species, including guinea pigs (Ruth, 1937; Talmage, 1947a-b; Wahl et al., 1977), mice (Gardner, 1936; Hall, 1947; Crelin, 1954; Storey, 1957; Kroc et al., 1958; Steinetz et al., 1957) and bats (Crelin and Newton, 1969; Crelin, 1969a). Modest relaxation of the pelvic ligaments occurs during pregnancy in women (Crelin, 1969b; Vix, 1971; Gamble et al., 1986). It does not occur in species such as rat (Crelin and Brightman, 1957) and sheep (Bassett and Phillipps, 1955). The lateral forces on the pelvic girdle could pull the pelvic bones apart and give the fibrogelatinous connective tissue joining them the aspects of a ligament (Sherwood, 1994).

Pubic symphysis of post-partum mice rapidly resume an appearance and dimension quite similar to those of nulliparous mice with the dynamic involution of the interpubic ligament (Hall, 1947; Crelin, 1954; Storey, 1957; Kroc et al., 1958; Steinetz et al., 1957; Horn, 1960).

Tissue contraction (i.e., the shortening of tissue) plays an important role in the healing of many tissues by facilitating wound closure. In structures such as ligaments and tendons, recovery of the original length and the restoration of tension after injury are critical if normal mechanical performance is to be achieved (Weiss et al., 1991; Faryniarz et al., 1996), but little attention has been paid to this process at physiological situations such as pregnancy.

The pregnant mouse pubic symphysis has been shown a good model to studies of extracellular matrix turnover, since the alterations in matrix components occurs in a short time. This model already was the basis for many biochemical and morphological works. Recently, using molecular biology tools, it was possible to obtain a relaxin null mutant female mice (knockout of relaxin gene). It was histologically demonstrated that these animals fail to suckle their young, because they had extremely small and hard nipples, and do not promote significant enlargement of the interpubic ligament at the end of pregnancy,

although they were fertile, producing normal litters and delivering normally (Zhao et al., 1999, 2000). However the phenotypic modulation of the cellular compartment has not hitherto been reported. The predominant cell types in the interpubic ligament are active fibroblasts and fibroblast-like cells (Hall, 1947; Storey, 1957), that owning a large amount of relaxin receptors (Yang et al, 1992).

In many physiological or pathological situations, marked alterations in the structural and biochemical architecture of fibroblast and fibroblast-like cells were observed, suggesting an important phenotypic plasticity that permits adaptation (Gown, 1990; Schmitt-Graff et al., 1994, Varayoud et al., 2001). Some cytokines and components of the extracellular matrix of connective tissue may exert important influences on the differentiation of fibroblasts. Such influences represents a heterogeneous cell population in terms of their content of cytoskeletal proteins, that reflects a different functional feature for these cells (Sappino et al., 1990; Holstein et al, 1996). During the fibroblast differentiation, the cytoskeletal elements, such as desmin, vimentin and α-smooth muscle actin (α-SMA) are temporarily or more permanently synthesized in a specific pathway of the process and display cell-specific localization (Darby et al., 1990; Cintorino et al., 1991; Can et al., 1995). This contractile apparatus suggest that these cells in some tissues may form a contractile network capable of resisting mechanical stress (Joyce et al., 1987).

The aim of this study was to investigate the expression of desmin, vimentin and α-smooth muscle actin (α-SMA) and the ultrastructural features of cells present in the virgin mouse pubic symphysis and at mouse interpubic ligament during pregnancy, at parturition and in post-partum.

MATERIALS AND METHODS

Animals

Virgin female Swiss mice, 3 month old and over 25-30g of body weight (Center for Animal Care of State University of Campinas, SP, Brazil) were used. In this study animals were maintained in a controlled environment (25 ± 2 °C; exposed to light daily cycle of 12 hours) and had free access to pellet laboratory chow and tap water.

To obtain pregnant specimen, virgin females were caged overnight with males (in proportion of 2:1). A "plug" in the vagina the following morning indicated successful mating and this day was designated the first day of pregnancy (D1). The delivery could be expected on day 19.

Pubic symphysis samples were achieved from animals during pregnancy (D12, D15 and D18), immediately after delivery (D19), and during post-partum involution (D20, D22 and D24). Virgin mice in estrus were also used as control. Estrus was determined by vaginal smears according to Shorr (1941).

Animal's studies were conducted in accordance with the Guide for the Care and Use of Laboratory Animals (National Academy of Science, 1996).

Tissue Sample Collection

For immunohistochemistry four animals were used for each experimental point and two animals per group were studied in electron microscopy. The animals were sacrificed by cervical dislocation. The pubic symphysis were dissected clean and fixed *in situ* with appropriate fixate for 10 minutes. Them the specimens were removed and immediately submerged in the same fixate.

Histology and Immunohistochemistry

Specimens were fixed in Carnoy's mixture, i.e. (ethanol: chloroform: acetic acid 60:30:10 by volume) for 24 hours at 4 °C, decalcified in 7% EDTA with 2% paraformaldehyde in 0.1M phosphate buffer pH 7.4 for 5 days at 4 °C, dehydrated and embedded in paraffin. Serial sections (7 μm in thickness) were cut in frontal plane through the symphyseal region and mounted on poly-l-lysine solution 0.1% w/v in water (Sigma Chemical, St. Louis, MO, U.S.A.) coated slides and dried for 24h at 37 °C.

Routine hematoxylin and eosin slides were prepared for each sample. Immunohistochemistry was performed as following: serial sections were deparaffinated and submitted to a microwave pre-treatment for optimal antigen retrieval (Hopwood, 1992). All incubations were performed in a humidity chamber. The slides were incubated with 0.3% hydrogen peroxide for 30 minutes to quench endogenous peroxidase. Nonspecific sites were blocked with normal serum in PBS 0.01M for 20 minutes. Excess serum was gently blotted off and the sections were incubated with primary antibodies diluted in PBS 0.01M with 0.1% BSA (Sigma Chemical, St. Louis, MO, U.S.A.) overnight at 4 ℃. The following mouse monoclonal antibodies were used: anti-α-SMA (clon αSM-1, Novocastra, UK) 1:125, anti-vimentin (clon V9, Novocastra, UK) 1:50 and anti-desmin (clon DER-11, Novocastra, UK) 1:125. Negative control staining was performed by omitting the primary antibody. The sections were washed for 10 minutes in the same buffer and incubated with biotinylated universal secondary antibody solution (Novostain Super ABC kit, Novocastra) for 30 minutes at room temperature. This was followed by another rinsing with buffer and incubation with Novostain ABC kit contain avidin DH and biotinylated horseradish peroxidase H reagents diluted in PBS 0.01M saline (pH 7.4) for 30 minutes at room temperature. After rinsing with TRIS/HCl buffer peroxidase activity was visualized using diaminobenzidine tetrahydrochloride (DAB–SIGMA) as substrate. The sections were incubated with 500μl of diaminobenzidine (aliquoted in 5 ml of Milli-Q water and 5 ml of TRIS/HCl 0.1M pH 7.6) in 4,5ml of TRIS/HCl buffer, activated with 10μl of 30% hydrogen peroxide for approximately 5-10 minutes.

Sections were counterstained with Ehrlich's hematoxylin for 20 seconds, rinsed and mounted with Canada balsam. Sections were observed and photographed with an ECLIPSE E800 Nikon microscope.

Transmission Electron Microscopy

Small segments (approximately $1mm^3$ in size) of the pubic symphysis or interpubic ligament were fixed in a solution containing 0.1% tannic acid in 2.5% glutaraldehyde dissolved in 0.1M sodium cacodylate buffer (pH 7.2) for 2 hours at room temperature, followed by post fixation in 1% osmium tetroxide for 1h at 4°C. Tannic acid added to the fixative enhanced the staining of various connective tissue components, including elastic system fibers (Montes, 1992).

Fixed materials were stained *en bloc* in 1% aqueous uranyl acetate overnight. They were then dehydrated through graded ethanols. The samples were immersed in propylene oxide and then placed in a 1:1 dilution of EPON 812 (Electron Microscope Science) in propylene oxide overnight. Next they were placed in pure resin and were put into molds containing fresh resin to polymerize for 72 hours at 60°C.

Semithin sections (1μm) were cut in LEICA ultratome and stained with toluidine blue. Representative areas of the pubic symphysis were selected. Ultrathin sections were obtained with the same ultratome and were double-stained by 2.0% uranyl acetate and 0.5% lead citrate. Ultrathin sections were studied and micrographed in a LEO 906 transmission electron microscope.

RESULTS

Immunohistochemistry

In all physiological stages analyzed (i.e.: virgin, pregnant and post-partum) a strong immunoreactivity for the α-SMA antibody was observed within some fibrocondrocites (Figs.1A, B) and fibroblast-like cells (Figs.1C, D, E, F) at the mouse pubic symphysis fibrocartilage and interpubic ligament, respectively. The diffuse reaction product was visible in the perinuclear cytoplasm, as well as in the cellular processes.

The staining of desmin and vimentin varied in fibroblast-like cells during pregnancy and after delivery. Sections at D15, D18 (Fig.2A), D19 (Fig.2B) and D20, labeled with anti-desmin, showed that the staining pattern corresponded to a halo surrounding the nucleus at high expression and the remaining cytoplasm lacked staining, while the fibroblast-like cells in virgin, D12, D22 and D24 were consistently unreactive. The division cells in all experimental points studied also remained negative for anti-desmin. Positive reaction on the pericytes and smooth-muscle cells presented in small vessels served as internal control to anti-desmin.

The vimentin immunoreactivity was observed in the same days that showed positive staining to desmin, but the fibroblast-like-cells displayed an anti-vimentin homogenous staining pattern throughout the cytoplasm, which extended into the cellular protrusions (Figs.3A, B). In these days, the positive reaction was observed even in division cells (Fig.3A). A dramatic decline in the level of vimentin immunoreactivity was noticed by the almost complete absence of immunoperoxidase staining with anti-vimentin antibody at D22 and D24.

Negative control staining for all immunoreactions showed no positivity.

Ultrastructural Features

Electron microscopic examination of the mouse pubic symphysis showed that in virgin and at D12 the cells displayed fibroblast features: fusiform shape, a smooth nuclear outline and well-developed rough endoplasmic reticulum, Golgi apparatus and mitochondria (Fig.4).

The fibroblast-like cells are embedded in a connective tissue matrix rich in collagen fibrils and elastic system fibers (Figs.5, 6). In all experiments points, elastic fibers are often

distributed along the cell processes and it is not uncommon to observe intimate contact between the cell membrane and the elastic fibers (Figs. 5, 6, 8, 11).

From D15 until post-partum, a large proportion of the cells showed myofibroblastic-like characteristics, often possessing large bundles of citoplasmic filaments (Figs. 7, 12). The presence of junctional complexes between the interior of the cells and the adjacent extracellular matrix, forming a structure so-called "fibronexus" is evident (Figs. 8, 9). These junctions are believed to be concerned with the transmission of contractile force within the tissue and are one of the distinctive characteristics of myofibroblastic cells. The cell membrane displayed numerous pinocytosis vesicles (Fig.7, 9) and the nucleus showed multiple indentations (Fig.10). Other ultrastructural features found in pubic symphysis cells are ramified long cellular processes, which contain only few organelles (Fig.11). The abundant citoplasmic filaments are almost disposed in the cellular periphery (Fig.12), while the prominent rough endoplasmic reticulum, Golgi apparatus and the mitochondrias are arranged in perinuclear cytoplasmic regions (Figs.13, 14). Many well-packed thin filaments were observed in close proximity to the cytoplasmic cortex aligned parallel to extracellular matrix microfibril elements (Fig15).

These ultrastuctural features, taken together, resemble the classically myofibroblastic phenotype defined in previous morphological descriptions that suggested a secretory and contractile role (Gabbiani et al., 1971; Desmoulière and Gabbiani, 1996; Powell et al., 1999; Varayoud et al., 2001).

DISCUSSION

In pregnant mice, the enlargement of the birth canal to facilitate the passage of the fetus comprises a separation of the pubic bones. This process results mainly from two events: a gradual growth of the interpubic ligament under estrogen stimulation and the softening of this structure attributed to the action of relaxin, required for the breakdown of fibrous connective tissue. After parturition the ligament involution also involves a rapid extracellular matrix (ECM) turnover and adaptation of the cellular compartment (Sherwood, 1994).

Studies about the pubic symphysis movement during pregnancy generally broach the ECM rearrangement (Storey, 1957; Zhao et al., 1999, 2000; Weiss et al., 1991), while the cell plasticity and cell-ECM interaction are rarely described. The present study revealed, by immunohistochemistry, the presence of contractile proteins in the fibroblastic-like cells of the pubic symphysis in cycling, pregnant and post-partum mice. The ultrastructural features of these cells also were analyzed.

It is increasingly recognized that the chondrocytes and fibroblasts appears to have a plastic phenotype and are capable of fulfilling distinct functions in normal and pathological situations as well as in different locations (Komuro, 1990; Sappino et al., 1990; Povysil et al., 1997; Wang et al., 2000; Nielsen et al., 1999; Langelier et al., 2000;).

It is well accepted that the ECM represents a structural support for cellular constituents, but evidence exists showing that the matrix plays a central role as a source of signals, which are capable of influencing the growth and the differentiation of several cell types, including fibroblast (for review, see Juliano and Haskill, 1993). This suggests that each organ owns characteristic cells containing specific features and that most of them are relatively undifferentiated with the ability to assume a particular phenotype, such as expressing specific cytoskeletal proteins according to the physiological needs and/or the microenvironmental stimuli (Sappino et al., 1990; Shimizu and Yoshizato, 1992; Komuro, 1990).

Currently it has been suggested that chondrocytes can be subdivided in subtypes according to their characteristic immunophenotype (Povysil, 1995; Wang et al., 2000;). Komuro (1990) has proposed to categorize fibroblasts into subtypes depending on their main functions: in fibrogenesis, tissue skeleton or barrier; intercellular communication system; gentle contractile machinery; endocrine activity; and vitamin A-storing. Among

these functions, at least, contractility and maintenance of tissue shape are directly related to cytoskeletal activities.

Cytoskeletal proteins have been extensively used as markers of differentiation, as well as marker of adaptation to physiologic and pathologic events. In some of these situations the fibroblasts are equipped with smooth muscle structures and are called myofibroblasts. Cells with morphological features similar to those of myofibroblasts have been found in a variety of organs such as rat intestinal villi (Güldner et al., 1972; Joyce, 1987), periodontal ligament (Beertsen et al., 1974; Yamasaki et al., 1987), human seminiferous tubules (Holstein et al., 1996), external theca of ovarian follicles of rats (O'Sheal, 1970), etc (for review, see Powell et al., 1999).

In cycling virgin, pregnancy and post-partum, our results showed a permanent and homogeneous α-SMA expression in the fibroblast-like cells at the time course of high ECM changes. Prominent actin filaments have been associated with matrix deposition by fibroblasts/myofibroblasts of granulation tissue (Gabbianni, 1994; Horiba and Fukuda, 1994; Desmouliere, 1995; Berry et al., 1998), including that of healing and remodeling ligament scar (Faryniarz et al., 1996). This data suggests that the α-SMA can participate in movements associate with endocytosis and exocytosis processes during ECM turnover and ECM-cell interaction in formation and involution of the interpubic ligaments. This cytoskeletal protein also can be involved in cell morphology such cell shape alteration and emission of cell prolongations as it was observed in our electron microscopy study.

The transient patterns of vimentin and desmin expressions in interpubic ligament cells were particularly interesting. Desmin and vimentin, as two distinctive types of intermediate filaments are recognized as the most stable components of the cytoskeleton of many cells. Both display cell-specific localization and are frequently expressed in a specific pathway of differentiation (for review, see Steinert and Roop, 1988; Albers and Fuchs, 1992). The vimentin and desmin expressions were observed in the same period (D15 from D20), but anti-vimentin showed a homogenous staining pattern throughout the cytoplasm and cellular protrusions, while the desmin label has restricted to a halo surrounding the nucleus. The perinuclear arrangement of desmin may be important to guide the modification of the nuclear size and shape, which has reinforced by observation of a negative desmin label in division cells. Our findings are consistent with Oliveira et al. (2000) and Varayoud et al. (2001). The temporal increase of vimentin expression, even in

division cells, together with permanent α-SMA expression, could account for the rapid transformation of cell shape and organelle distribution as a consequence of increased synthetic activity of ECM components.

The transient observation of intermediate filaments in the late pregnant and a term interpubic ligament seems to be likely the sequence of events observed during the annulus fibrous development, where the intermediate filament vimentin does not seem to be involved in the cell and matrix orientation process, because it appears too late during the development, but with adaptation to the mechanical stress (Hayes , 1999).

In addition, it is interesting to emphasize that desmin and vimentin labeling were observed in the same time when the plasma levels of relaxin and estrogen increased in mice pregnants (Sherwood, 1994). The cytoskeletal expression can be modulated in some cell by sex hormones (Ross and Klebanoff, 1967; Bo et al., 1968; Kawaguchi et al., 1985; Glasser and Julian, 1986; Hsu and Frankel, 1987). The action of these hormones in ECM of pubic symphysis is extensively described in literature (Sherwood, 1994) but their participation in mechanisms of the cytoskeletal protein expression control remain to be investigated.

Our ultrastructural analysis revealed cell features that agree with the myofibroblasts descriptions by others authors (Gabbiani et al., 1971; Desmoulière and Gabbiani, 1996; Powell et al., 1999; Varayoud et al., 2001). In the early pregnancy the chondrocytes released from their lacunae arranged themselves along the transversely oriented collagen fibers and appeared as fibroblasts-like cells (Crelin, 1954). During the growth of interpubic ligament, the fibroblast-like cells displayed remarkable alteration in their shape from fusiform with smooth nuclear outline (in cycling and D12 mice) to round-ovoid with indented nucleus (from D15). In the late pregnancy the cells contained prominent Golgi, endoplasmic reticulum and several pinocytotic vesicles, which are indicators of a higher synthetic and secretory activity. Our results also showed the close contact between elastic system fibers and cell prolongation and the presence of structures like fibronexus, doing the cell-ECM interaction. After parturition these cells then could revert back to their original chondrocyte state. This is evidence that the chondrocytes of the pubic symphysis are not fixed, differentiated cells, but merely in a state of modulation (Crelin, 1954; Linck et al., 1975; Linck et al., 1976).

The morphologic and ultrastructural findings suggest that these cells have a putative contractile apparatus forming a cell-ECM network capable of transmitting the

forces generated from the movement of pubic bones during pregnancy, both to neighboring cells and to elements of ECM. The reduction in tension and thus disassembly of intermediate filaments in ligament fibroblast after delivery is suggestive that they are no longer required for cell adaptation to mechanical stress, but on the other hand, the maintenance of actin expression during pregnancy, deliverance and post-partum suggest that thin microfilaments are required for cell orientation and matrix organization. Therefore, the presence of cells with these particular features in the pubic symphysis can contribute to the structural integrity of the interpubic connective tissue architecture.

FIGURE LEGENDS

FIG.1A, B, C, D, E, F. Photomicrographs of a coronal section through the pubic symphysis of the virgin mouse (A), at D12 (B), D18 (C), D19 (D), D20 (E) and D24 (F). Immunohistochemistry of sections of the fibrocartilage (A, B) and interpubic ligament central area (C, D, E, F), demonstrating cells with positive staining for the α-smooth muscle actin isoform, as shown by the peroxidase on the cytoplasm. The scale bar (in panel A) represents 5μm (for A), 7μm (B), 10μm (C), 7,5μm (D), 8μm (E) and 6μm (F).

FIG.2A, B. Photomicrographs of a coronal section through the pubic symphysis at D18 (A) and during the parturition – D19 (B), submitted to immunoperoxidase staining with anti-desmin antibody. A, B, Note the intense and homogenous staining in the cytoplasm pericitic cells (arrows) and in the fibroblast-like cells, where the stain pattern corresponds to a halo surrounding the nucleus (arrowheads). B, division cells are negatively stained (asterisk). Bar, 12μm.

FIG.3A, B. Photomicrographs of a coronal section through the mouse pubic symphysis at D19, submitted to immunoperoxidase staining with anti-vimentin antibody. Homogeneous staining was noted in the fibroblast-like cells (arrows), even in division cells (arrowheads). Bar, 13μm (A) and 6μm (B).

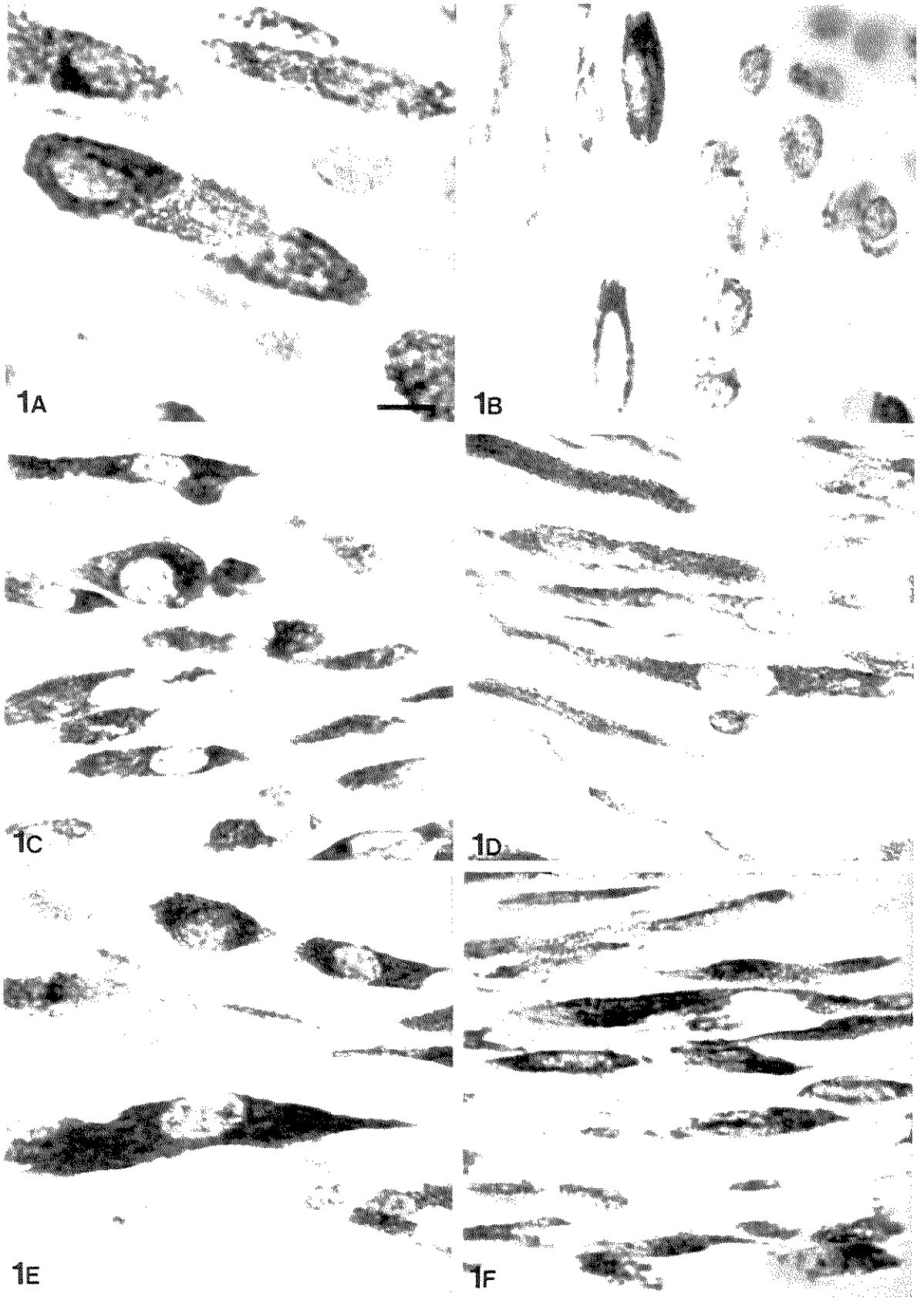
FIG.4, 5, 6. Transmission electron micrographs of pubic symphysis at D12. 4, a fibroblast-like characterized by a fusiform shape, a smooth nuclear outline and cytoplasm rich in rough endoplasmic reticulum, Golgi apparatus and mitochondria. Note an elaunin fiber (arrowheads) in oblique (5) and transversal (6) section in close contact with the long cellular processes (arrow). Bar, 150nm (4), 250nm (5) and 200nm (6).

FIG.7, 8, 9. Transmission electron micrographs of pubic symphysis of D15, showing portions of cytoplasm fibroblast-like-cell. 7, Note the presence of a peripheral bundle of cytoplasmic filaments (arrow) and pinocytotic vesicles (arrowheads). 8, Cross-section of elastic system fiber (arrow) rich in elastin (E), located near to cellular process (asterisk). 9, The extracellular matrix forms organized plates in the region directly opposite to the

plasmalemma-associated density (arrows) forming a hemidesmosome-like structure so-called "fibronexus". Bar, 300nm (7), 250nm (8) and 200nm (9).

FIG.10. Transmission electron micrograph of a thin cross section of pubic symphysis at D18. The fibroblast-like cell shows an indented nucleus (N). Bar, 400nm.

FIG.11, 12, 13, 14, 15. Transmission electron micrographs of pubic symphysis mouse during the parturition (D19). 11, an elaunin fiber (arrow) cross-sectioned in close contact with the long cellular process (asterisk). 12, Note the numerous microfilaments bundles (arrow) parallel to the main axis of the cell. 13, 14, a well-developed rough endoplasmic reticulum (RER) and Golgi complex (G) are located within the perinuclear cytoplasm. Note the presence of mitochondrias (M). 15, Observe the distribution of bundles of cytoplasmic filaments (arrowheads) that insert in regions of higher electron density at the cell membrane. Note the extracellular fibrils in close contact with cellular processes (arrows). Bar, 300nm (11), 400nm (12), 600nm (13), 500nm (14) and 750nm (15).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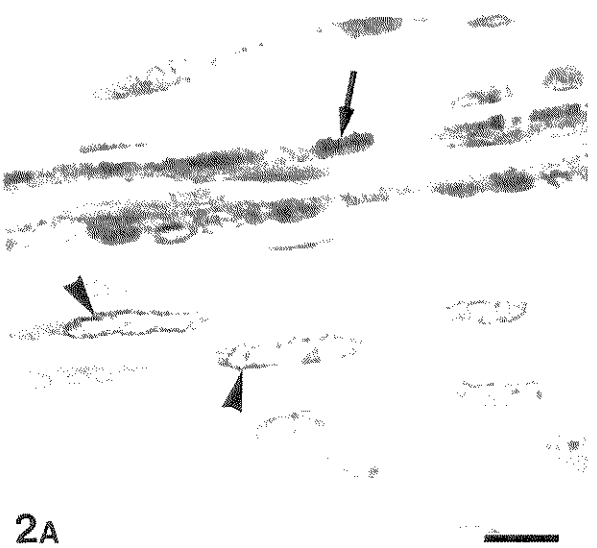

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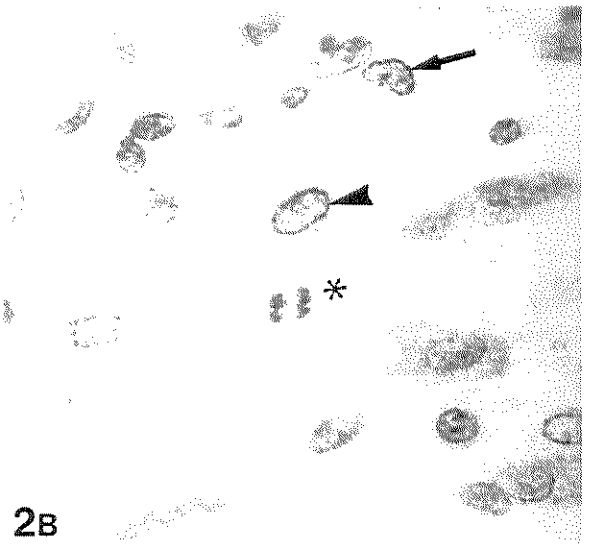

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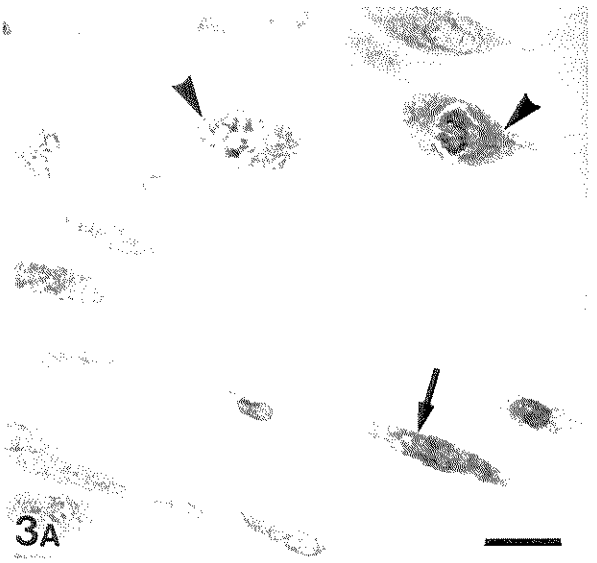

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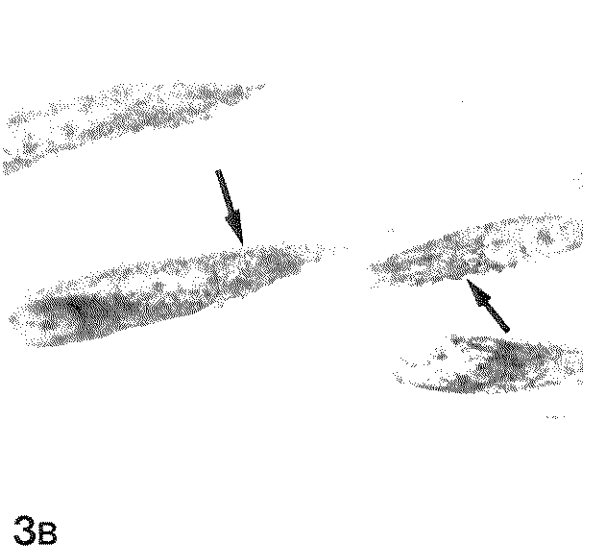

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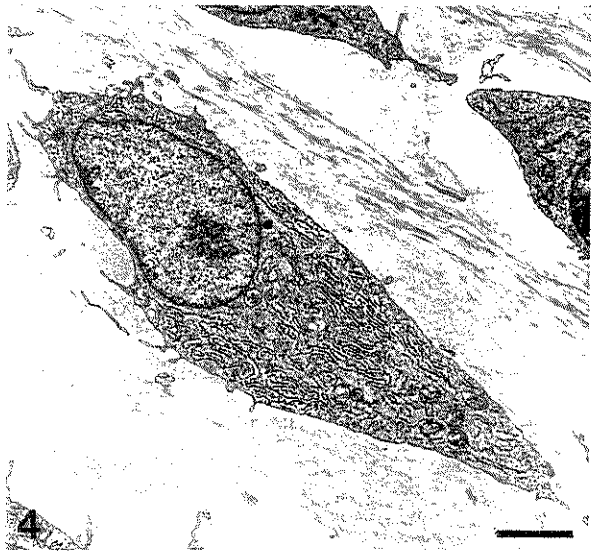

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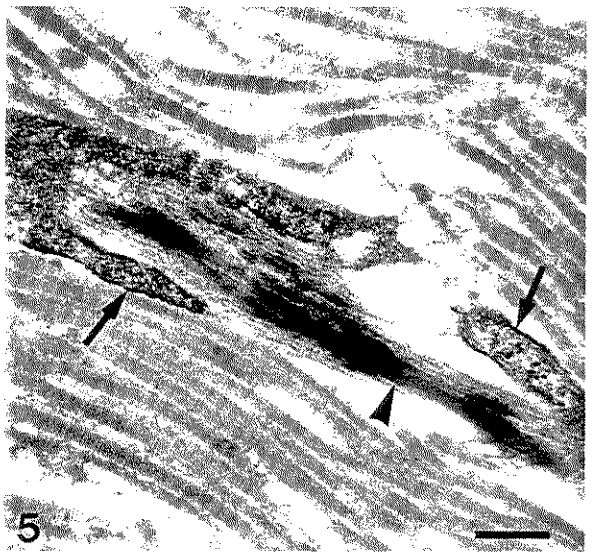

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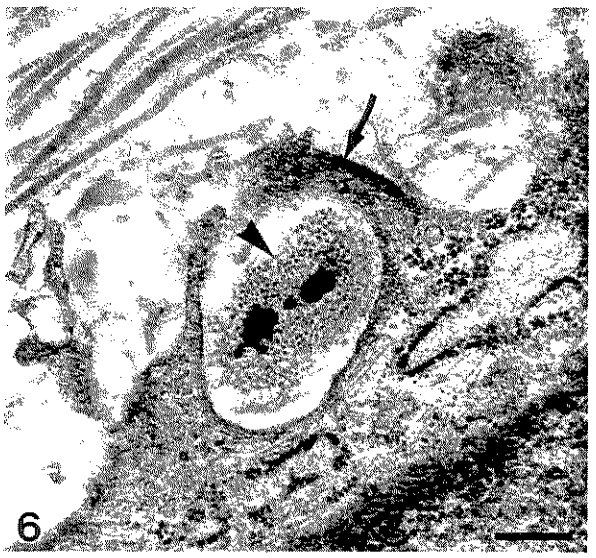

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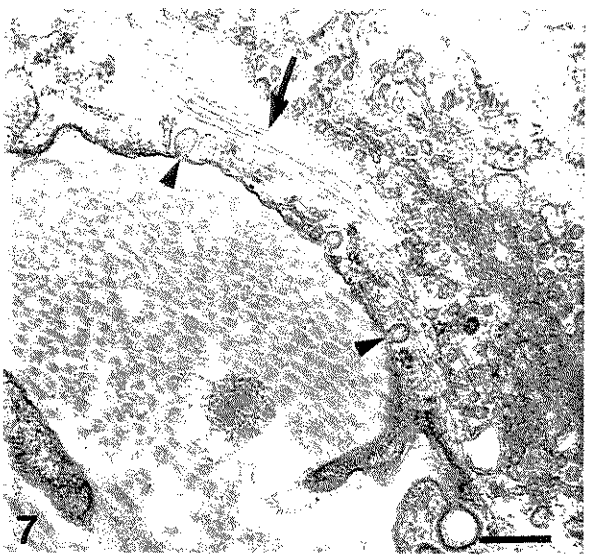

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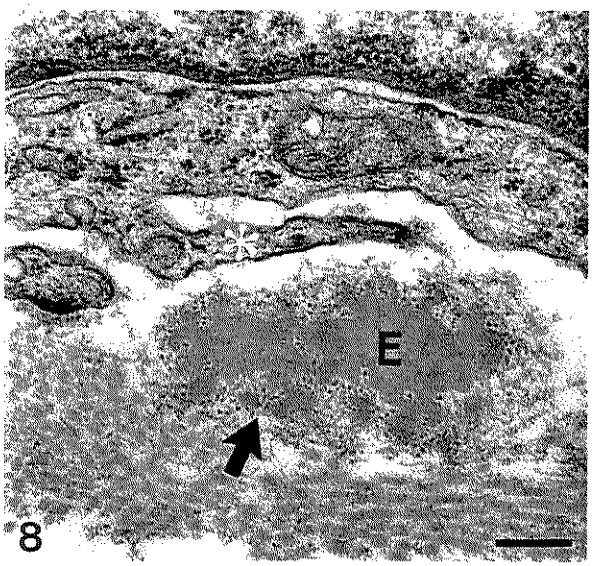

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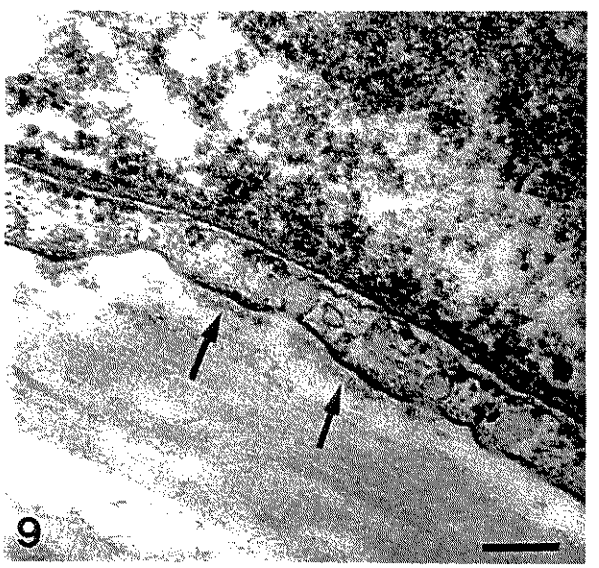

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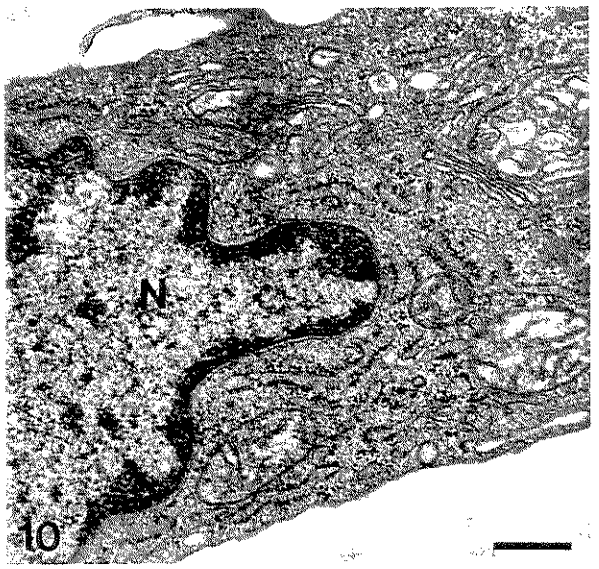
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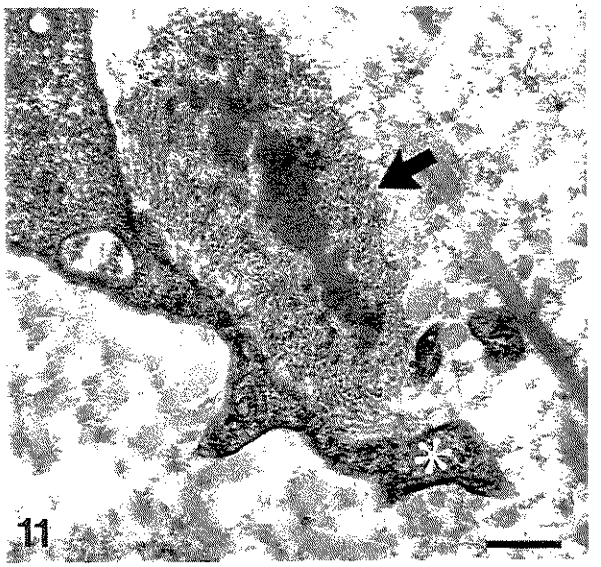
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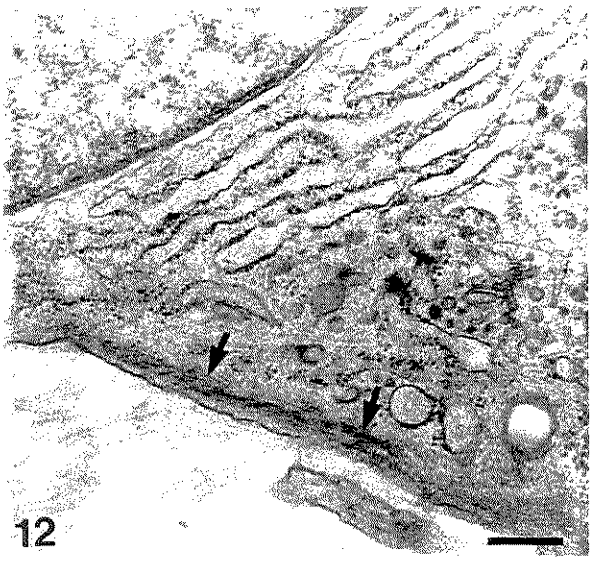
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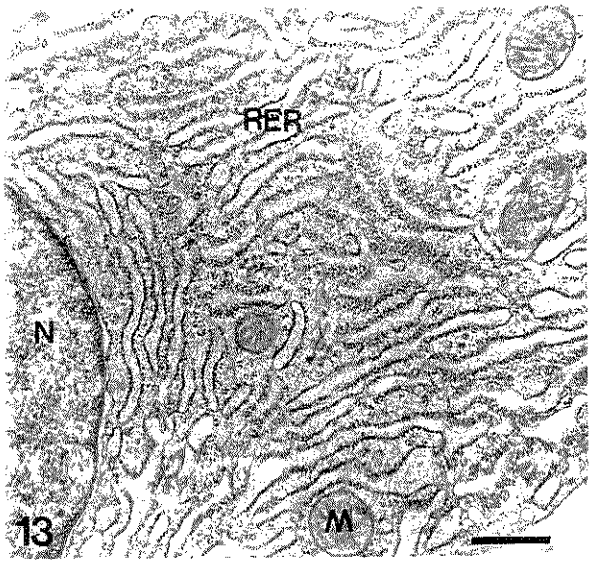
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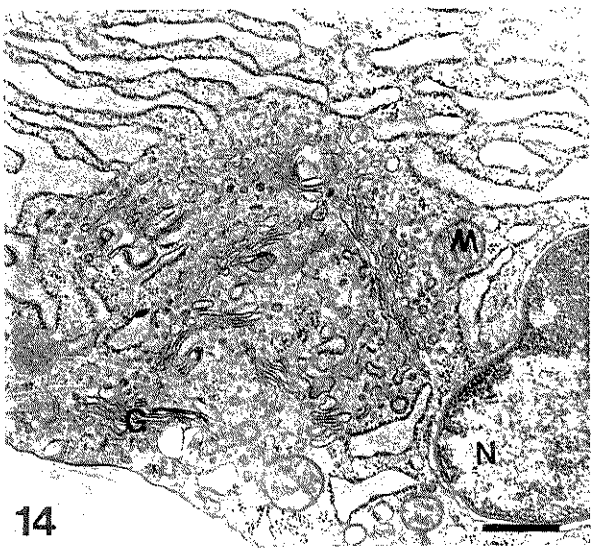
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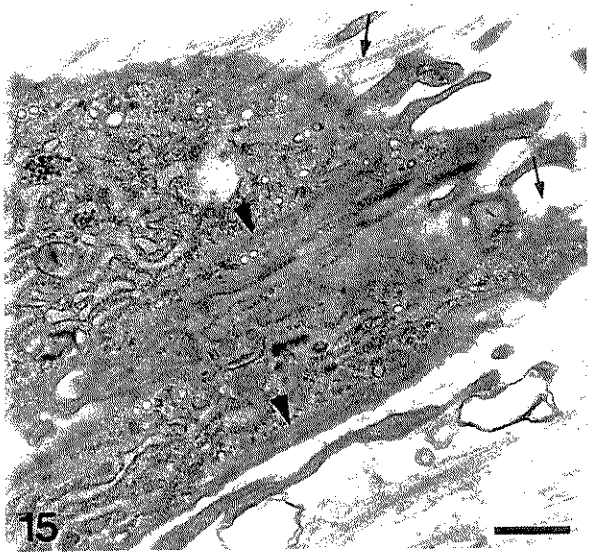
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REFERENCES

ALBERS, K., FUCHS, E. 1992. The molecular biology of intermediate filaments proteins. *International Review of Cytology* ***134***, 243-279.

BASSET, E.G., PHILLIPPS, D.S.M. 1955. Changes in the pelvic region of the ewe during pregnancy and parturition. *N. Z. Vet. J.* ***3***, 20-25.

BEERTSEN, W., EVENTS, V., VAN DEN HOOF, A. 1974. Fine structure of fibroblasts in the periodontal ligament of the rat incisor and their possible role in tooth eruption. *Arch. Oral Biol.* ***19***, 1097-1098.

BERRY, D.P., HARDING, K.G., STANTON, M.R., JASANI, B., EHRLICH, H.P. 1998. Human wound contraction, collagen organization, fibroblasts, and myofibroblasts. *Plast. Reconstr. Surg.* ***102***, 124-131.

BO, W.J., ODOR, D.L., ROCKROCK, M.L. 1968. Ultrastructure of uterine smooth muscle following progesterone or progesterone-estrogen treatment. *Ann. Rec.* ***163***, 121-132.

CAN, A., TEKELIOGLU, M., BALTACI, A. 1995. Expression of desmin and vimentin intermediate filaments in human decidual cells during first trimester pregnancy. *Placenta* ***16***, 261-275.

CINTORINO, M, BELLIZZI de MARCO, E., LEONCINI, P., TRIPODI, S.A, RAMAEKERS, F.C., SAPPINO, A., SCHMITTI-GRÄFF,A, GABBIANI, G. 1991. Expression of α-smooth-muscle actin in stromal cells of the uterine cervix during epithelial neoplastic changes. *Int. J. Cancer* ***47***, 843-846.

CRELIN, E.S. 1954. The effect of estrogen and relaxin on intact and transplanted pubic symphyses in mice. *Anat. Rec.* ***118***, 380-381.

CRELIN, E.S., BRIGHTMAN, M.W. 1957.The pelvis of the rat, its response of estrogen and relaxin. *Anat. Rec.* ***128***, 467-484.

CRELIN, E.S., NEWTON, E.V. 1969. The pelvis of the free-tailed bat, Sexual dimorphism and pregnancy changes. *Anat. Rec.* ***164,*** 349-358.

CRELIN, E.S. 1969. Interpubic ligament, elasticity in pregnant free-tailed bat. *Science* ***164***, 81-2. a.

CRELIN, E.S. 1969. The development of bony pelvis and its changes during pregnancy and parturition. *Trans. NY Acad. Sci.* ***31***, 1049-1058, b.

DARBY, I., SKALLI, O., GABBIANI, G. 1990. α-Smooth muscle actin is transiently expressed by myofibroblasts during experimental wound healing. *Lab. Invest.* ***63,*** 21-29.

DESMOULIÈRE, A. 1995. Factors influencing myofibroblast differentiation during wound healing and fibrosis. *Cell Biol Int.* ***19***, 471-6.

DESMOULIÈRE, A. 1996. The role of the myofibroblast in wound healing and fibrocontractive diseases. In, Clark RAF (ed.), The Molecular and Cellular Biology of Wound Repair. New York, Plenum Press, 391-423.

FARYNIARZ, D.A., CHARPONNIER, C., GABBIANI, G., YANNAS, I.V., SPECTOR, M. 1996. Myofibroblasts in the healing lapine medial collateral ligament, possible mechanism of contraction. *J. Orthop. Res.* ***14***, 228-237.

GABBIANI, G., RYAN, G., MAJNE, G. 1971. Presence of modified fibroblasts in granulation tissue and their posible role in wound contraction. *Experientia* ***27***, 549-550.

GABBIANI, G. 1994. Modulation of fibroblastic cytoskeletal features during wound healing and fibrosis. *Pathol. Res. Pract.* ***190***, 851-853.

GAMBLE, J.G, SIMMONS, S.C., FREEDMAN, M. 1986. The symphysis pubis, anatomic and pathologic considerations. *Clinical Orthopaedics and Related Research* ***203,*** 261-72.

GARDNER, W.U. 1936. Sexual dimorphism of the pelvis of the mouse, the effect of estrogenic hormones upon the pelvis and upon the development of scrotal hernias. *Am. J. Anat.* ***59***, 459-483.

GLASSER, S.R., JULIAN, J. 1986. Intermediate filament protein as a marker of utrine stromal cell decidualization. *Biol. Reprod.* ***35,*** 463-474.

GOWN, A. 1990. The mysteries of the myofibroblast (partially) unmasked. *Lab. Invest.* ***63***, 1-3.

Guide for the Care and Use of Laboratory Animals (National Academy of Science, 1996)

GÜLDNER, F.H., WOLFF, J.R., KEYSERLINK, D. 1972. Fibroblasts as part of the contractile system in duodenal villi of rat. *Z. Zellforsch* ***135***, 349-360.

HALL, K. 1947. The effects of pregnance and relaxin on the histology of the pubic sysphysis in the mouse. *J. Endocrinol.* ***5,*** 174-82.

HORN, E.H. 1960. Interpubic ligament regression in relaxin-treated virgin and primiparous, post-partum mice. *Endocrinology* ***67***, 668-673.

HOLSTEIN, A., MAEKAWA, M., NAGANO, T., DAVIDOFF, M.S. 1996. Myofibroblasts in the lamina propria of human seminiferous tubules are dynamic structures of heterogeneous phenotype. *Arch. Histol. Cytol.* ***59***, 109-125.

HORIBA, K., FUKUDA, Y. 1994. Synchronous appearance of fibronectin, integrin alpha 5 beta 1, vinculina and actin in epithelial cells and fibroblasts during rat tracheal wound healing. *Virchows Arch.* ***425***, 425-434.

HSU, C.Y., FRANKEL, F.R. 1987. Effect of estrogen on the expression of mRNAs of different actin isoforms in immature rat uterus. *J. Biol. Chem.* ***262***, 9594-9600.

JOYCE, 1987. Morphologic and biochemical evidence for a contractile cell network within the rat intestinal mucosa. *Gastroenterology* ***92***, 68-81.

JULIANO, R.L., HASKILL, S. 1993. Signal transduction from the extracellular matrix. *J. Cell Biol.* ***120***, 577-585.

KAWAGUCHI, K., FUJII, S., KONISHI, I., OKAMURA, H., MORI, T. 1985. Ultrastructural study of cultured smooth muscle cells from uterine leiomyoma and myometrium under the influence of sex steroids. *Gynecol. Oncol.* ***21***, 32-41.

KROC, R.L., STEINETZ, B.G., BEACH, V.L. 1958. The effects of estrogens, progestagens, and relaxin in pregnant and nonpregnanat laboratorory rodents. *Ann. N. Y. Acad. Sci.* ***75***, 942-80.

KOMURO, T. 1990. Re-evaluation of fibroblasts and fibroblast-like cells. *Anat. Embryol.* ***182***, 103-112.

LANGELIER, E., SUETTERLIN, R., HOEMANN, C.D., AEBI, U., BUSCHMANN, M.D. 2000. The chondrocyte cytoskeleton in mature articular cartilage, structure and distribution of actin, tubulin, and vimentin filaments. *J. Histochem. Cytochem.* ***48***, 1307-20.

LINCK, G., OUDET, C., PETROVIC, A. 1975. Multiplication of the different varieties of cells of the mouse pubic symphysis during growth and early gestation, radioautographic study with the use of tritiated thymidine. *Bull Assoc. Anat. (Nancy)* ***59,*** 467-78.

LINCK, G., PETROVIC, A., STOECKLE, M.E., PORTE, A. 1976. Fine structure of the public symphysis in the mouse. *Bull Assoc. Anat. (Nancy)* ***60***, 201-9.

MONTES, G.S. 1992. Distribution of oxytalan, elaunin and elastic fibres in tissues. *Cienc. Cult.* ***44***, 224-33.

NIELSEN, G.P., KEEL, S.B., DICKERSIN, G.R., SELIG, M.K., BHAN, A.K., ROSENBERG, A.E. 1999. Chondromyxoid fibroma, a tumor showing myofibroblastic, myochondroblastic, and chondrocytic differentiation. *Mod. Pathol.* ***12***, 514-517.

OLIVEIRA, S.F., GRECA, C.P.S., ABRAHAMSOHN, P.A., REIS, M.G., ZORN, T.M.T. 2000. Organization of desmin-containing intermediate filaments during differentiation of mouse decidual cells. *Histochem. Cell Biol.* ***113***, 319-327.

O'SHEAL, J.D. 1970. An ultrastructural study of smooth muscle-like cells in theca externa of ovarian follicles in the rat. *Anat. Rec.* ***167***, 127-140.

POVYSIL, C. 1995. Actin-positive chondroblasts (myochondroblasts) in benign chondroblastoma. *Cesk. Patol.* ***31***, 77-78.

POVYSIL, C., TOMANOVA, R., MATEJOVSKY, Z. 1997. Muscle-specific actin expression in chondroblastomas. *Hum. Pathol.* ***28***, 316-320.

POWELL, D.W., MIFFLIN, R.C., VALENTICH, J.D., CROWE, S.E., SAADA, J.I., WEST, A.B. 1999. Myofibroblasts. II. Intestinal subepithelial myofibroblasts. *Am. J. Physiol.* ***277***, 183-201

ROSS, R., KLEBANOFF, S.J. 1967. Fine structural changes in uterine smooth muscle and fibroblasts in response to estrogens. *J. Cell Biol.* ***32***, 155-169.

RUTH, E.B. 1937. Metamorphosis of the pubic symphysis. III. Histological changes in the symphysis of the pregnant guinea pig. *Anat. Rec.* ***67,*** 409-421.

SAPPINO, A.P., SCHÜRCH, W., GABBIANI, G. 1990. Differentiation repertoire of fibroblastic cells, Expression of cytoskeletal proteins as marker of phenotypic modulations. *Lab. Invest.* ***63***, 144-161.

SCHMITT-GRAFF, A., DESMOULIERE, A., GABBIANI, G. 1994. Heterogeneity of myofibroblast phenotypic features, an example of fibroblastic cell plasticity. *Virchows Archiv.* ***425***, 3-24.

SHERWOOD, O. D. 1994. Relaxin. En, Knobil E, Neill j. d. (eds). The physiology of reproduction. Raven Press, New York. Chapter 16, pp.861-1009.

SHIMIZU, K., YOSHIZATO, K. 1992. Organ-dependent expression of differentiated states in fibroblasts cultured in vitro. *Dev. Growth Differ.* ***34***, 43-50.

SHORR, E. 1941. A new technique for staining vaginal smears. II. Single differencial stain. Science. **5**, 545-546.

STEINERT, P.M., ROOP, D.R. 1988. Molecular and cellular biology of intermediate filaments. *Annu. Rev. Biochem.* ***57***, 593-625.

STEINETZ, B.G., BEACH, V.L., KROC, R.L. 1957. The influence of progesterone, relaxin and estrogen on some structural and functional changes in the pre-parturient mouse. *Endocrinology* ***61,*** 271-280.

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.

TALMAGE, R.V. 1947. Changes produced in the symphyseal pubis of the guinea pig by the sex steroids and relaxin. *Anat. Record* ***99,*** 91-113, a.

TALMAGE, R.V. 1947. A histological study of the effects of relaxin on the symphysis pubis of the guinea pig. *J. Exp. Zool.* ***106,*** 281-92, b.

VARAYOUD, J., RAMOS, J.G., JOAZEIRO, P.P., MONTES, G.S., TORO, M.M., LUQUE, E.H. 2001. Characterization of fibroblastic cell plasticity in the lamina propria of the rat uterine cervix at term. *Biol. Reprod.* ***65***, 101-118.

VIX, V.A., RYU, C.Y. 1971. The adult symphysis pubis, normal and abnormal. *Am J Roentgenol Radium Ther Nucl Med.* ***112***, 517-25.

WAHL L.M., BLANDAU, R.J., PAGE, R.C. 1977. Effects of hormones on collagen metabolism and collagenase activity in the pubic sysmphysis ligament of the guinea pig. *Endocrinology* ***100,*** 571-9.

WEISS, G. 1991. The physiology of human relaxin. *Contrib. Gynecol. Obstet.* ***18***, 130-146.

YAMASAKI, A., ROSE, G.G., PINERO, G.J., MAHAN, C.J. 1987. Microfilaments in human cementoblasts and periodontal fibroblasts. *J. Periodontol.* ***58***, 40-45.

YANG, S., REMBIESA, B., BÜLLESBACH, E.E., SCHWABE, C. 1992. Relaxin receptors in mice, Demonstration of ligant binding in symphyseal tissue and uterine membrane fragments. *Endocrinology* ***130***, 179-85.

ZHAO, L., ROCHE, P.J., GUNNERSEN, J.M., HAMMOND, V.E., TREGEAR, G.W., WINTOUR, E.M., BECK, F. 1999. Mice without a functional relaxin gene are unable to delivery milk to their pups. *Endocrinology* ***140***, 445-453.

ZHAO, L., SAMUEL, C.S., TREGEAR, G.W., BECK, F., WINTOUR, E.M. 2000. Collagen studies in late pregnant relaxin null mice. *Biol. Reprod.* ***63***, 697-703.

CONCLUSÕES

Através dos resultados obtidos neste trabalho, foi possível concluir que:

1. As técnicas de coloração seletiva, imunohistoquímica, o estudo morfométrico e ultra-estrutural foram adequados para demonstrar os componentes do sistema elástico e as características fenotípicas das células na sínfise e no ligamento interpúbico de camundongo.
2. Até o 12°dia de prenhez os componentes do sistema elástico conservam características semelhantes as dos animais fêmeas virgens.
3. As fibras do sistema elástico variam em espessura e orientação durante a última semana de prenhez e no pós-parto.
4. As fibras do sistema elástico devem servir como um guia para a orientação das células presentes na sínfise púbica fibrocartilaginosa e no ligamento interpúbico.
5. As fibras do sistema elástico desempenham importante papel na sínfise púbica durante a formação e involução do ligamento interpúbico na prenhez, evitando o estiramento e/ou rompimento desta articulação e ajudando na recuperação da arquitetura da mesma após o parto.
6. As células presentes no ligamento interpúbico possuem um aparato contráctil e características ultra-estruturais coincidentes com miofibroblastos, sendo capazes de se adaptar ao estresse mecânico o qual são submetidas durante a prenhez. Além disso, elas formam uma rede de interações com a MEC (a exemplo das fibras do sistema elástico), podendo transmitir a força gerada pela movimentação dos ossos púbicos durante a prenhez, tanto para células vizinhas quanto para outros elementos da MEC.
7. Estas células desempenham uma função relevante depositando, organizando e orientando os componentes da MEC.
8. As características das células e das fibras do sistema elástico observadas no presente trabalho, contribuem para a integridade mecânica e estrutural da sínfise púbica e do ligamento interpúbico durante o estresse mecânico no momento do parto.

REFERÊNCIAS BIBLIOGRÁFICAS

BATTLERHNER, C.N., CARNEIRO FILHO, M., FERREIRA, J.M.C., SALDIVA, P.H.N., MONTES, G.S. 1996. Histochemical and ultrastructural study of the extracellular matrix fibers in patellar tendon donor site scars and normal controls. *J.Submicrosc. Cytol. Pathol.* ***28,*** 175-186.

BRAVERMAN, I.M., FONFERKO, E. 1982. Studies in cutaneous aging: I. The elastic fiber network. *J. Invest. Dermatol.* ***78***, 434-43.

BRESSAN, G.M., DAGA-GORDINI, D., COLOMBATTI, A., CASTELLANI, I., MARIGO, V., VOLPIN,D. 1993. Emilin, a component of elastic fibers preferentially located at the elastin-microfibrils interface.*J Cell Biol.****121***, 201-12.

BÜLLESBACH, E.E., SCHWABE, C. 1995. A novel Leydig cell cDNA-derived protein is a relaxin-like factor. *J. Biol. Chem.* ***270***, 16011-15.

BÜLLESBACH, E.E., SCHWABE, C. 1996. The chemical synthesis of the rat relaxin in the unexpected high potency of the synthetic hormone in the mouse. *Eur. J. Biochem.* ***241***, 533-7.

CALDINI, E. G., CALDINI, N. De-PASQUALE, V., STROCCHI, R., GUIZZARDI, S., RUGGERI, A., MONTES, G.S. 1990. Distribution of elastic system fibers in the rat tail tendon and its associated sheaths. *Acta Anat.,* ***139,*** 341-8.

CARVALHO, H.F., NETO, J.L, TABOGA, S.R. 1994. Microfibrils, neglected components of pressure-bearing tendons. *Ann. Anat.* ***176***, 155-59.

CARVALHO, H.F.& VIDAL, B.C. 1995. The elastic system of a pressure-bearing tendon of bullfrog *Rana catesbeiana*. *Ann. Anat.* ***177***, 397-404.

CARVALHO, H.F. & TABOGA, S.R. 1996a. Fluorescence and confocal laser scanning microscopy imaging of elastic fibers in hematoxylin-eosin stained sections. *Histochem. Cell Biol.* ***106***, 587-92.

CARVALHO, H.F. & TABOGA, S.R. 1996b. The applicability of hematoxylin-eosin staining plus fluorescence or confocal laser scanning microscopy to the study of elastic fibers in cartilages. *Cell Biology* ***319,*** 991-6.

CHIHAL, H.J., ESPEY, L.L. 1973. Utilization of the relaxed symphysis pubis of guinea pigs for clues to mechanism of ovulation. *Endocrinology* ***93***, 1441-5.

CLEARY, E.G., FANNING, J.C., PROSSER, I. 1981. Possible roles of microfibrils in elastogenesis. *Conn. Tiss. Res.* ***8,*** 161-6.

CLEARY, E.G., GIBSON, M.A. 1983. Elastin-associated microfibrils and microfibrilar proteins. *Int. Rev. Connect. Tiss. Res.* ***10***, 97-109.

COTTA-PEREIRA, G., RODRIGO, F.G., BITTENCOUT-SAMPAIO, S. 1976. Oxytalan, elaunin and elastic fibers in the human skin. *J. Invest. Dermatol.* ***66***, 143-8.

COTTA-PEREIRA, G., RODRIGO, F.G., DAVID-FERREIRA, J.F. The elastic system fibers. In, SANBERG, L.G. GRAY, W.R., FRANZBLAU, C. *Elastin and elastic tissue. Advances in Experimental medicine and Biology*. v. 79, New York, Plenum Press, 1977, p. 19-30.

COTTA-PEREIRA, G., DEL-CARO, L. M., MONTES, G.S. 1984. Distribution of elastic system fibres in hyaline and fibrous cartilages of the rat. *Acta Anat.* ***119,*** 80-5.

CRELIN, E.S. 1954. The effect of estrogen and relaxin on intact and transplanted pubic symphyses in mice. *Anat. Rec.* ***118***, 380-381.

CRELIN, E.S., HAINES, A.L. 1955. The effects of locally applied estrogen on the pubic symphysis and knee joint in castrated mice. *Endocrinology* ***56***, 461-470.

CRELIN, E.S., LEVIN.J. 1955. The prepuberal pubic symphysis and uterus in the mouse their response estrogen and relaxin. *Endocrinology* ***57***, 730-747.

CRELIN, E.S., BRIGHTMAN, M.W. 1957. The pelvis of the rat, its response of estrogen and relaxin. *Anat. Rec.* ***128***, 467-484.

CRELIN, E.S. 1960. The development of bony pelvic sexual dimorphism in mice. *Ann. NY Acad. Sci.* ***84***, 481-511.

CRELIN, E.S., NEWTON, E.V. 1969. The pelvis of the free-tailed bat, Sexual dimorphism and pregnancy changes. *Anat. Rec.* ***164***, 349-358.

CRELIN, E.S. 1969a. Interpubic ligament, elasticity in pregnant free-tailed bat. *Science.* ***164***, 81-2.

CRELIN, E.S. 1969b.The development of bony pelvis and its changes during pregnancy and parturition. *Trans. NY Acad. Sci.* ***31***, 1049-1058.

DÂNGELO, J.G., FATTINI, C.A. Anatomia humana sistêmica e segmentar. 2ª edição, Ed. Ateneu. Rio de Janeiro, 1988.

DEBELLE, L., ALIX, A.J.P. 1999. The structures of elastin and their function. *Biochimie* ***81***, 981-994.

DEBELLE, L., TAMBURRO, A.M. 1999. Elastin, molecular description and function. *Int. J. Biochem. Cell Biol.* ***31***, 261-272.

DORRINGTON, K.L., McCRUM, N.G. 1977. Elastin as a rubber.*Biopolymers.* ***16***, 1201-22.

DOWNING, S.J., SHERWOOD, O.D. 1986. The physiological role of relaxin in the pregnant rat. IV. The influence of relaxin on cervical collagen and glycosaminoglycans. *Endocrinology* ***118***, 471-9.

FAZIO, M.J., MATTEI,M.G., PASSAGE, E., CHU, M.L., BLACK, D., SOLOMON, E., DAVIDSON,J.M., UITTO,J. 1991. Human elastin gene: new evidence for localization to the long arm of chromosome 7.*Am J Hum Genet.* ***48***, 696-703.

FERRAIOLO, B.L., CRONIN, M., BAKHIT, C., ROTH, M. CHESTNUT, M., LYON, R. 1989. The pharmacokinetics and pharmacodynamics of a human relaxin in the mouse symphysis bioassay. *Endocrinology* ***125***, 2922-6.

FERREIRA, J. M. C., CALDINI, E.G., MONTES, G.S. 1987. Distribution of elastic system fibers in the peripheral nerves of mammals. *Acta Anat.* ***130***,168-73.

FRIEDEN, E.H., HISAW, 1953.The biochemistry of relaxin. *Recent Prog. Horm. Res.* ***8***, 333-78.

FUKUDA, Y, FERRANS, V.J., CRYSTAL, R.G. 1984. Development of elastic fibers of nuchal ligament, aorta, and lung of fetal and postnatal sheep,an ultrastrucutral and electron microscopic immunohistochemical study. *Am. J. Anat.* ***170***, 597-629.

FULLMER, H.M. & LILLE, R.D. 1958. The oxytalan fiber, a previously underscribed connective tissue fibers. . *J. Histochem. Cytochem.* ***6***, 425-30.

FULLMER, H.M. 1960. A comparative histochemical study of elastic, pre-elastic and oxytalan connective tissue fibers. *J. Histochem. Cytochem.* ***8***, 290-5.

FULLMER, H.M., SHEETZ, J.H., NARKATES, A.J. 1974. Oxytalan connective tissue fibres, a review. *J. Oral. Pathol.* ***3***, 291-316.

GAMBLE, J.G, SIMMONS, S.C., FREEDMAN, M. 1986. The symphysis pubis, anatomic and pathologic considerations. *Clinical Orthopaedics and Related Research* ***203,*** 261-72.

GARDNER, W.U. 1936. Sexual dimorphism of the pelvis of the mouse, the effect of estrogenic hormones upon the pelvis and upon the development of scrotal hernias. *Am. J. Anat.* ***59***, 459-483.

GAWLIK, Z. 1965. Morphological and morphochemical properties of the elastic system in the motor organ of the man. *Folia Histochem. Cytochem.* **3**,233-251.

GHADIALLY, F.N. Elastic fibres, elaunin fibres and oxytalan fibres. In. *Ultrastructural pathology of the cell and matrix.* London, Butterworths, 1982. p. 908-15.

GHADIALLY, F.N., LALONDE, J.-M.A., WEDGE, J.H. 1983. Ultrastructure of normal and torm menisci of the human knee joint. *J. Anat.* ***136***, 773-91.

GIBSON, M.A., CLEARY, E.G. 1987. The immunohistochemical localisation of microfibril-associated glycoprotein (MAGP) in elastic and non-elastic tissues. *Immunol Cell Biol.* ***65***, 345-56.

GIBSON, M.A., SANDBERG,L.B., GROSSO, L.E., CLEARY, E.G 1991. Complementary DNA cloning establishes microfibril-associated glycoprotein (MAGP) to be a discrete component of the elastin-associated microfibrils. *J Biol Chem.* ***266***, 7596-601.

GOLICHOWSKI, A.M., KING, S.R., MASCARO, K. 1980. Pregnancy-related changes in rat cervical glycosaminoglycans. *Biochem. J.* ***192***, 1-8.

GOSLINE, J.M. 1978. Hydrophobic interaction and a model for the elasticity of elastin. *Biopolymers*. **17**, 677-95.

GREENLEE, T.K., ROSS, R., HARTMAN, J.C. 1966. The fine structure of elastic fibers. *J.Cell Biol.* ***30,*** 59-71.

GUNJA-SMITH, Z., LIN, J., WOESSNER JR., J.F. 1989. Changes in desmosine and Pyridinoline crosslinks during rapid synthesis and degradation of elastin and collagen in the rat uterus. *Matrix* ***9***, 21-27.

HALL, K. 1947. The effects of pregnancy and relaxin on the histology of the pubic symphysis in the mouse. *J. Endocrinol.* ***5***, 174-82.

HALL, K. J. Endocrinol. **11**, 210.

HAM, A.W. Histologia. 6 ed. Ed. Guanabara Koogan. Rio de Janeiro 1972.

HANDFORD, P.A., DOWNING, A.K., REINHARDT, D.P., SAKAI, L.Y. 2000. Fribrillin: from domain structure to supramolecular assembly. *Matrix Biology* ***19***, 457-470.

HART, D. A, SCIORE, P., BOYKIW, R., RENO, C. 1998. Pregnancy induces changes in the pattern of mRNA expression in knee ligaments of the adolescent rabbit. *Matrix Biology* ***17,*** 21-3.

HESS, I. 1987. The occurrence of elastic system fibers in the matrix of normal articular cartilage. *Cell Tissue Res.* ***248,*** 589-593.

HINEK, A., RABINOVITCH, M. 1994. 67-kD elastin-binding protein is a protective "companion" of extracellular insoluble elastin and intracellular tropoelastin.*J Cell Biol.* ***126***, 563-74.

HINEK, A. 1997. Elastin receptor and cell-matrix interactions in heart transplant-associated arteriosclerosis. .*Arch Immunol Ther Exp (Warsz).* ***45***, 15-29.

HORN, E.H. 1960. Interpubic ligament regression in relaxin-treated virgin and primiparous, post-partum mice. *Endocrinology* ***67***, 668-673.

HORRIGAN, S.K., RICH, C.B., STREETEN, B.W., LI, Z.Y. FOSTER, J.A. 1992. Characterization of an associated microfibril protein through recombinant DNA techniques. *J Biol Chem.* ***267***, 10087-95.

JUNQUEIRA, L.C.U., ZUGAIB, M., MONTES, G.S., TOLEDO, O.M.S., KRISZTAN, R.M., SHIGIHARA, K.M. 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***, 273-81.

KAGAN, H.M., VACCARO,C.A., BRONSON, R.E., TANG, S.S., BRODY, J.S. . 1986 Ultrastructural immunolocalization of lysyl oxidase in vascular connective tissue.*J Cell Biol.* ***103***, 1121-8.

KIELTY, C.M., BERRY, L., WHITTAKER, S.P., GRANT, M.E., SHUTTLEWORTH, C.A. 1993. Microfibrillar assemblies of foetal bovine skin. Developmental expression and relative abundance of type VI collagen and fibrillin. *Matrix*. ***13***, 103-12.

KOBAYASHI, R., TASHIMA, Y., MASUDA, H., SHOZAWA, T., NUMATA, Y., MIVAUCHI, K. 1989. Isolation and characterization of a new 36-kDa microfibril-associated glycoprotein from porcine aorta. *J Biol Chem.* ***264***, 17437-44.

KOO, H.P., MACARAK, E.J., CHANG, S.L., ROSENBLOOM,J., HOWARD, P.S. 1998. Temporal expression of elastic fiber components in bladder development. *Connect Tissue Res.****37***, 1-11.

KROC, R.L., STEINETZ, B.G., BEACH, V.L. 1958. The effects of estrogens, progestagens, and relaxin in pregnant and nonpregnant laboratorory rodents. *Ann. N. Y. Acad. Sci.* ***75***, 942-80.

KUMARATILAKE, J.S., GIBSON, M.A., FANNING, J.C., CLEARY, E.G. 1989. The tissue distribution of microfibrils reacting with a monospecific antibody to MAGP, the major glycoprotein antigen of elastin-associated microfibrils.*Eur J Cell Biol.* ***50***, 117-27.

LEE, B., GODFREY, M., VITALE, E., HORI, H., MATTEI, M.G., SAFARAZI, M., TSIPOURAS, P., RAMIREZ, F., HOLLISTER, D.W. 1991. Linkage of Marfan syndrome and a phenotypically related disorder to two different fibrillin genes. *Nature*. ***352***, 330-4.

LEICK-MALDONADO, E.A, LEMOS, M., TIBÉRIO, I.F.L.C., CALDINI, E.G., MONTES, G.S., MARTINS, M.A , SALDIVA, P.H.N. 1997. Differential distribution of elastic system fibers in control and bronchoconstricted intraparenchymatous airways in the guinea-pig lung. *J. Submicrosc. Cytol. Pathol.* ***29,*** 427-434.

LEMOS, M., POZO, R.M.K., MONTES, G.S., SALDIVA, P.H.N. 1997. Organization of collagen and elastic fibers studied in stretch preparations of whole mounts of human visceral pleura. *Annals of Anatomy,* ***179,*** 447-452.

LEPPERT, P.C., YU, S.Y. 1991. Three-dimensional structures of uterine elastic fibers: scanning electron microscopic studies. *Connect Tissue Res.* ***27***, 15-31.

LUQUE, E. H., MONTES., G.S. 1989. Progesterone promotes a massive infiltration of the rat uterine cervix by the eosinophilic polimorphonuclear leukocytes. *Anat. Rec.* ***223***, 257-65.

MARIENCHECK, M.C., DAVIS, E.C., ZHANG, H., RAMIREZ, F., ROSEMBLOOM, J., GIBSON, M.A., PARKS, W.C., MECHAM, R. 1995. Fribrillin-1 and fibrillin-2 show temporal and tissue-specific regulation of expression in developing elastic tissues. *Connect. Tissue Res.* ***31***, 87-97.

McDONALD, J.K., SCHWABE, C. 1982. Relaxin-induced elevations of catepsin B and dipeptidyl peptydase I in the mouse pubic symphysis, with localization by fluorecence enzyme hsitochemistry. *Ann N. Y. Acad. Sci.* ***380***, 178-86.

MECHAN, R.P., FOSTER, J.A. 1978. A structural model for desmosine cross-linked peptides. *Biochem J.* ***173***, 617-25.

MECHAN, R.P. 1981. ELastin biosynthesis: a look at the current scene. *Connect Tissue Res.* ***8***, 155-60.

MECHAM, R.P. 1991. Elastin synthesis and fiber assembly.*Ann N Y Acad Sci.*. ***624***,137-46.

MECHAM, R.P. & HEUSER, J.E. The elastic fiber In, HAY, E.D. *Cell Biology of Extracelular Matrix*. New York. Plenum Press. 1991. p.79-101

MECHAM, R.P., DAVIS, E.C. 1994. Elastic fiber structure and assembly. In Yurchenco, P.D., Birk, D.E., Mecham, R.P. (Eds), Extracellular Matrix Assembly and Structure, Academic Press, San Diego. pp. 281-314.

MONTES, G.S. 1992. Distribution of oxytalan, elaunin and elastic fibres in tissues. *Cienc. Cult.* ***44***, 224-33.

MONTES, G.S. 1996. Structural biology of the fibres of the collagenous and elastic systems. *Cell Biology Int.* ***20***,15-27.

OLLIVER, L., LUVALLE, P.A., DAVIDSON, J.M., ROSENBLOOM, J., MATHEW, C.G., BESTER, A.J., BOYD, C.D. 1987. The gene coding for tropoelastin is represented as a single copy sequence in the haploid sheep genome. *Coll Relat Res.* ***7***, 77-89.

PANIAGUA, R., REGADEIRA, J., NISTAL, M., SANTAMARIA, L. 1983. Elastic fibres of the human ductus deferens. *J. Anat.* ***137,*** 467-476.

PARRY, D.A.D., CRAIG, A.S. Collagen fibrils during developmemt an maturation and their contribution to the mechanical attributes of connective tissue. In, *Collagen*. V. II. Ed.,NIMNI, M.E., Boca Ratón, CRC Press, 1988, p. 1-23.

PARTRIDGE, S.M. 1962. Elastin. *Adv. Prot. Chem.* ***17,*** 227-302.

PASQUALI-RONCHETTI, I., BACCARANI-CONTRI, M. 1997. Elastic fiber during development and aging. *Microsc. Res. Tech.* ***38***, 428-35.

PINHEIRO, M.C. Tese de mestrado, Biologia estrutural das fibras de colágeno da sínfise e do ligamento pubianos de camundongos durante a prenhez, parto e pós-parto. Campinas, SP, 1998.

RAMIREZ, F., PEREIRA, L. 1999. The fibrillins. *Int J Biochem Cell Biol.* ***31***, 255-9.

ROSENBLOOM, J., ABRAMS, W.R., MECHAM. R. 1993. Extracellular matrix 4, the elastic fiber. *FASEB J.* ***7***, 1208-18.

ROSS, R., BORNSTEIN, P. 1969. The elastic fiber. I. The separation and partial characterization of its macromolecular components. *J. Cell Biol.* ***40***, 366-381.

ROSS, R. 1973. The elastic fiber. A review. *J. Histochem. Cytochem.* **21**, 199-208.

RUTH, E.B. 1937. Metamorphosis of the pubic symphysis. III. Histological changes in the symphysis of the pregnant guinea pig. *Anat. Rec.* ***67***, 409-421.

SAKAI, L.Y., KEENE, D.R., ENGVALL, E. 1986. Fibrillin, a new 350-kD glycoprotein, is a component of extracellular microfibrils. *J Cell Biol. Dec* ***103***, 2499-509.

SAMUEL, C.S., COGHLAN, J.P., BATEMAN, J.F. 1998. Effects of relaxin, pregnancy and parturition on collagen metabolism in the rat pubic symphysis. *J. Endocrinol* ***159***, 117-25.

SANDBERG, L.B., SOSKEL, N., LESLIE, J.G. 1981. Elastin structure, byosinthesis and relation to disease state. *New Engl. J. Med.* ***304***, 566-79.

SCHWABE, C., STEINETZ, B., WEISS, G., SEGALOFF, A., McDONALD, J.K., O'BYRNE, E., HOCHMAN, J., CARRIERE, B., GOLDSMITH, L. 1978. Relaxin. *Recent. Prog. Horm. Res.* ***34***,123-211.

SEAR, C.H.J., GRANT, M.E., JACKSON, D.S. 1981. The nature of microfibrillar glycoproteins of elastic fibers. A biosynthetic study. *Biomech. J.* ***194,*** 587-598.

SHAPIRO, S.D., CAMPBELL, E.J., WELGUS, H.G., SENIOR, R.M. 1991. Elastin degradation by mononuclear phagocytes. *Ann N Y Acad Sci.* ***624,*** 69-80.

SHERWOOD, O. D. Relaxin. En, Knobil E, Neill j. d. (eds). The physiology of reproduction. Raven Press, New York, 1994.

SOUZA,A.D., MONTES, G.S., VILLELA, B.T.X., CRUZ, A C., COTTA-PEREIRA, G. 1991. Elastic-related fibers in the annular ligament of the stapes-oval window articulation. Na electron microscopic and histochemical study in the rat and the rabbit. *Rev. Larynggol.* ***112,*** 7-10.

STARCHER, B., PERCIVAL, S. 1985. Elastin turnover in the rat uterus. *Conn. Tiss. Res.* ***13***, 207-15.

STEINETZ, B.G., BEACH, V.L., KROC, R.L. 1957. The influence of progesterone, relaxin and estrogen on some structural and functional changes in the pre-parturient mouse. *Endocrinology* ***61***, 271-280.

STEINETZ, B.G., MANNING, J.P., BUTLER M., BEACH, V.L. 1965. Relationships of growth hormone, steroids and relaxin in the transformation of pubic joint cartilage to ligament in hypophysectomized mice. *Endocrinology* ***76***, 876-882.

STEINETZ, B.G., O'BYRNE, E.M. GOLDSMITH, L.T., ANDERSON, M.B. 1988. The source of relaxin in pregnant syrian hamster. *Endocrinology* ***122***, 795-98.

STONE, P.J., FRANZBLAU, C., KAGAN, H.M. 1982. Proteolysis of insoluble elastin. *Methods Enzymol.* ***82***, 588-605.

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.

STREETEN, B.W., LICARI, P.A. 1983. The zonules and the elastic microfibrillar system in the ciliary body. *Invest. Ophtalmol. Vis. Sci.* ***24***, 667-681.

TAKAGI, M., KAZAMA, T., SHIMADA, K., HOSOKAWA, Y., HISHIKAWA, H. 1989. Differential distribution and ultrastructural staining of oxytalan and elastic fibers in the periodontal ligament of Alligator mississippiensis. *Anat Rec.* ***225***, 279-87.

TALMAGE, R.V. 1947_a. Changes produced in the symphyseal pubis of the guinea pig by the sex steroids and relaxin. *Anat. Record* ***99***, 91-113.

TALMAGE, R.V. 1947_b. A histological study of the effects of relaxin on the symphysis pubis of the guinea pig. *J. Exp. Zool.* ***106***, 281-92.

VASILENKO, P., MEAD, J.P., WEIDMANN, J.E. 1986. Uterine growth-promoting effects of relaxin, a morphometric and histological analysis. *Biol. Reprod.* ***35***, 988-995.

VASILENKO, P. & MEAD, J.P. 1987. Growth-promoting effects of relaxin and related compositional changes in the uterus, cervix, and vagina of the rat. *Endocrinology* ***120***, 1370-6.

VERHOEFF, F.H. 1908. Some new staining methods of wide applicability. Including a rapid and differential stain for elastic tissue. *J. Am. Med. Ass.* ***50***, 876-7.

VIELL, B., STRUCK, H. 1987. Effects of the hormone relaxin on teh matabolism of glycosaminoglycans in the mouse symphysis pubis. *Horm. Metabol. Res.* ***19***, 415-18.

VIX, V.A., RYU, C.Y. 1971. The adult symphysis pubis, normal and abnormal. *Am J Roentgenol Radium Ther Nucl Med.* ***112***, 517-25.

VRHOVSKI, B., WEISS, A.S. 1998. Biochemistry of tropoelastin. *Eur J Biochem.* ***258***, 1-18.

WAHL L.M., BLANDAU, R.J., PAGE, R.C. 1977. Effects of hormones on collagen metabolism and collagenase activity in the pubic symphysis ligament of the guinea pig. *Endocrinology* ***100***, 571-9.

WEIGERT, C. 1898. Über eine Method zur Färbung elasticher Fasern. *Zentbl.Allg. Pathol. Pathol. Anat.* ,***9***, 289-92.

WEIS-FOGH, T., ANDERSON, S.O. 1970. New molecular model for the long-range elasticity of elastin. *Nature* ***227***, 718-21.

YANG, S., REMBIESA, B., BÜLLESBACH, E.E., SCHWABE, C. 1992. Relaxin receptors in mice, Demonstration of ligand binding in symphyseal tissue and uterine membrane fragments. *Endocrinology* ***130***, 179-85.