UNIVERSIDADE ESTADUAL PAULISTA
FACULDADE DE MEDICINA VETERINÁRIA E ZOOTECNIA

ANORMALIDADES ULTRASSONOGRÁFICAS E RADIOGRÁFICAS EM JOELHOS DE CAVALOS DA RAÇA QUARTO DE MILHA DA LINHAGEM DE APARTAÇÃO

LUIS EMILIANO CISNEROS ÁLVAREZ

BOTUCATU - SP
Julho, 2013

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LUIS EMILIANO CISNEROS ÁLVAREZ

Tese apresentada junto ao Programa de Pós-Graduação em Medicina Veterinária para obtenção do título de Doutor.

Orientador: Prof. Dr. Luiz Carlos Vulcano

BOTUCATU - SP
Julho, 2013

FICHA CATALOGRÁFICA ELABORADA PELA SEÇÃO DE AQUIS. E TRAT. DA INFORMAÇÃO
DIVISÃO TÉCNICA DE BIBLIOTECA E DOCUMENTAÇÃO - CAMPUS DE BOTUCATU - UNESP
BIBLIOTECÁRIA RESPONSÁVEL: ***ROSEMEIRE APARECIDA VICENTE***

Cisneros Álvarez, Luis Emiliano.
Anormalidades ultrassonográficas e radiográficas em joelhos de cavalos da raça Quarto de Milha da linhagem de apartação / Luis Emiliano Cisneros Álvarez. – Botucatu, 2013

Tese (doutorado) - Universidade Estadual Paulista, Faculdade de Medicina Veterinária e Zootecnia
Orientador: Luiz Carlos Vulcano
Capes: 50501038

1. Equino. 2. Claudicação em equino. 3. Joelhos – Ferimentos e lesões. 4. Radiografia veterinária. 5. Radiologia veterinária. 6. Ultrassonografia.

Palavras-chave: Claudicação; Equino; Joelho; Radiografia; Ultrassom.

Nome do Autor: Luis Emiliano Cisneros Álvarez

Título: Anormalidades ultrassonográficas e radiográficas em joelhos de cavalos da raça Quarto de Milha da linhagem de apartação.

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Data da defesa: 22 de Julho de 2013.

A mi mamá y a mi hermano

In Lak'ech Hala Ken

Agradecimentos:

Ao Professor Luiz Carlos Vulcano pela oportunidade de trabalhar neste projeto.

À Professora Ana Liz Garcia Alves e ao Professor Carlos Alberto Hussni por abrir as portas dos seus grupos de pesquisa.

Ao Professor Roberto Calderon Gonçalves sem cujo apoio solidário e logístico este projeto simplesmente não teria sido consolidado.

Ao Professor Alexandre Secorun Borges: sem palavras.

Ao meu amigo Peres Ramos Badial por sempre trabalhar junto e por não me deixar desistir.

Aos amigos e colegas Dietrich Pizzigatti, Luiz Henrique Lima de Mattos e Murilo Charlier pelo seu trabalho árduo, sempre realizado com um bom sorriso.

Ao Médico Veterinário Marcelo Pyles pela amizade, boa fé e por permitir incluir os seus casos no meu estudo.

Aos Médicos Veterinários Giovani Olivo e Leandro Américo Rafael pela amizade e pelo seu apoio para poder viajar até os haras e obter as imagens necessárias para a realização do estudo.

Ao Médico Veterinário e amigo, sempre companheiro no trabalho, Armando de Mattos Carvalho.

Ao pessoal do que foi a minha verdadeira casa durante três anos: a biblioteca. Marluci, Enilze, Luciana, Rosemary, Diva, Rosemeire, Márcia, Tais, Dino, Quim, Sulamita, Regina, Silene, Márcia, Bruna, Darcila, Marlene, Rosangela, Nivaldete, Luzia, Sayuri, Márcia Cristina e Élida.

Ao Heraldo André Catalan Rosa pela sua sempre expedita colaboração, não importando o horário.

Ao Didier e ao César! Pela força e pela ajuda constante, que foi muito importante na reta final.

Ao Professor José Pantoja pela mente aberta.

Aos Senhores criadores Haroldo Pessoa de Araújo Sobrinho e Francisco Carlos Taboga por ter aberto as portas das suas casas e me permitir trabalhar com os seus cavalos.

À CAPES pela concessão da bolsa de doutorado através do programa PEC/PG.

Aos cavalos.

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RESUMO

A apartação é uma das competições mais populares na qual o cavalo Quarto de Milha é utilizado. Embora as lesões nas articulações femorotibial e femoropatelar sejam frequentes nessa modalidade esportiva, não foram publicados trabalhos que relatem os achados ultrassonográficos e radiográficos em animais submetidos a treinamento intensivo ou competição. Assim, o objetivo deste trabalho foi investigar a ocorrência de anormalidades ultrassonográficas e radiográficas nas articulações femorotibial e femoropatelar em cavalos de apartação da raça Quarto de Milha submetidos a treinamento intensivo. Foram realizados exames ultrassonográficos, radiográficos e testes de flexão bilaterais dos joelhos de cavalos Quarto de Milha de apartação sem claudicação aparente para o treinador, com idades entre 38-47 meses (grupo treinamento, TG) e maiores do que 47 meses (grupo competição, CG). As anormalidades ultrassonográficas, radiográficas e presença de claudicação antes e após teste de flexão foram categorizadas e as frequências calculadas e comparadas entre os grupos. Foram identificadas anormalidades ultrassonográficas em 100% dos animais avaliados, sendo a sinovite a entidade nosológica mais comum (89,3%, dos animais avaliados), seguida pela rarefação de menisco medial (64,3%) e desmite de ligamento colateral medial (33,9%). Nas radiografias foram identificadas anormalidades em 59,5% dos animais, sendo os animais jovens os que apresentaram maior ocorrência de lesões ($P<0,05$). Houve também diferença significativa entre o número total de animais com claudicação antes e após o teste de flexão. Não foi detectada associação entre o tipo de lesões nos meniscos e o grau de claudicação. Conclui-se que existe uma proporção alta de anormalidades ultrassonográficas e radiográficas nos joelhos de cavalos Quarto de Milha de apartação, sendo a ocorrência de achados radiográficos maior entre os animais mais jovens. Ao mesmo tempo, foi identificado que uma quantidade significativa de animais realiza os treinamentos tolerando algum grau de dor musculoesquelética.

Palavras chave: Quarto de Milha; apartação; ultrassonografia; radiografia; claudicação; joelho.

CISNEROS, L. E. **Ultrasonographic and radiographic abnormalities in the stifle joint of cutting quarter horses**. Botucatu, 2013, 101 p. Tese (Doutorado) - Faculdade de Medicina Veterinária e Zootecnia, Campus de Botucatu, Universidade Estadual Paulista.

ABSTRACT

Cutting is one of the most popular uses of the Quarter Horse. Although lesions to the femorotibial and femoropatellar joints are common in this sport, no studies have been published on the ultrasonographic and radiographic findings in cutting Quarter Horses. The aim of this study was to investigate the occurrence of ultrasonographic and radiographic changes in the stifles of cutting horses, either training or competing. Ultrasonographic, radiographic and flexion test examinations were performed in the stifles of cutting horses, aging 38-47 months (training group, TG) and older (competition group, CG). Lameness should not have been realized by the horse's trainer/owner. Ultrasonographic, radiographic and lameness findings after and before flexion test were categorized and their frequencies compared between groups. Ultrasonographic changes were identified in 100% of the horses. The most frequent finding was synovitis (89.3% of the animals), followed by rarefaction of the medial meniscus (64.3%) and desmitis of the medial collateral ligament (33.9%). Radiographic changes were identified in 59.5% of the animals, with the younger horses suffering from a higher number of abnormalities ($P<0.05$). There was also a significant difference between the overall number of lame horses before and after flexion test. Association between type of meniscal lesion and degree of lameness was not observed. We concluded that there is a high proportion of ultrasonographic and radiographic changes in the stifle of cutting Quarter Horses. Likewise, radiographic changes are most commonly found among younger horses. A considerable number of cutting horses perform while tolerating some degree of musculoskeletal pain.

Key words: Quarter Horse; cutting; ultrasound; radiograph; lameness; stifle.

CAPÍTULO 1

1. INTRODUÇÃO

Estudos da evolução dos equinos têm sinalado o alto grau de adaptação cursorial destes animais, que é representado pelo deslocamento proximal dos feixes musculares dos membros e a diminuição de tamanho dos músculos distais (HILDEBRAND, M., 1987) acompanhada do alongamento relativo e fusão dos elementos ósseos (SIMPSON, G. G., 1951; SMITH, J. M. e SAVAGE, R. J. G., 1956; HILDEBRAND, M., 1960). Devido a essas características, a musculatura do cavalo, está constituída para gerar movimentos rápidos e amplos em um plano cranial e caudal (HILDEBRAND, M., 1987).

Ao mesmo tempo, esses animais são únicos na sua característica de permanecer em pé durante períodos de tempo que vão de 20 a 23 horas por dia (BOYD, L. E. et al., 1988). Assim, o membro pélvico do *Equus ferus caballus* se distingue da maioria dos animais domésticos pela presença de um mecanismo de trava composto por ossos, músculos e ligamentos que facilitam ao animal a permanência de longos períodos em estação com mínima atividade muscular e baixo consumo de energia (HERMANSON, J. W. e MACFADDEN, B. J., 1996). Este mecanismo, chamado aparelho de sustentação, está intimamente relacionado com o complexo articular do joelho[1].

A tendência natural da articulação do joelho quando o animal está em estação é à flexão, que é impedida ativamente pela força muscular do grupo quadríceps femoral e passivamente pelo aparelho de sustentação. No membro pélvico, este aparelho está formado pelos ligamentos patelares, a superfície patelar medial e a tróclea medial do fêmur, que é significativamente maior que a tróclea lateral, nos equinos modernos[2] (HERMANSON, J. W. e MACFADDEN, B. J., 1996).

Os membros dos equinos sofreram adaptações que o possibilitaram a manter o equilíbrio entre a força de contração e o baixo peso relativo das massas musculares, bem como a poupar energia muscular. Entretanto, estas vantagens têm custo: as extremidades do cavalo, altamente adaptadas para se movimentar cranial e caudalmente, são menos eficiente para estabilizar as articulações quando se realizam movimentos em planos diferentes, por exemplo abdução e adução (HILDEBRAND, M., 1987). Desse

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1 O joelho no equino está constituído pela articulação femorotibial e a articulação femoropatelar. Para efeitos práticos, este complexo articular será denominado simplesmente como "joelho" no restante do texto exceto quando seja necessária alguma descrição particular referente aos compartimentos articulares específicos.

2 O *Protohippus* é hoje considerado como o primeiro clado equino em apresentar aumento nas dimensões da crista medial da tróclea femoral a partir de espécimes datados de 12 milhões de anos atrás (HERMANSON, J. W. e MACFADDEN, B. J., 1996). Um clado ou clade (do grego klados, ramo) é um grupo de organismos originados de um único ancestral comum exclusivo.

modo, quando interferências humanas impõem ao cavalo tarefas que divergem da sua dinâmica natural, eles podem sofrer lesão gradual ou súbita (HILDEBRAND, M., 1987) o que, por outro lado, tem como consequência a redução temporária ou permanente da função locomotora (WALMSLEY, J. P. et al., 2003).

O desenvolvimento da raça Quarto de Milha (QM) data do século XV, quando os colonizadores anglo-americanos cruzaram cavalos ingleses com cavalos nativos e ferais. A população equina americana consistia de exemplares descendentes de cavalos das raças Andaluz, Árabe e Berbere, trazidos à América do Norte pelos conquistadores espanhóis. O cruzamento do Puro Sangue Inglês (PSI) com esses exemplares, trousse como resultados indivíduos relativamente pequenos, resistentes e rápidos. O nome da raça deriva do fato de que estes animais são particularmente rápidos em corridas lineares em distâncias de até um quarto de milha (aproximadamente 402 metros) (SCOTT, M., 2008).

Existem alguns fenótipos raciais no QM, dependendo da atividade para a qual são utilizados. Assim, semelhante ao PSI, os animais QM de corrida e conformação são geralmente mais altos e de musculatura mais alongada, enquanto que os exemplares utilizados para trabalho com gado são geralmente mais compactos e musculosos (SCOTT, M., 2008). É importante salientar que a raça QM é a mais utilizada tanto para os esportes de rodeio como para as atividades e esportes de lida de gado incluindo rédeas e apartação (BLACK, J. B., 1999; SCOTT, M., 2008). Neste sentido, os cavalos QM de lida de gado (menores e geralmente mais jovens que os cavalos de rodeio) tendem a ser afetados por lesões agudas, condições subagudas e doenças do desenvolvimento (SCOTT, M., 2008).

Dentre as atividades de lida de gado, a modalidade de apartação consiste em um exercício onde cavalo e cavaleiro separam um bovino do resto do rebanho e o mantem isolado, utilizando para isto paradas súbitas e corridas curtas (SCOTT, M., 2008). Este esporte utiliza primariamente animais das raças QM e Paint Horse, de linhagens selecionadas pela sua capacidade de atuar sob pressão, com baixa estatura relativa (142-152 centímetros) e com agilidade para realizar paradas súbitas e movimentos de pivô de alto torque (JACKMAN, B. R., 2001). O treinamento da maioria destes animais começa entre o final do primeiro ano e início do segundo ano de idade, objetivando sua estreia em competições oficiais ("Potro do Futuro" ou "Futurity") dentro do outono ou inverno boreal do seu terceiro ano de idade (JACKMAN, B. R., 2001; SCOTT, M., 2008).

Cavalos pequenos e jovens com baixa densidade óssea e de massa nos membros são mais propensos a sofrer lesões (SCOTT, M., 2008). Na apartação então, observa-se a somatória de três fatores que são considerados predisponentes para lesões locomotoras: (1) utilização de animais jovens (2) realização de treinos extenuantes (3) realização de exercícios que fogem da dinâmica natural dos membros dos equinos, tais como paradas súbitas e viradas de alto torque que exercem forças rotacionais e colocam estresse sobre as articulações dos membros pélvicos, incluindo os tarsos e os joelhos (SCOTT, M., 2008; CONTINO, E. K. et al., 2012).

Tem sido afirmado que a maioria de casos de queda no desempenho atlético em cavalos de apartação é provocada por claudicação associada à articulação tarsometatarsiana (JACKMAN, B. R., 2001). Assim mesmo, existe um estudo mostrando a prevalência de achados radiográficos em animais de um e dois anos destinados à prova de apartação (CONTINO, E. K. et al., 2012). Porém, não existem trabalhos descrevendo os achados ultrassonográficos nestes animais após períodos de treinamento intensivo com o objetivo de serem apresentados pela primeira vez na competição do "Potro do Futuro".

É nossa hipótese que os animais adultos-jovens (até 47 meses) treinados intensamente para apartação, têm alta ocorrência de lesões que não provocam claudicação ou queda no desempenho e que esta ocorrência é maior que nos animais mais velhos (maiores que 48 meses), realizando o mesmo treinamento. Assim, o objetivo deste trabalho é investigar a frequência de anormalidades ultrassonográficas e radiográficas nas articulações femorotibial e femoropatelar em cavalos de apartação antes e após a estreia no “Potro do Futuro”. Especificamente objetiva-se: (1) determinar e comparar a frequência de alterações ultrassonográficas e radiográficas em dois grupos de animais com distinta faixa etária: cavalos entre 38-47 meses e cavalos com idade acima de 47 meses, ambos os grupos sob regimes de treinamento semelhantes; (2) classificar o tipo e número de alterações nos meniscos; e (3) associar as lesões nos meniscos com a presença de claudicação.

2. REVISÃO DE LITERATURA

2.1. Anatomia do joelho de equino

O joelho[3] nos equinos (*articulatio genus*) é composto pelas articulações femoropatelar (*articulatio femoropatellaris*), entre o fêmur e a patela, e a femorotibial (*articulatio femorotibialis*), entre o fêmur e a tíbia. Este complexo é classificado como articulação composta, incongruente e em gínglimo (GOUGH, M. R. et al., 2002; SCHALLER, O. e CONSTANTINESCU, G. M., 2007; GETTY, R., 2008; KÖNIG, H. E. e LIEBICH, H. G., 2009).

2.1.1. Articulação femoropatelar (AFP)

Esta articulação é a mais extensa do corpo dos equinos. Suas superfícies de contato são a tróclea do fêmur e a face articular da patela (SCHALLER, O. e CONSTANTINESCU, G. M., 2007). A tróclea é composta por duas cristas, com um sulco separando-as, e está revestida por cartilagem articular. A crista medial é maior que a lateral, especialmente em sua parte proximal (LATIMER, F. G. et al., 2001; SCHALLER, O. e CONSTANTINESCU, G. M., 2007). A superfície articular da patela prolonga-se medialmente através de uma placa suplementar de fibrocartilagem (fibrocartilagem parapatelar), a qual se curva e entra em contato com a face proximal da crista medial da tróclea. Um segmento estreito desta cartilagem também está presente ao longo da borda patelar lateral (GETTY, R., 2008).

A cápsula articular femoropatelar insere-se na margem articular da patela. No fêmur, a cápsula é observada a uma distância da cartilagem articular que varia de 2,5 cm na face proximomedial a 1 cm na face lateral (GETTY, R., 2008). Na extremidade proximal forma um saco sobre o quadríceps, o qual está separado do músculo por uma fina camada de gordura. Cranialmente, a cápsula está afastada dos ligamentos patelares pelo corpo adiposo infrapatelar (*corpus adiposum infrapatellare*) e distalmente está em contato com a cápsula femorotibial (GETTY, R., 2008) (Figura 1). A AFP comunica-se com a parte medial da articulação femorotibial em aproximadamente 65% dos cavalos através de uma fenda na parte mais distal da crista medial da tróclea femoral. Uma fenda adicional contígua à margem distal da crista lateral comunica a AFP com a porção

[3] Nomenclatura segundo a Nomina Anatomica Veterinaria (*Nomina Anatomica Veterinaria* 2012)

lateral da AFT em aproximadamente 17,5% dos indivíduos (REEVES, M. J. et al., 1991).

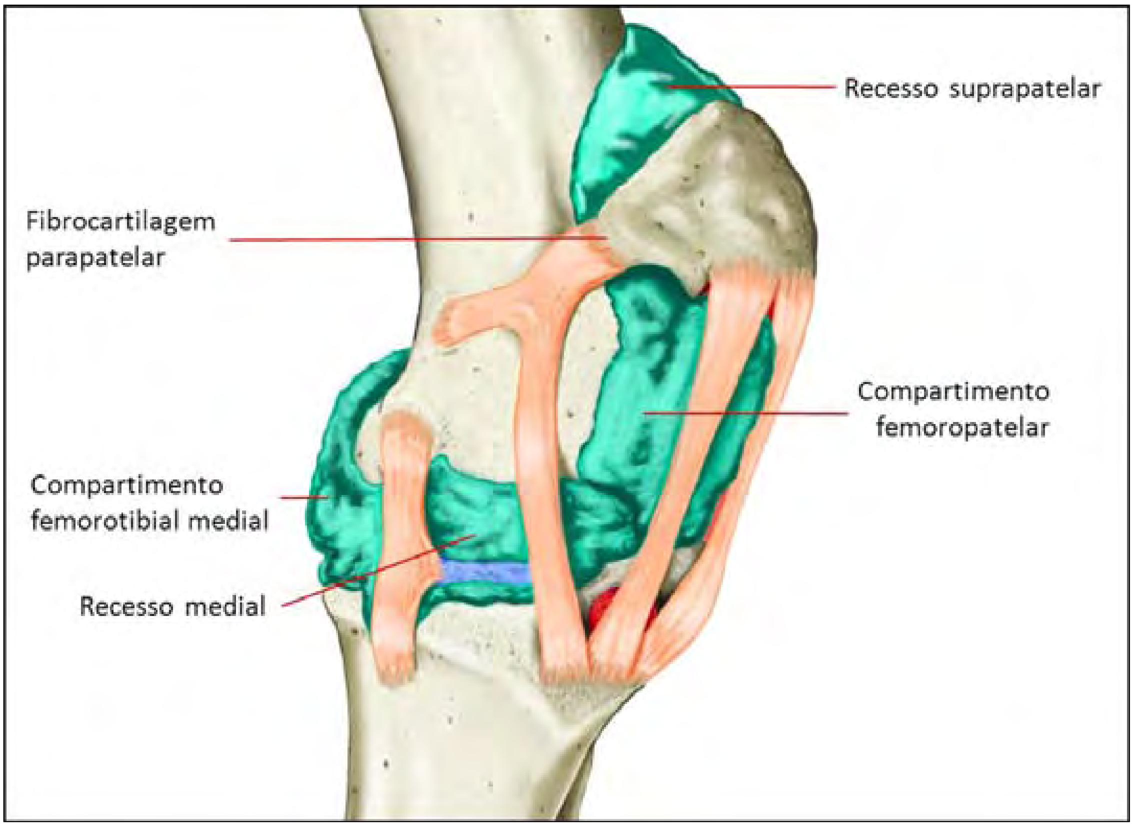

FIGURA 1. Imagem esquemática da face medial do joelho equino com as cápsulas articulares. Modificada de Köning, H. E. e Liebich, H. G., 2009.

2.1.1.1. Ligamentos patelares e femoropatelares

O ligamento patelar medial (LPM) origina-se na fibrocartilagem parapatelar e está unido ao músculo vasto medial. Distalmente, o LPM se insere na face medial da tuberosidade tibial e sua margem caudal é confluente com a aponeurose comum dos músculos grácil e sartório (KÖNIG, H. E. e LIEBICH, H. G., 2009). O ligamento patelar intermédio (LPI) tem origem na superfície craniodistal (ápice) da patela e corre através do sulco troclear inserindo-se, craniodistalmente, na tuberosidade da tíbia (KÖNIG, H. E. e LIEBICH, H. G., 2009). Já o ligamento patelar lateral (LPL) tem origem na face craniolateral da patela e se insere na margem lateral da tuberosidade tibial. O LPL compartilha fibras com o tendão de inserção do músculo bíceps femoral

(KÖNIG, H. E. e LIEBICH, H. G., 2009) e com a aponeurose do músculo tensor da fáscia lata (BUDRAS, K. D. et al., 2011)[4] (Figuras 2 e 3).

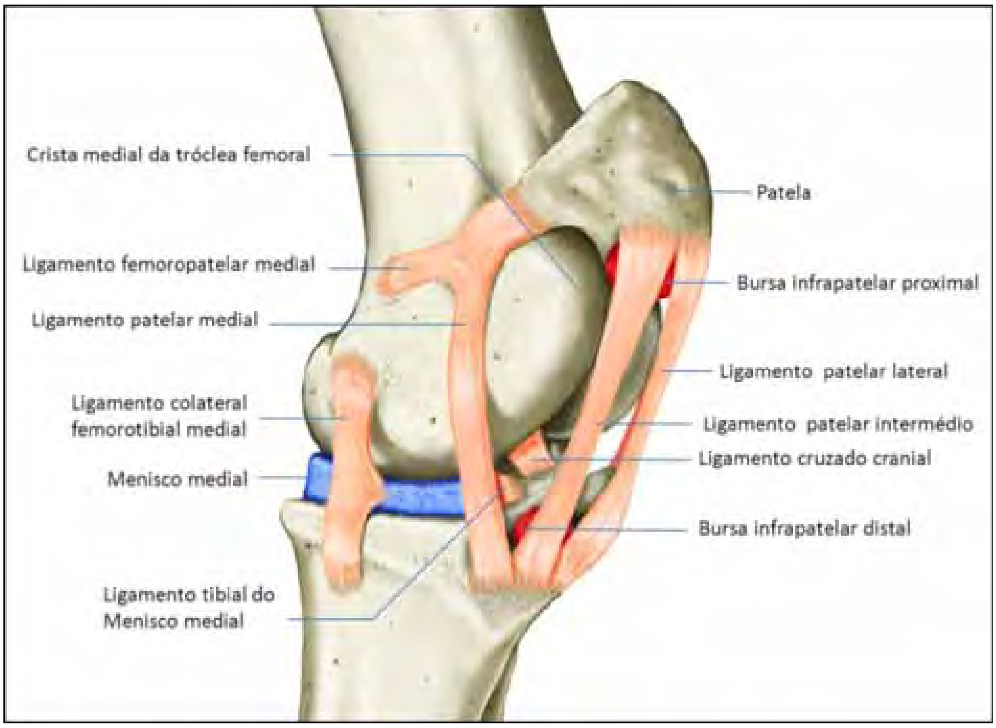

FIGURA 2. Imagem esquemática da face medial do joelho equino sem as cápsulas articulares. Modificada de Köning, H. E. e Liebich, H. G., 2009.

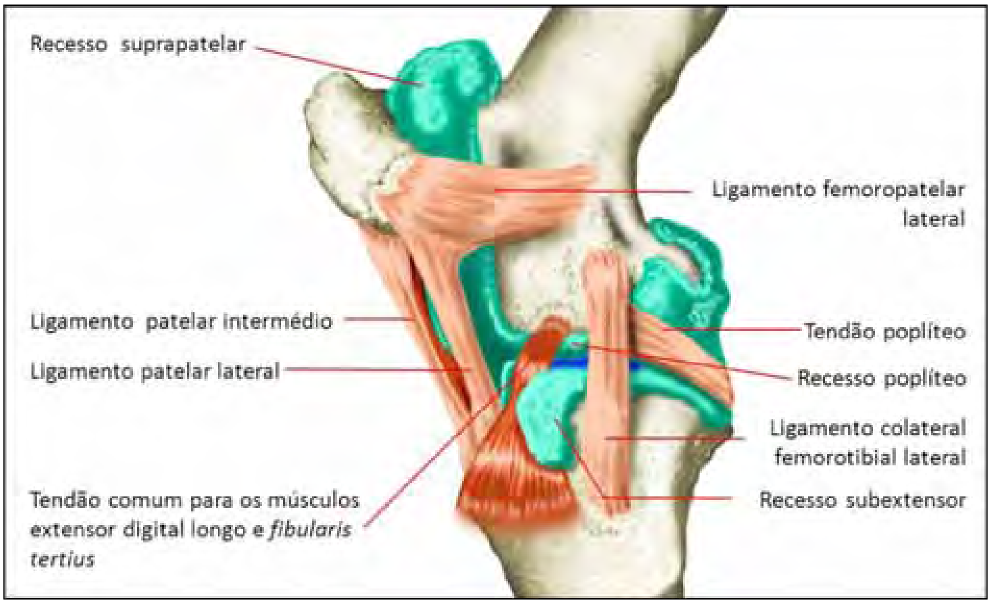

FIGURA 3. Imagem esquemática da face lateral do joelho equino com as cápsulas articulares. Modificada de Köning, H. E. e Liebich, H. G., 2009.

[4]Os ligamentos patelares são extracapsulares e formam parte do aparelho de sustentação do membro pélvico nos equinos (LATIMER, F. G. et al., 2000). Quando a articulação é flexionada, o quadríceps femoral contrai primeiro, levantando e desencaixando a patela desde a crista medial, permitindo assim o deslizamento desta pela tróclea femoral (TNIBAR, M. A., 2002).

Os ligamentos femoropatelares medial e lateral são feixes de fibras unidas em parte aos retináculos patelares (*retinacula patellae*)[5]. O ligamento femoropatelar medial origina-se proximalmente ao epicôndilo medial do fêmur e se insere na fibrocartilagem parapatelar. As suas fibras não se diferenciam facilmente daquelas da cápsula, pelo que é considerado como pericapsular. O ligamento femoropatelar lateral é extracapsular. Tem origem no epicôndilo ipsilateral do fêmur, proximal ao ligamento colateral lateral e se insere na margem lateral da patela (KÖNIG, H. E. e LIEBICH, H. G., 2009) (Figuras 2 e 3).

2.1.2. Articulação femorotibial (AFT)

A articulação femorotibial forma-se entre as superfícies articulares dos côndilos do fêmur e da tíbia. O côndilo medial do fêmur conflui, cranialmente, com a face distal da crista medial da tróclea, enquanto a crista estreita que liga o côndilo lateral à tróclea é não articular. As superfícies articulares tibiais se adaptam pobremente aos côndilos femorais e estão em contato apenas com uma pequena área deles (GETTY, R., 2008; DYCE, K. M. et al., 2010).

Na tíbia, a cápsula da AFT está inserida na margem da superfície articular. No fêmur, a linha de inserção acha-se, em sua maioria, a 1 cm da margem articular e insere-se igualmente nas bordas convexas dos meniscos (GETTY, R., 2008; KÖNIG, H. E. e LIEBICH, H. G., 2009). Cranialmente, a cápsula consiste basicamente na membrana sinovial. Caudalmente, a membrana é reforçada pelo ligamento poplíteo oblíquo (*ligamentum popliteum obliquum*). Este é uma fita plana que surge do fêmur, justaposta lateralmente à origem da porção medial do músculo gastrocnêmio e estende-se lateroproximal-mediodistalmente até a borda caudal do côndilo medial da tíbia (SCHALLER, O. e CONSTANTINESCU, G. M., 2007; GETTY, R., 2008; KÖNIG, H. E. e LIEBICH, H. G., 2009). A membrana sinovial envolve os ligamentos cruzados, formando um septo entre a porção medial e lateral da articulação, que geralmente não se comunicam (REEVES, M. J. et al., 1991; LATIMER, F. G. et al., 2000; KÖNIG, H. E. e LIEBICH, H. G., 2009). O compartimento medial apresenta um recesso (recesso medial) que contém líquido em cavalos sadios. Este se encontra proximal ao menisco e craniomedial ao côndilo do fêmur, entre o ligamento colateral medial e o ligamento patelar medial (HOEGAERTS, M. e SAUNDERS, J. H., 2004; GETTY, R., 2008)

[5]Fibras de tecido conectivo com origem na fáscia regional que dão congruência e formam uma unidade entre o tendão do músculo quadríceps, a patela, os côndilos femorais e a tróclea da tíbia.

(Figura 1). Lateralmente, a AFT possui dois recessos. O primeiro, ou recesso subextensor (GETTY, R., 2008), reveste o tendão comum de origem do músculo extensor digital longo (*extensor digitorum longus*) (EDL) e *fibularis [peroneus] tertius* (FT), a partir da fossa extensora e se estende 4 a 7 centímetros distalmente. O segundo recesso consiste em uma saculação que cobre o tendão de origem do músculo poplíteo (KÖNIG, H. E. e LIEBICH, H. G., 2009; BUDRAS, K. D. et al., 2011) (Figura 3). Os recessos laterais são espaços virtuais e não contém fluido em cavalos hígidos (HOEGAERTS, M. e SAUNDERS, J. H., 2004; GETTY, R., 2008).

2.1.2.1. Meniscos e ligamentos meniscais

Os meniscos lateral (ML) e medial (MM) encontram-se entre os côndilos femorais e tibiais e dão congruência à AFT (LATIMER, F. G., 2004). São placas semilunares de fibrocartilagem, com uma superfície proximal côncava adaptada ao côndilo femoral e uma superfície distal que corresponde ao côndilo tibial. O ML não abrange as áreas lateral e caudal do côndilo tibial, sobre as quais desliza o tendão de origem do músculo poplíteo (KÖNIG, H. E. e LIEBICH, H. G., 2009).

Os meniscos estão unidos à tíbia pelos ligamentos tibiais craniais e caudais (KÖNIG, H. E. e LIEBICH, H. G., 2009). Os ligamentos craniais (lateral e medial) unem a face cranial dos meniscos com a respectiva região intercondilar lateral e medial da tíbia (Figura 4). Os ligamentos caudais medial e lateral se originam nos ângulos caudais dos meniscos e se inserem na tíbia na área intercondilar caudal e na incisura poplítea, respectivamente (KÖNIG, H. E. e LIEBICH, H. G., 2009). O ligamento meniscofemoral que se origina também no ângulo caudal do menisco lateral se insere na extremidade caudal da fossa intercondilar do fêmur (KÖNIG, H. E. e LIEBICH, H. G., 2009).

2.1.2.2. Ligamentos femorotibiais colaterais e cruzados

Estes ligamentos estabilizam funcionalmente a AFT e são extracapsulares (LATIMER, F. G., 2004). O ligamento femorotibial colateral medial (*ligamentum collaterale mediale*) (LCM) tem origem no epicôndilo medial do fêmur e insere-se distal à margem articular do côndilo medial da tíbia. O ligamento femorotibial colateral lateral (*ligamentum collaterale laterale*) (LCL) (mais espesso) se origina no epicôndilo lateral do fêmur e se insere no côndilo lateral da tíbia e, com um ramo mais importante, na cabeça da fíbula (KÖNIG, H. E. e LIEBICH, H. G., 2009). Os ligamentos cruzados (*ligamenta cruciata genus*) estão localizados na fossa intercondilar do fêmur, entre os sacos sinoviais femorotibiais[6]. O ligamento cruzado cranial estende-se desde a face interna do côndilo lateral do fêmur, até a área intercondilar central da tíbia (SCHALLER, O. e CONSTANTINESCU, G. M., 2007). O ligamento cruzado caudal origina-se na superfície axial do côndilo medial do fêmur cranial à fossa intercondilar e, na tíbia, liga-se num tubérculo na borda caudomedial da incisura deste osso (KÖNIG, H. E. e LIEBICH, H. G., 2009) (Figura 4).

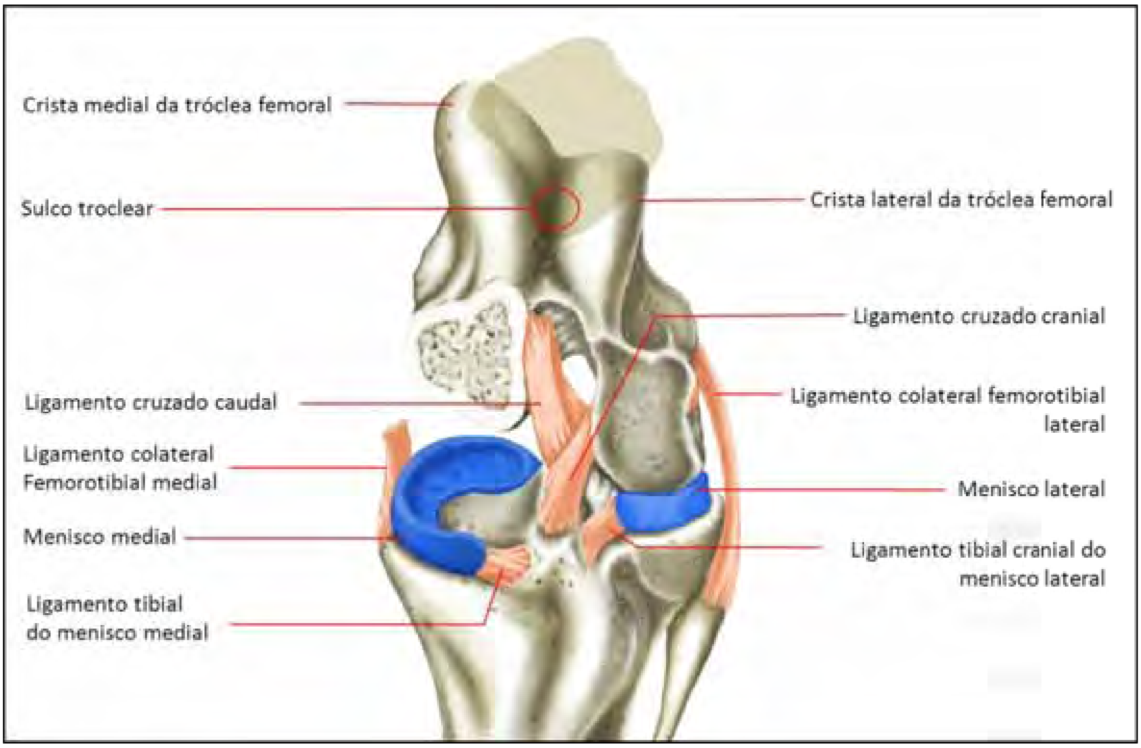

FIGURA 4. Vista cranial da articulação femorotibial. O côndilo medial foi removido para melhor observar a disposição do menisco medial. Modificada de Köning, H. E. e Liebich, H. G., 2009.

2.2. Técnica ultrassonográfica

O exame ultrassonográfico é uma técnica diagnóstica não invasiva, importante para avaliação de tecidos moles dos membros torácicos e pélvicos dos equinos (PENNINCK, D. G. et al., 1990; DENOIX, J. M., 1998) que permite a visualização de tendões, ligamentos, enteses, bursas, bainhas sinoviais e algumas superfícies articulares e nervos (GENOVESE, R. L. et al., 1986; DENOIX, J. M., 1998; REEF, V., 1998).

[6] A disposição dos ligamentos cruzados formando um "X" assimétrico (GETTY, R., 2008) que produz o mecanismo de Tchebychev onde o ponto de intersecção entre ambos coincide com o centro de rotação da articulação estabilizando-a cranial e caudalmente (BADOUX, D. M., 1984).

2.2.1. Preparação para o exame

A preparação para o exame ultrassonográfico é crucial para a boa formação de imagem e pode ser responsável pelo sucesso ou fracasso na visualização das estruturas desejadas. Em hipótese nenhuma deve ser negligenciada a importância de minimizar a diferença de impedância acústica entre o meio externo (ar) e a principal barreira orgânica (pele), visando o correto deslocamento dos feixes ultrassonográficos nos tecidos moles a serem investigados. Dessa maneira, a preparação do exame inclui tricotomia ampla das regiões medial, cranial e lateral do joelho, começando cinco centímetros acima da patela até a crista tibial e aproximadamente cinco centímetros caudais aos ligamentos colaterais femorotibiais medial e lateral (Figura 5). Se há interesse na avaliação da face caudal do joelho, realiza-se tricotomia da musculatura caudal, 10 cm proximais e 10 cm distais à prega formada na flexão da coxa. Preferencialmente a tricotomia deverá ser efetuada utilizando-se máquina de tosa e pente No. 40, após a qual se realiza lavagem da região com água e detergente, tantas vezes quanto for necessário, para limpeza da região. Nos animais sem feridas na pele é ideal a utilização de uma solução de álcool 70% após a lavagem para eliminar restos de gordura. A seguir, molha-se a região com água durante 2-5 minutos. Em hipótese nenhuma se aplica álcool quando existem lacerações, solução de continuidade ou ferimentos abertos. Nos casos de tricotomia com navalha, recomenda-se realizar o exame somente depois da diminuição da sensibilidade na pele, o que melhorará a qualidade no exame, em função da facilidade de manuseio do animal sem dor. Na sequência, aplica-se gel acústico, em quantidade suficiente para formar uma camada uniforme na área total a ser examinada (Figura 6). Se houver ferida perfurante, é recomendável a utilização de gel estéril. Em alguns casos, o proprietário pode recusar a realização de tricotomia. É evidente que o Médico Veterinário será responsável por mostrar a importância de tirar o pelo, porém, em casos excepcionais pode ser utilizado álcool (REEF, V., 1998). Claramente, é mais correto realizar tricotomia. Já que as lesões no joelho não são emergências de modo geral, tome tempo na preparação do paciente. Como mencionado antes, isto é de relevância para o resultado do exame.

Por último, e dependendo da estrutura avaliada, do formato e frequência do transdutor, e das características do equipamento, pode ser considerada a utilização de um anteparo de silicone. É comum a geração de ecos intensos na porção mais superficial da zona de fresnel (também chamada zona de campo próximo) devido a reverberações do transdutor, o que provoca maior interferência e menor definição das imagens das estruturas mais superficiais. O anteparo de silicone diminui estes ecos de reverberação quando colocado entre a pele e o transdutor (BILLER, D. S. e MYER, W., 1988). Assim, as estruturas mais superficiais são melhor visualizadas com a utilização deste acessório, principalmente quando se utilizam transdutores de frequência média (por exemplo, de 5 MHz). Atualmente, alguns equipamentos regulam os feixes de maior intensidade na região superficial da zona de campo próximo, o que reduz a zona de interferência a 1-2 mm.

2.2.2. Transdutores

Na prática podem ser utilizados transdutores lineares multifrequênciais de 3,5 até 12 MHz (WALMSLEY, J. P., 2005). Cauvin et al. (1996) utilizaram transdutores convexos e setoriais de 3,5 e 7,5 MHz. Os transdutores convexos com menor frequência (3,5 MHz) são utilizados para avaliação das partes mais profundas da cavidade articular como, por exemplo, a origem do ligamento cruzado na tíbia. A técnica em "cinco etapas" (HOEGAERTS, M. e SAUNDERS, J. H., 2004) recomenda a utilização de um transdutor linear de 6-9 MHz, outro de 7-12 MHz e um transdutor convexo de 4-6 MHz. Flynn e Withcombe (2002) descreveram o uso de transdutores lineares de 7,5 e 10 MHz; e de transdutores convexos de 5-6 MHz.

O posicionamento do transdutor realiza-se geralmente perpendicular à estrutura avaliada. Uma variação tão sutil como dois graus na angulação do feixe provocará perda da definição da imagem por efeito anisotrópico (CONNOLLY, D. J. A. et al., 2001).

2.3. Topografia ultrassonográfica

Vários autores têm descrito técnicas para avaliação ultrassonográfica das estruturas do joelho equino (PENNINCK, D. G. et al., 1990; CAUVIN, E. R. J. et al., 1996; DENOIX, J. M. et al., 1996; HOEGAERTS, M. e SAUNDERS, J. H., 2004; COUDRY, V. e DENOIX, J. M., 2005). Em síntese, três áreas topográficas podem ser identificadas: a face medial, lateral e cranial. Através destas janelas acústicas é possível avaliar:

1. Medialmente o LCM, MM e o recesso medial da articulação femorotibial medial.

2. Craniomedial e cranialmente: o LPM, LPI, ápice e superfície cranial da patela, recesso suprapatelar femorotibial, coxim gorduroso infrapatelar, bursas infrapatelares

proximal (que é exclusiva dos equinos) e distal, bem como a superfície condral da crista medial e sulco da tróclea femoral.

3. Craniolateral e lateralmente: o LPL, o tendão comum de origem dos músculos extensor digital longo e *fibularis tertius*, o menisco lateral, LCL, tendão de origem do músculo poplíteo, e os recessos subextensor e poplíteo[7] da articulação femorotibial lateral.

Existem algumas limitações técnicas para realizar imagens dos ligamentos craniais dos meniscos (BARR, E. D. et al., 2006). Na nossa prática, estas dificuldades estão relacionadas com a flexão do membro avaliado, que nem sempre é bem tolerada pelo animal. A visualização dos ligamentos cruzados pode ser um desafio pela dificuldade para posicionar o transdutor perpendicularmente às fibras (HOEGAERTS, M. e SAUNDERS, J. H., 2004).

Alguns protocolos descrevem a avaliação da face caudal do joelho para identificação de anormalidades no ligamento femoromeniscal do menisco lateral e os ligamentos caudais e craniais dos meniscos (CAUVIN, E. R. J. et al., 1996; HOEGAERTS, M. e SAUNDERS, J. H., 2004). Porém, a avaliação destas estruturas é pouco realizada na prática (FLYNN, K. A. e WHITCOMB, M. B., 2002). Caudalmente, as massas musculares dos animais geralmente obrigam à utilização de transdutores de baixa frequência com o que se ganha em profundidade, mas se perde em definição. Desse modo podem ser identificadas unicamente lesões grosseiras, enquanto lesões sutis são pouco visualizadas.

2.4. Anatomia ultrassonográfica

2.4.1. Articulação femoropatelar

O espaço articular femoropatelar, com exceção da sua face lateral, não é visualizado. Embora este espaço seja constituído por uma câmara ampla, possui quantidade mínima de líquido para que possa ser detectada em animais clinicamente sadios, em regime de treinamento ou de trabalho moderado. A detecção desses espaços preenchidos com líquido pode ser indicativa de lesão. Em animais atletas podem-se observar imagens anecóicas comumente laterais, mas também mediais à patela (DENOIX, J. M. et al., 1996), provocadas por efusão sinovial.

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[7] O termo recesso poplíteo é utilizado pelo autor para denominar a saculação femorotibial lateral entre o tendão poplíteo e o ligamento colateral lateral.

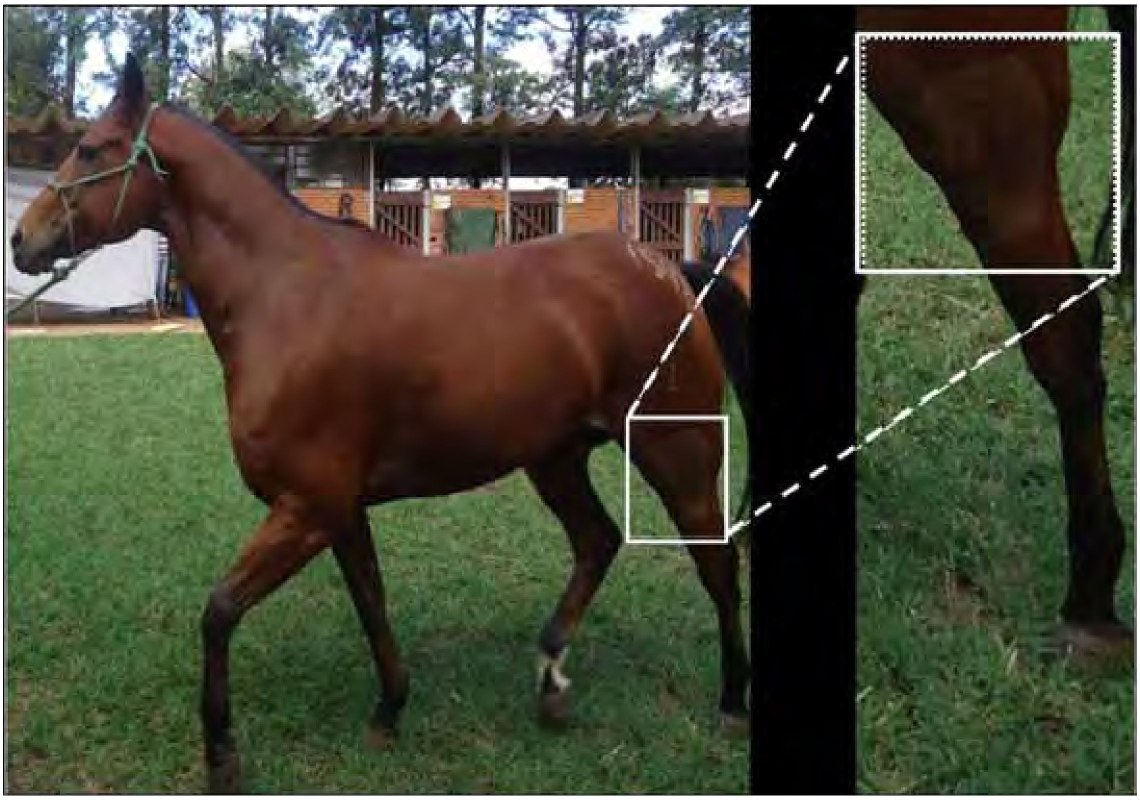

FIGURA 5. Tricotomia da face lateral e cranial do joelho. **Imagens: Arquivo pessoal.**

FIGURA 6. Tricotomia e aplicação de gel para realização de exame ultrassonográfico em uma égua com aumento de volume cranial do joelho. **Imagem: Arquivo pessoal.**

2.4.1. Tróclea femoral

As imagens transversais se realizam colocando o transdutor cranial e distal à patela, efetuando a varredura até a metade do espaço articular femorotibial para avaliação das cristas e do sulco. A cartilagem da tróclea femoral é facilmente visualizada como uma linha anecóica bem definida e uniforme de 2-3 mm por cima da margem óssea, sendo mais espessa (aproximadamente duas vezes) na crista lateral do que na crista medial. O osso subcondral é visto como uma linha hiperecóica contínua que pode apresentar alguma irregularidade no sulco troclear (DENOIX, J. M. et al., 1996) (Figura 7). Longitudinalmente é possível realizar imagens deslocando-se o transdutor, de medial a lateral, para obter cortes sagitais da crista medial, sulco e crista lateral da tróclea (HOEGAERTS, M. et al., 2005).

2.4.1.2. Ligamentos patelares

Os ligamentos podem ser examinados longitudinalmente para avaliação da integridade dos feixes e presença de entesófitos e transversalmente para observar ecotextura, margens e formato. Imagens dos três ligamentos patelares são obtidas facilmente com a utilização de um transdutor linear de 7,5-10 MHz. Para melhor visualização da pele e tecido subcutâneo pode ser utilizado anteparo de silicone e profundidade de 4-6 cm (REEF, V., 1998). Podem ser utilizados até dois focos devido ao pouco movimento das estruturas avaliadas. O anteparo não é obrigatório para avaliação, porém aprimora o exame quando existe suspeita de fibrose ou aderência entre a pele e fáscia patelar, por exemplo, em casos de ferida perfurante com cicatriz.

O ligamento patelar medial está coberto pela fáscia patelar e pela pele (DENOIX, J. M. et al., 1996). Origina-se na fibrocartilagem parapatelar, na borda medial da patela. Para formação da imagem, se coloca o transdutor sobre a origem do ligamento em um ângulo de aproximadamente 45° craniomedial-caudolateralmente e se faz varredura em direção distal, em cortes transversais e longitudinais. A região de origem aparece hipoecóica devido à orientação das fibras da fibrocartilagem que passam por cima da crista medial da tróclea do fêmur. Em sentido distal localiza-se medial à tróclea e forma uma imagem homogênea em feixes paralelos em direção à fase medial da crista da tíbia (DENOIX, J. M. et al., 1996) (Figura 8). Transversalmente, o LPM tem formato triangular na sua origem e se confunde com a fibrocartilagem parapatelar dando um aspecto hipoecóico (Figura 9). O LPM adquire formato arredondado/oval que se transforma em triangular na sua região de inserção (DENOIX, J. M. et al., 1996) e exibe ecotextura heterogênea levemente hipoecóica, provocada pelo arranjo e tipo de fibras de colágeno nas regiões de enteses. Caudalmente está unido com a aponeurose terminal dos músculos grácil e sartório (WRIGHT, I., 1995).

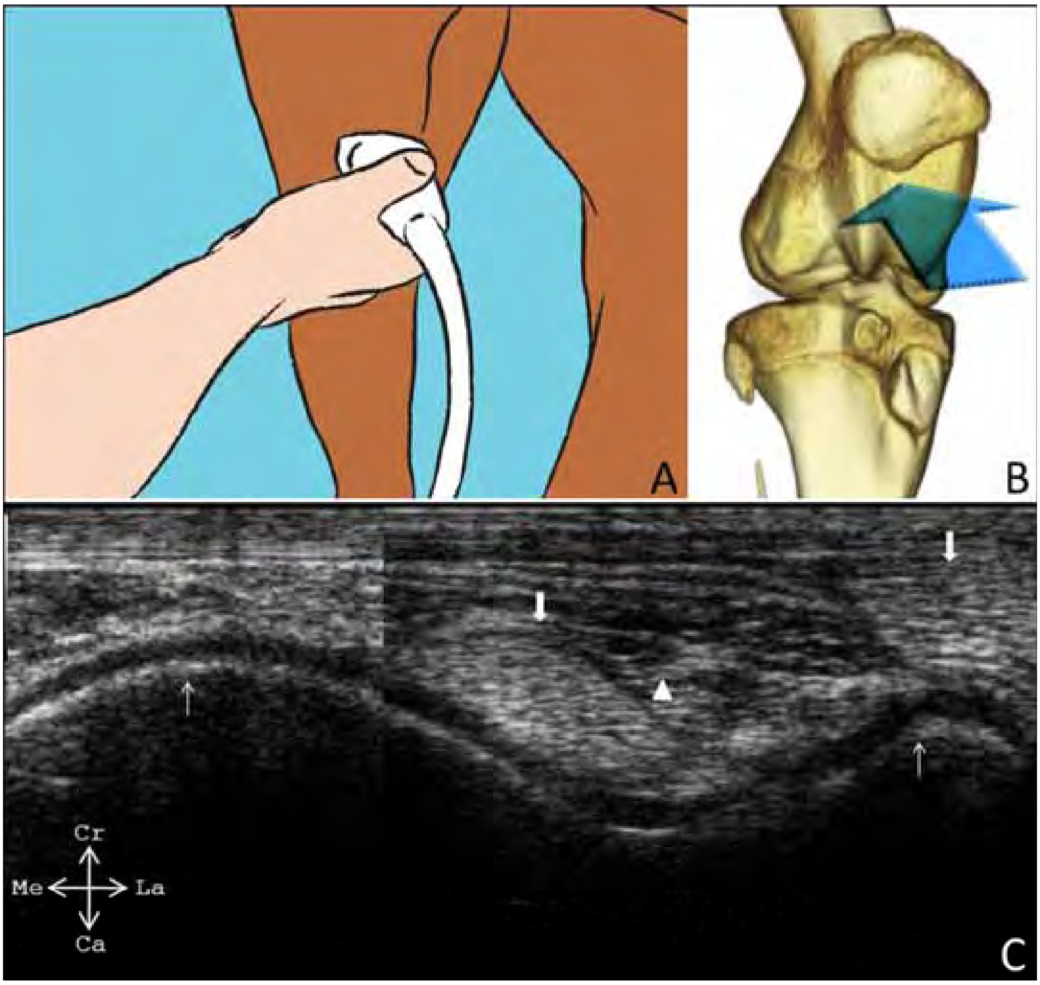

FIGURA 7. Desenho topográfico de posicionamento do transdutor (A) e de incidência do feixe de ultrassom (B) para realizar imagens ultrassonográficas em corte transversal do ligamento patelar intermédio (LPI) e da crista medial, sulco e crista lateral da tróclea femoral. (C) A crista medial (seta esquerda) é consideravelmente maior que a crista lateral (seta direita). Visualiza-se a cartilagem como uma banda hipo/anecóica regular por cima da margem troclear (linha hiperecóica). É evidente também o LPI perto da sua origem na patela (seta sólida) com formato ovalado. A seta sólida à direita indica o ligamento patelar lateral e a cabeça de seta o coxim gorduroso infrapatelar. Cr=cranial; Ca=caudal; Me=medial; La=lateral. Imagem obtida com aparelho MyLab 30, Esaote™ e transdutor linear multifrequência a 10 MHz, 2 focos e 4 cm de profundidade. **Imagens: Arquivo pessoal.**

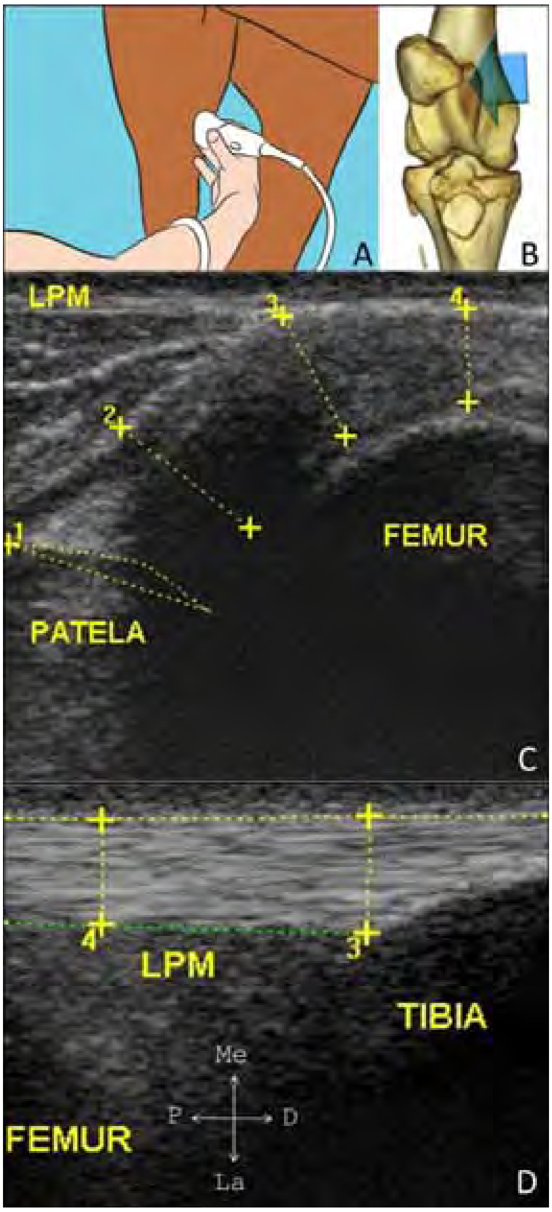

FIGURA 8. Desenho topográfico de posicionamento do transdutor (A) e de incidência do feixe de ultrassom (B) para realizar imagens ultrassonográficas em corte longitudinal do ligamento patelar medial (LPM). (C) Origem do LPM. A fibrocartilagem parapatelar (linhas pontilhadas 1, 2, 3 e 4) observa-se hipoecóica, por causa da falta de incidência perpendicular do feixe de ultrassom. (D) Inserção do LPM na tíbia. O LPM (entre linhas pontilhadas) tem arranjo homogêneo e paralelo das fibras e possui ecogenicidade normal. Me= medial; la=lateral; P=proximal; D=distal. Imagens obtidas com aparelho Logic 3, General Electric™, e transdutor linear multifrequência operando a 10 MHz, 2 focos e 4 cm de profundidade. **Imagens: Arquivo pessoal.**

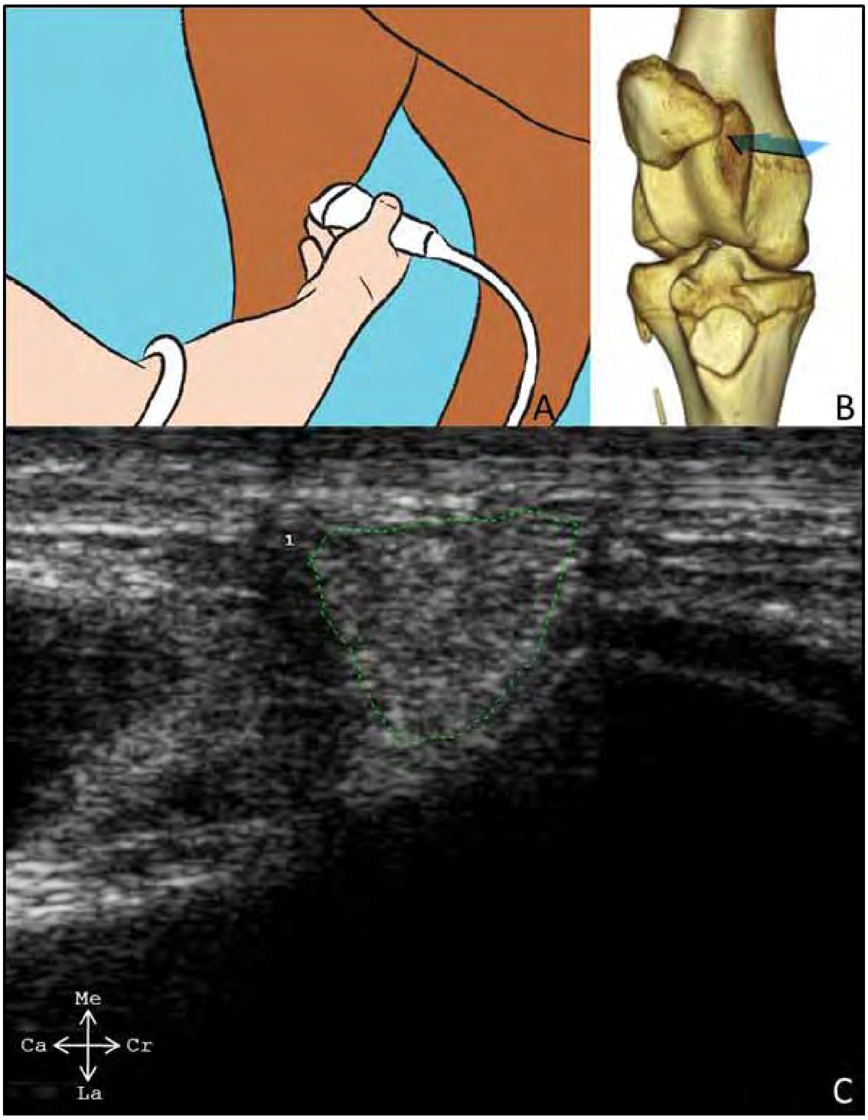

FIGURA 9. Desenho topográfico de posicionamento do transdutor (A) e de incidência do feixe de ultrassom (B) para realizar imagens ultrassonográficas em corte transversal da origem do ligamento patelar medial (LPM). (C) O LPM (linha pontilhada) tem formato triangular na sua origem e se observa medial à crista da tróclea femoral. Me= medial; la=lateral; Ca=caudal; Cr=cranial. Imagem obtida com aparelho MyLab 30, Esaote™ e transdutor linear multifrequência a 10 MHz, 2 focos e 4 cm de profundidade. **Imagens: Arquivo pessoal.**

O ligamento patelar intermédio está coberto também pela fáscia patelar e a pele. Para formação da imagem, se coloca o transdutor na superfície cranial da patela por cima do ligamento, e se realiza varredura distal em cortes sagitais e transversais. Longitudinalmente o ligamento e osso estão claramente delimitados na região de origem na face cranial e ápice da patela (Figura10). Transversalmente o LPI é fusiforme na sua origem. Conforme se examina com direção à sua inserção, o ligamento fica arredondado e levemente triangular até terminar na forma de um círculo bem definido que passa através do sulco[8] para ligar-se à crista tibial, onde aparece ecóico heterogêneo e oval (Figura 11). Mais profundamente ao LPI se localiza o coxim gorduroso infrapatelar. Quando o animal está em estação, o coxim gorduroso se projeta cranialmente formando uma fina linha hipoecóica entre a origem do LPI e a fáscia patelar. Entre a patela e o LPI se encontra a bursa infrapatelar proximal, que é uma particularidade dos equinos (BUDRAS, K. D. et al., 2011) e na região de inserção na tíbia se encontra a bursa infrapatelar distal. A presença de líquido nestas bursas é virtual, e assim, são visíveis na ultrassonografia somente em casos de proliferação provocada por trauma ou atrito repetitivo.

O LPL é o mais curto dentre os ligamentos patelares. Visualiza-se posicionando o transdutor na região craniolateral (DENOIX, J. M. et al., 1996) e orientado craniolateral-caudomedial. Ele está separado da pele pela aponeurose da fáscia lata com a qual se une distalmente, junto com um forte tendão do músculo bíceps femoral (DENOIX, J. M. et al., 1996; REEF, V., 1998). Esta união aponeurose-tendão-LPL faz com que as margens deste último sejam mais difíceis de serem visualizadas nas imagens longitudinais, principalmente no terço distal do ligamento. Mesmo assim, o LPL é observado como uma faixa homogênea de feixes fusiformes e paralelos, com aparência frouxa quando comparados com aqueles do LPI e LPM. Transversalmente, o LPL passa por cima da crista lateral da tróclea medial do fêmur e forma uma imagem côncava. Na sua parte média se observa como uma faixa larga e fina que se insere distalmente na superfície lateral da crista tibial. Em cortes transversais na região de passagem pela crista lateral da tróclea, é possível observar uma área anecóica bem delimitada que corresponde ao recesso sinovial lateral da articulação femoropatelar, adjacente à margem caudal do LPL (DENOIX, J. M. et al., 1996) e que contém alguma quantidade de líquido em cavalos sadios (Figura 12).

2.4.2. Articulação femorotibial

O recesso medial da AFT se visualiza entre o LCM e o LPI, dorsal ao menisco medial. Um transdutor de matriz linear de 7,5-10 MHz e profundidade de 4-6 cm possibilitam sua avaliação. Este compartimento, que contém líquido em cavalos hígidos, é visualizado como uma "bexiga" anecóica ovalada, com margens bem definidas. Na

[8] Sulco para o ligamento patelar intermédio (GETTY, R., 2008)

formação da imagem é importante diminuir ao máximo a pressão exercida pelo transdutor no intuito de evitar a falsa impressão de colapso provocado por doença articular degenerativa ou sinovite séptica (Figura 13).

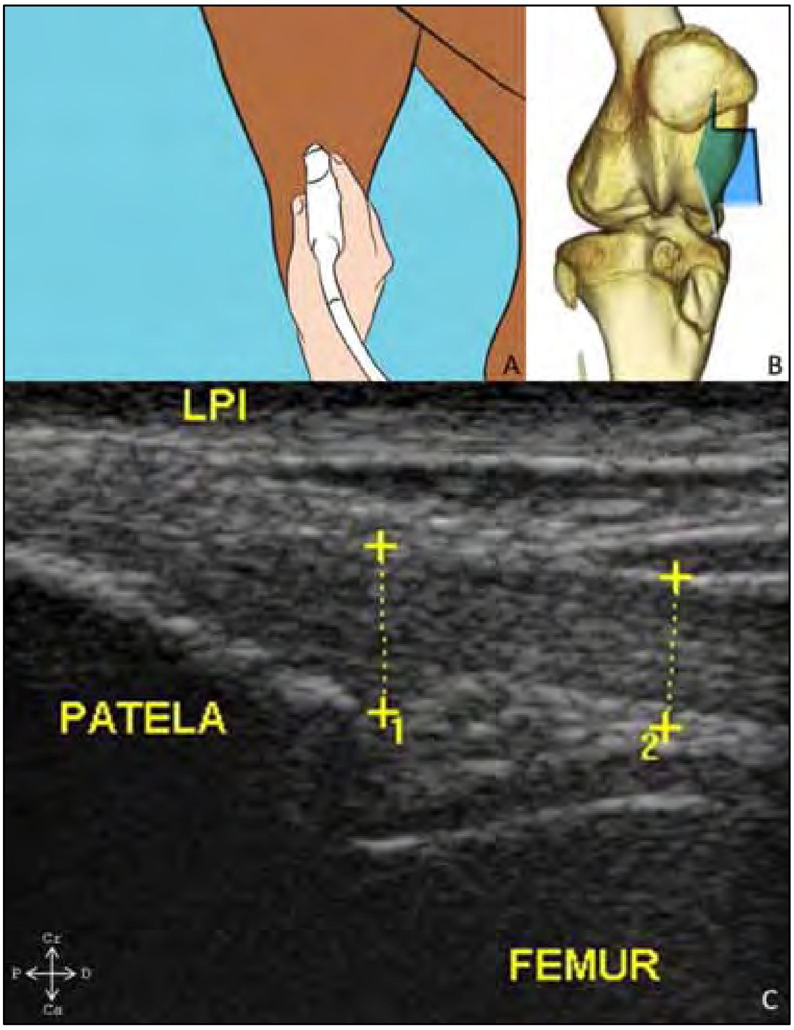

FIGURA 10. Desenho topográfico de posicionamento do transdutor (A) e de incidência do feixe de ultrassom (B) para realizar imagens ultrassonográficas em corte longitudinal do ligamento patelar intermédio (LPI). (C) O LPI está marcado com linhas de pontos. A sua ecogenicidade é homogênea e as fibras paralelas. A diferenciação entre a patela e o ligamento é clara. São evidentes também a margem óssea do sulco da tróclea femoral (linha hiperecóica) e uma linha hipo/anecóica definida por cima do fêmur que corresponde à cartilagem do sulco da tróclea femoral. O tecido hipoecóico difuso ao redor do LPI é o coxim gorduroso infrapatelar. Cr=cranial; Ca=caudal; P=proximal; D=distal. Imagem obtida com aparelho Logic 3, General Electric™, e transdutor linear multifrequência operando a 10 MHz, 2 focos e 4 cm de profundidade. **Imagens: Arquivo pessoal.**

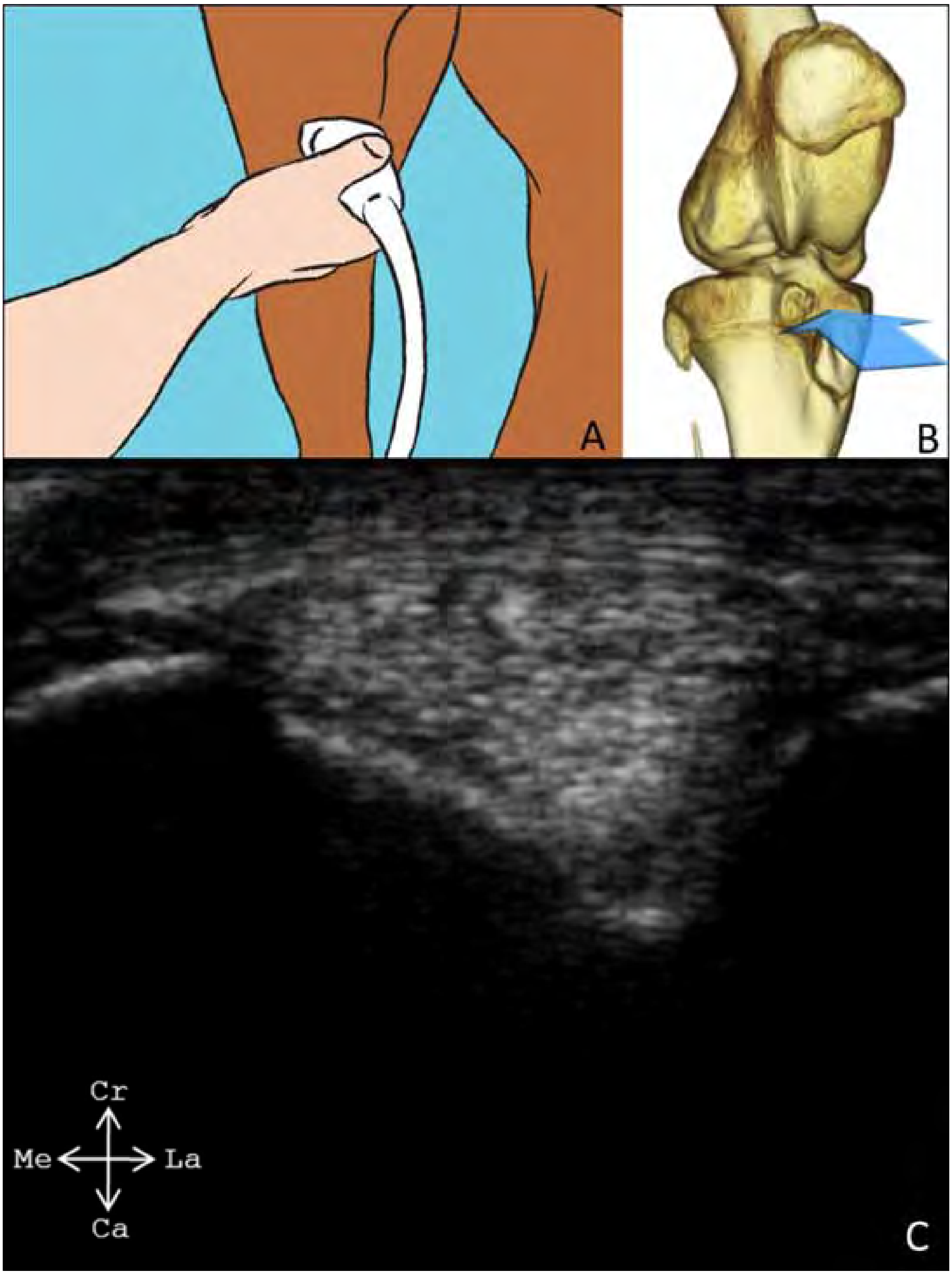

FIGURA 11. Desenho topográfico de posicionamento do transdutor (A) e de incidência do feixe de ultrassom (B) para realizar imagens ultrassonográficas em corte transversal do ligamento patelar intermédio (LPI) na região de inserção com a tíbia. (C) Visualiza-se o ligamento heterogêneo perto da região de inserção. Isto é uma imagem normal nas regiões de enteses e os pontos hipoecóicos não devem ser confundidos com lesão. Cr=cranial; Ca=caudal; Me=medial; La=lateral. Imagem obtida com aparelho MyLab 30, Esaote™ e transdutor linear multifrequência a 10 MHz, 2 focos e 4 cm de profundidade. **Imagens: Arquivo pessoal.**

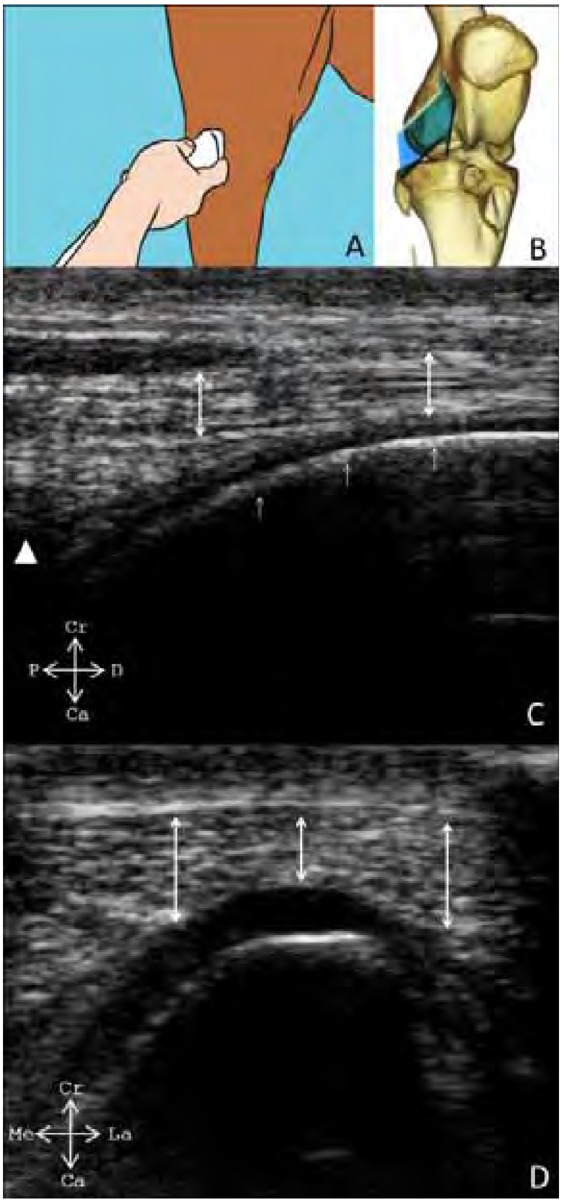

FIGURA 12. Desenho topográfico de posicionamento do transdutor (A) e de incidência do feixe de ultrassom (B) para realizar imagens ultrassonográficas em corte longitudinal e transversal do ligamento patelar lateral (LPL) na região da sua origem na patela. (C) Longitudinalmente, visualiza-se o ligamento (setas de duas cabeças) perto da região de origem na patela (cabeça de seta). As setas pequenas indicam a margem óssea da crista lateral da tróclea e por cima desta margem identifica-se a cartilagem (linha anecóica) da crista. (D) Mesma região em corte transversal. O LPL passa por cima da crista medial e aparece com formato convexo (setas de duas cabeças). Cr=cranial; Ca=caudal; Me=medial; La=lateral. P=proximal; D=distal. Imagens obtidas com aparelho MyLab 30, Esaote™ e transdutor linear multifrequência a 10 MHz, 2 focos e 4 cm de profundidade. **Imagens: Arquivo pessoal.**

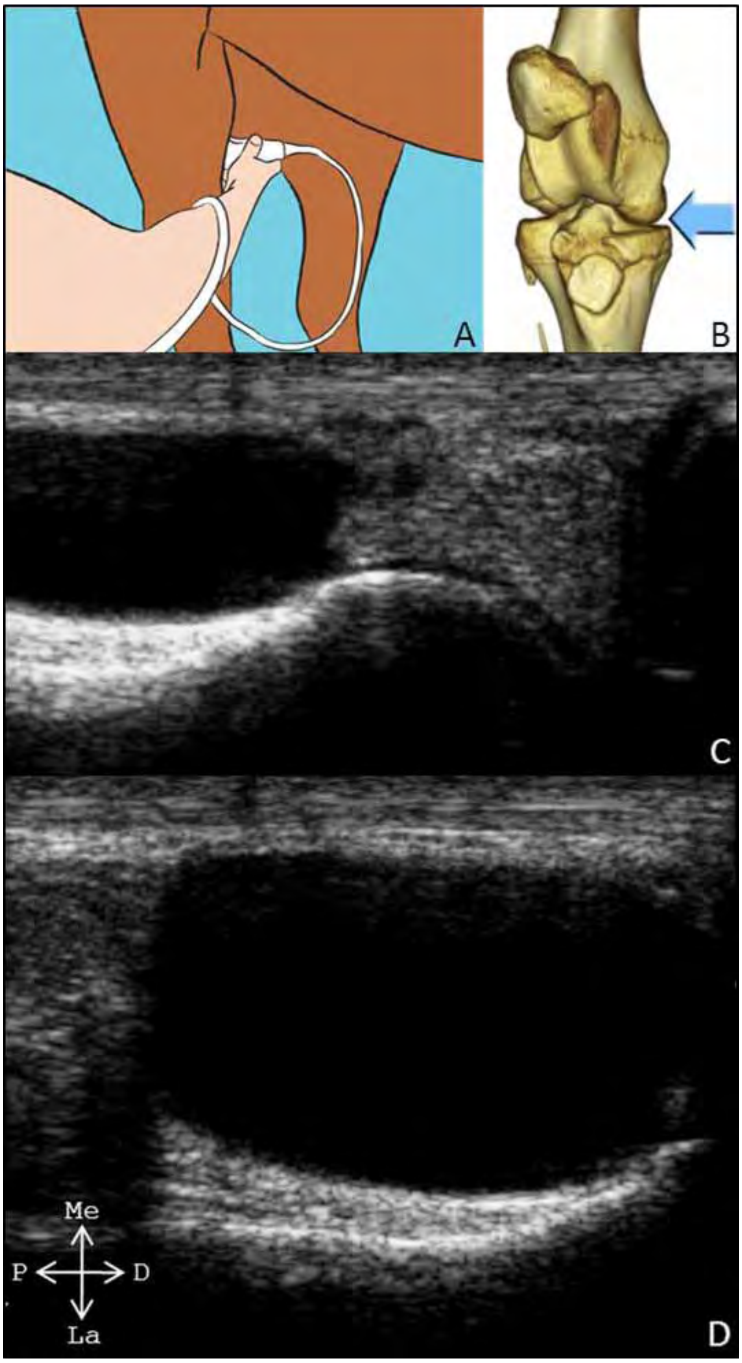

FIGURA 13. Desenho topográfico de posicionamento do transdutor (A) e de incidência do feixe de ultrassom (B) para realizar imagens ultrassonográficas em corte longitudinal do menisco medial e do recesso medial femorotibial. (C) O menisco medial observa-se como uma figura ecóica triangular entre as margens dos côndilos mediais do fêmur e da tíbia. O recesso medial (imagem anecóica à esquerda) tem formato normal. (D) O formato oval e a presença de líquido são normais neste espaço. Me= medial; la=lateral; p=proximal; D=distal. Imagens obtidas com transdutor linear multifrequência a 8 MHz e profundidade de 4 cm. Não foi utilizado anteparo de silicone. Imagens: **Imagens: Arquivo pessoal.**

O compartimento articular femorotibial lateral possui dois recessos: o recesso subextensor que não contém líquido em animais sadios (HOEGAERTS, M. e SAUNDERS, J. H., 2004), e o recesso poplíteo, que apresenta a mesma característica. Já que estes recessos são espaços virtuais, eles são visíveis unicamente quando existe aumento na quantidade de líquido sinovial. Para avaliação do recesso subextensor, coloca-se o transdutor na face craniolateral, deslocando-o pela fossa extensora da tíbia. Se distendido, este recesso tem aparência sacular anecóica ao longo do *fibularis tertius* que vai de 4-7 cm, distal ao platô tibial. Para avaliação do recesso poplíteo, coloca-se o transdutor na mesma orientação, mas na altura do espaço articular femorotibial lateral, entre o LPL e o LCL. O transdutor deve ser movimentado no sentido anti-horário no membro esquerdo, e horário no membro direito, conservando a angulação, até identificar o ML caudal ao tendão do *fibularis tertius.* Assim, caso exista distensão, o recesso será observado como uma "bexiga" proximal ao ML e em contato com o tendão poplíteo.

2.4.2.1. Meniscos

O menisco medial visualiza-se entre o LCM e o LPM. Para localizá-lo, o transdutor é colocado seguindo as especificações para o LCM percorrendo-se a sua superfície visível, em direção cranial. Transdutores entre 7,5 e 10 MHz e profundidade de 4 a 6 centímetros são adequados para visualização. O MM aparece longitudinalmente como uma figura triangular ecóica entre o fêmur e a tíbia (Figura 14). Transversalmente, apresenta-se como bandas paralelas de tecido fibroso, arranjadas em formato convexo (Figura 15).

O ML é mais profundo e, por vezes, mais difícil de localizar que o MM. Um jeito fácil de obter imagens é posicionar o transdutor na região craniolateral, apontando os feixes na direção craniolateral-caudomedial, imediatamente caudal ao LPL. O tendão comum de origem do extensor digital longo e *fibularis tertius* será a primeira estrutura visualizada; depois, continua-se a deslocar o transdutor no sentido caudal, mas conservando-se a angulação. Deste modo, o ML aparecerá como uma estrutura ecóica com formato triangular. O ML também pode ser localizado colocando-se o transdutor por cima do LCL. Em algumas ocasiões, principalmente em animais muito musculosos, é necessária a utilização de transdutores com frequências de 5 MHz ou menores, para visualizar o menisco lateral (Figura 16).

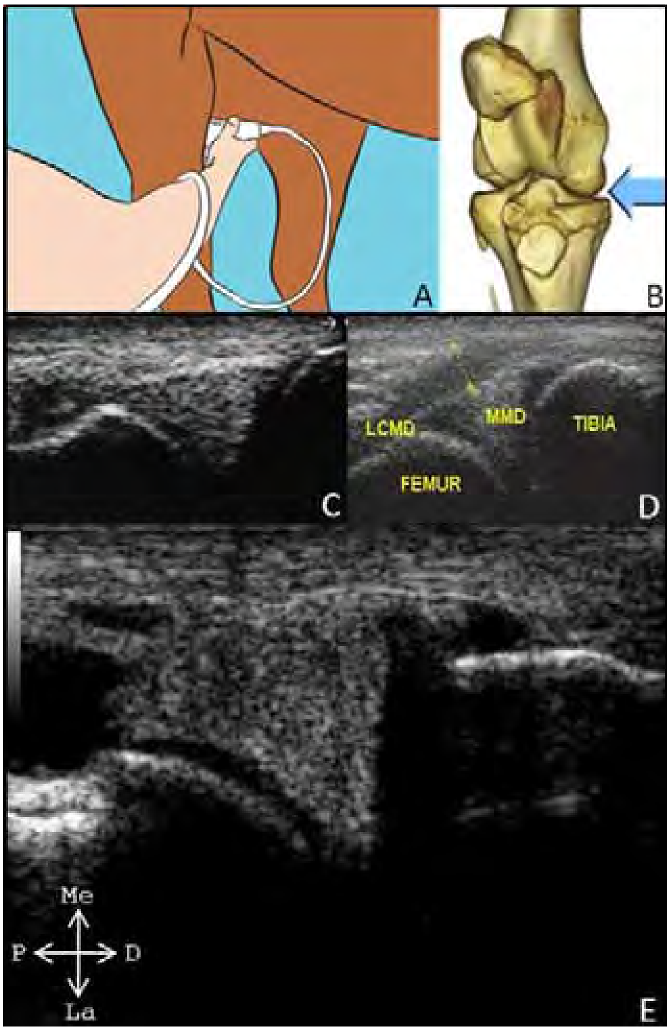

FIGURA 14. Desenho topográfico de posicionamento do transdutor (A) e de incidência do feixe de ultrassom (B) para realizar imagens ultrassonográficas em corte longitudinal do menisco medial. Nas três imagens (C, D e E) se observa o menisco como uma figura ecóica triangular entre as margens dos côndilos mediais do fêmur e da tíbia. Me= medial; la=lateral; p=proximal; D=distal. Não foi utilizado anteparo de silicone. (C) Imagem realizada com um transdutor linear de 7,5 MHz, e aparelho Hitachi EUB™ 405, modelo 1990. (D) Imagem obtida com aparelho Logic 3, General Electric™, e transdutor linear multifrequência operando a 10 MHz, 2 focos e profundidade de 4 cm. LCMD = ligamento colateral femorotibial medial; MMD = menisco medial. O LCMD é hipoecóico devido a que o feixe não está dirigido perpendicular às suas fibras. A linha pontilhada corresponde ao ligamento colateral femorotibial medial, que se observa hipoecóico devido a que o feixe de ultrassom não esta incidindo as fibras perpendicularmente (E) Imagem obtida com aparelho MyLab 30, Esaote™ e transdutor linear multifrequência a 8 MHz. **Imagens: Arquivo pessoal.**

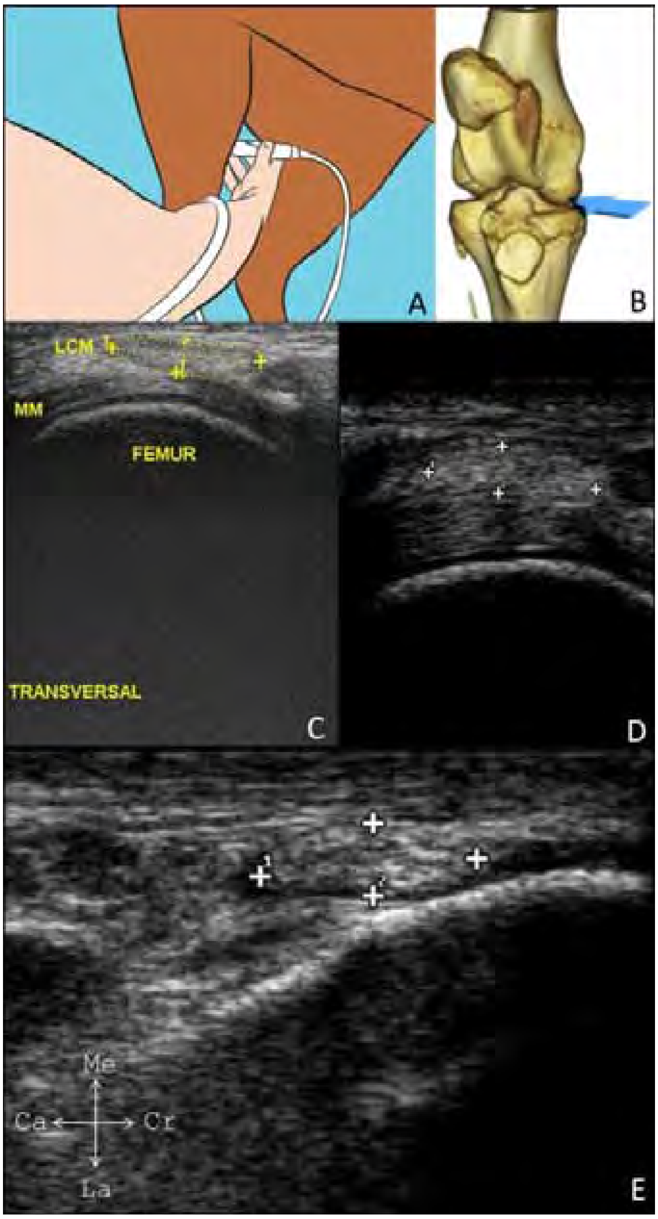

FIGURA 15. Desenho topográfico de posicionamento do transdutor (A) e de incidência do feixe de ultrassom (B) para realizar imagens ultrassonográficas em corte transversal do menisco medial (MM) e do ligamento colateral medial (LCM). (C) As fibras paralelas seguindo o contorno do fêmur correspondem ao MM. O LCM está entre cruzes (Ultrassom Logic 3, General Electric™, e transdutor linear multifrequêncial operando a 10 MHz, 2 focos e profundidade de 4 cm). (D) e (E) O LCM está entre marcas (D) na altura do MM e (E) na altura da margem óssea da tíbia (Ultrassom MyLab 30, Esaote™ e transdutor linear multifrequência a 10 MHz, 2 focos e 4 cm de profundidade). Me= medial; La=lateral; Ca=caudal; Cr=cranial; LCM=ligamento colateral femorotibial medial; MM=menisco medial. **Imagens: Arquivo pessoal.**

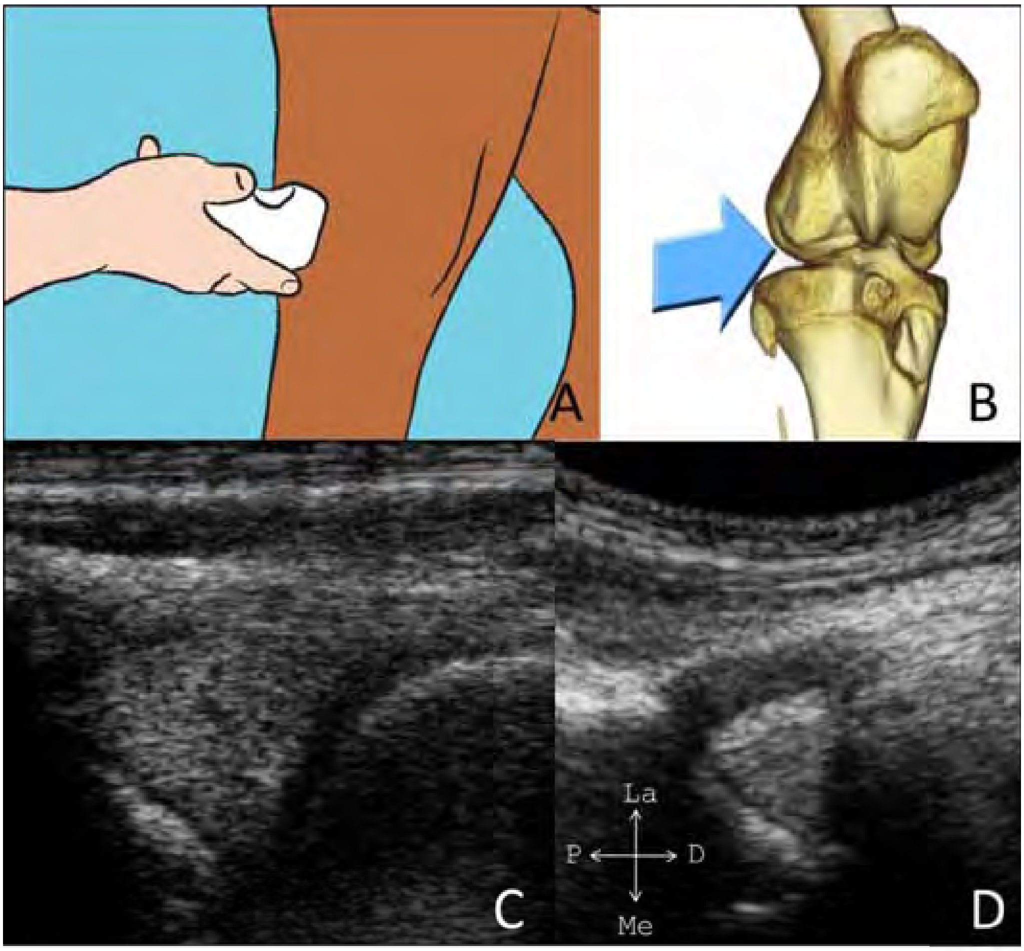

FIGURA 16. Desenho topográfico de posicionamento do transdutor (A) e de incidência do feixe de ultrassom (B) para realizar imagens ultrassonográficas em corte longitudinal do menisco lateral (ML). Em (C) e (D) o menisco é claramente visível como uma imagem triangular ecóica entre as margens ósseas do fêmur (esquerda) e a tíbia (direita). La=lateral; Me=medial; P=proximal; D=distal. Em (C) imagem obtida com aparelho MyLab 30, Esaote™ e transdutor linear multifrequência a 8 MHz, 2 focos e 6 cm de profundidade; e em (D) com transdutor multifrequência convexo a 5 MHz, 2 focos e 7 cm de profundidade. **Imagens: Arquivo pessoal.**

Os ligamentos tibiais craniais dos meniscos são visualizados com o membro em flexão (CAUVIN, E. R. J. et al., 1996; HOEGAERTS, M. e SAUNDERS, J. H., 2004). Deve-se posicionar o transdutor medial ou lateral ao LPI, de 2 a 4 cm, distal à patela. O feixe de ultrassom é orientado caudodistalmente e o transdutor (linear) girado no sentido horário ou anti-horário dependendo do ligamento a ser identificado (por exemplo, sentido anti-horário para o ligamento tibial cranial do menisco medial, no membro esquerdo) (HOEGAERTS, M. e SAUNDERS, J. H., 2004).

2.4.2.2. Ligamentos colaterais femorotibiais

As especificações técnicas para observação dos ligamentos colaterais são similares àquelas dos ligamentos patelares. A profundidade utilizada é de 2-4 cm. Quando utilizados transdutores de 7,5 MHz é recomendável utilização de anteparo de silicone, para realização de imagens do LCM (COUDRY, V. e DENOIX, J. M., 2005). No caso do LCL o anteparo não é necessário, pois a pele é mais espessa na face lateral e o ligamento está coberto parcialmente pelo músculo bíceps femoral (COUDRY, V. e DENOIX, J. M., 2005). Isto é particularmente evidente em animais sob treinamento intensivo e muito musculosos. Se existe inflamação e aumento de volume serão utilizados transdutores com menor frequência, para atingir profundidades maiores.

As imagens do LCM são facilmente obtidas colocando-se o transdutor transversal ou longitudinalmente sobre a sua origem no côndilo medial do fêmur e dirigindo-o na direção da face medial da tíbia. O LCM está coberto pela pele e fáscia patelar. As fibras musculares do adutor são observadas sobre o LCM na sua região de origem. O LCM situa-se superficial ao MM, e as suas fibras profundas se aderem ao MM e cápsula articular femorotibial. Tem aspecto ecóico e compartilha as mesmas características ultrassonográficas de outros ligamentos (Figura 15). Seu formato é ovalado em corte transversal e com largura de 4-5 mm (cavalo de 550 kg) (COUDRY, V. e DENOIX, J. M., 2005) Em equinos da raça Quarto de Milha (peso médio de 460 kg) foram observados largura e comprimento transversais de quatro e onze milímetros respectivamente. (CISNEROS, L. E., 2008).

O LCL é visualizado do epicôndilo lateral do fêmur até a sua inserção na cabeça da fíbula, logo abaixo da pele (COUDRY, V. e DENOIX, J. M., 2005). É separado do menisco medial pelas fibras do tendão poplíteo, que forma geralmente um espaço anecóico entre o LCL e o ML. Embora seja formado por duas bandas de fibras intimamente unidas, longitudinalmente é visto como uma faixa homogênea de fibras paralelas ecóicas. Em corte transversal, na altura do espaço femorotibial, foi relatada largura de 6-9 mm em um cavalo de 550 kg (COUDRY, V. e DENOIX, J. M., 2005). Largura e comprimento de cinco e onze milímetros, respectivamente, foram identificados em cavalos QM (Figura 17 e 18) (CISNEROS, L. E., 2008).

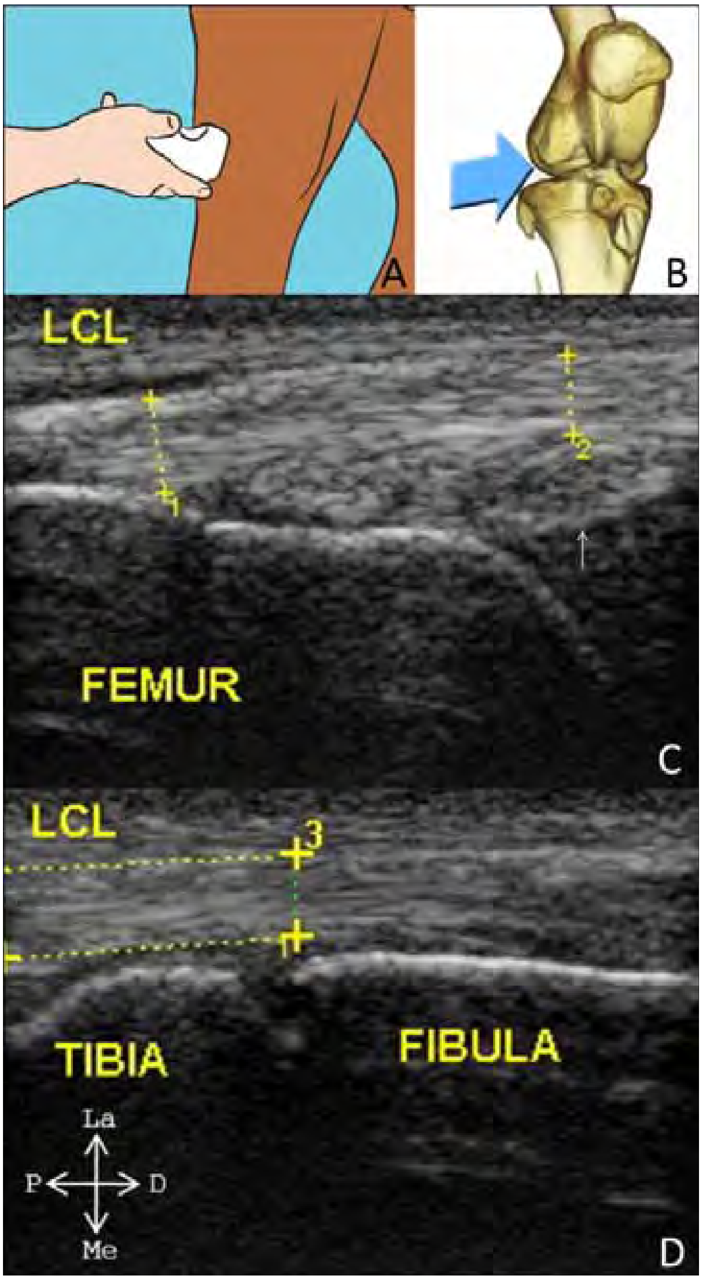

FIGURA 17. Desenho topográfico de posicionamento do transdutor (A) e de incidência do feixe de ultrassom (B) para realizar imagens ultrassonográficas em corte longitudinal do ligamento colateral femorotibial lateral (LCL). (C) Origem do LCL (linhas pontilhadas). A imagem marcada com a seta corresponde ao tendão poplíteo. (D) inserção do LCL na fíbula (linhas pontilhadas). La=lateral; Me=medial; P=proximal; D=distal. Imagens obtidas com aparelho Logic 3, General Electric™, e transdutor linear multifrequência operando a 10 MHz, 2 focos e 6 cm de profundidade. **Imagens: Arquivo pessoal.**

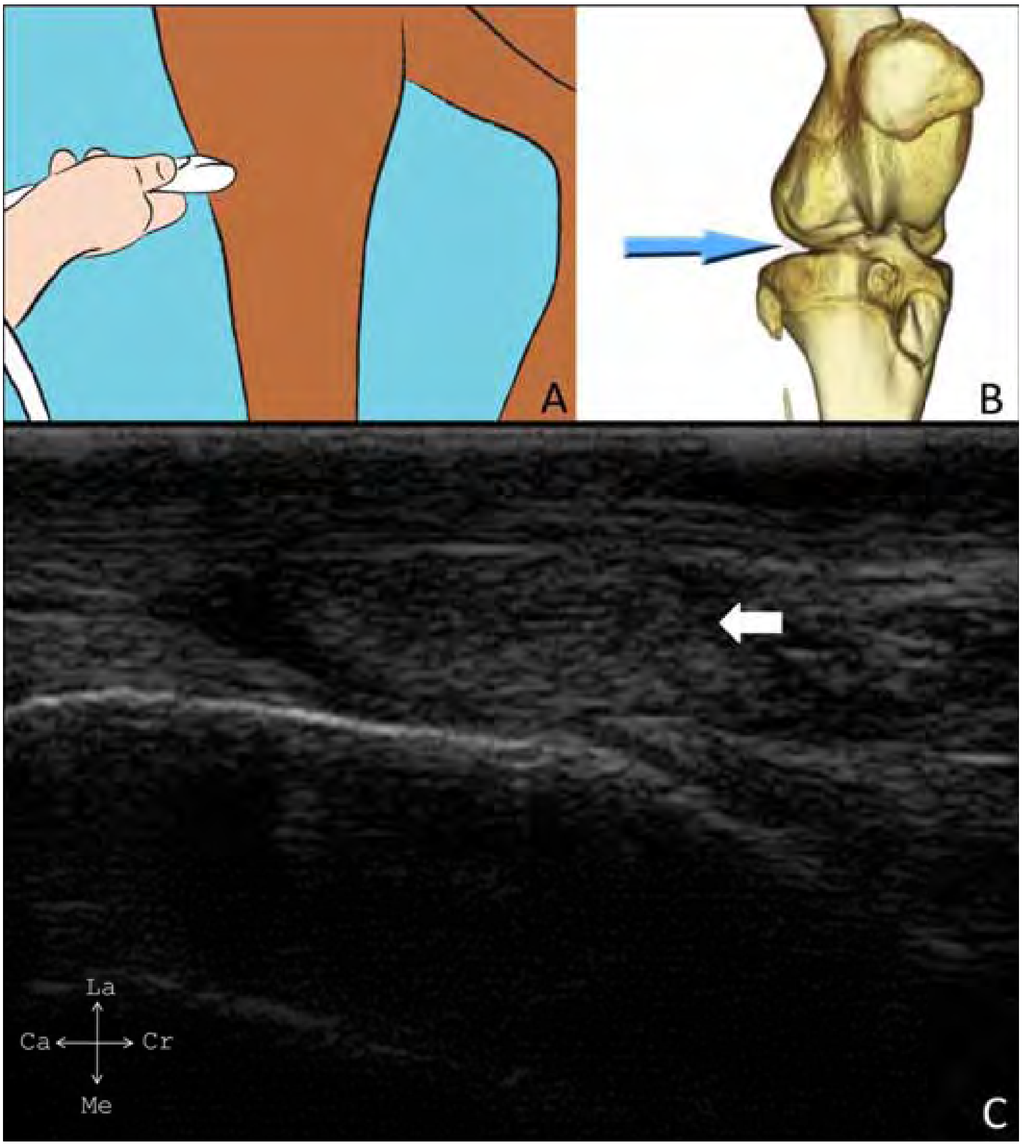

FIGURA 18. Desenho topográfico de posicionamento do transdutor (A) e de incidência do feixe de ultrassom (B) para realizar imagens ultrassonográficas em corte transversal do ligamento colateral femorotibial lateral (LCL). (C) O LCL é ecóico e regular (seta sólida). La=lateral; Me=medial; Ca=caudal; Cr=cranial. Imagem obtida com aparelho MyLab 30, Esaote™ e transdutor linear multifrequência a 10 MHz, 2 focos e 4 cm de profundidade. **Imagens: Arquivo pessoal.**

2.4.3. Ligamentos cruzados

Os ligamentos cruzados podem ser examinados pela face cranial do joelho com o membro flexionado. Como antes mencionado, a colocação do feixe de ultrassom perpendicular às fibras (vide topografia ultrassonográfica), pode representar alguma limitação na formação da imagem. O ligamento cruzado cranial (LCxCr) pode ser visualizado com a utilização de transdutores setoriais (7,5 MHz) (CAUVIN, E. R. J. et al., 1996) ou convexos (6-9 e 7-12 MHz) (HOEGAERTS, M. e SAUNDERS, J. H., 2004) tanto longitudinal, como transversalmente. O transdutor setorial deve ser posicionado aproximadamente dois centímetros distais à patela, medial ao LPI e seus feixes orientados cranioproximal-caudodistal e lateral (CAUVIN, E. R. J. et al., 1996), de tal forma que incidam a superfície da tíbia. Feito isto, se gira o transdutor 20-25° no sentido horário para o membro esquerdo ou no sentido anti-horário para o membro direito. Assim feito, a inserção do LCxCr na tíbia será visualizada como uma faixa ecóica homogênea de 1-2 cm de diâmetro (CAUVIN, E. R. J. et al., 1996). A técnica de "cinco passos" (HOEGAERTS, M. e SAUNDERS, J. H., 2004) utiliza o transdutor convexo, posicionado no sentido vertical acima do LPI, girando-se 20 graus no sentido horário ou anti-horário, dependendo do membro avaliado. Com ambas as técnicas é possível realização de imagens transversais do LCxCr no mesmo sentido.

Para visualizar a inserção femoral (cranial) de ligamento cruzado caudal (LCxCa) seguem-se as mesmas especificações descritas para o LCxCr. O transdutor deve ser posicionado de 2-3 cm proximal à crista da tíbia, entre o LPL e LPI, dirigindo-se em direção craniolateral-caudomedial e proximal (CAUVIN, E. R. J. et al., 1996).

2.4.4. Tendão comum para os músculos extensor digital longo e fibularis [peroneus] tertius

Essa estrutura tendínea pode ser avaliada com transdutor linear de 7,5-10 MHz posicionado entre o LPL e o LCL na altura do espaço femororotibial em direção craniolateral-caudomedial. Quando avaliado longitudinalmente, o tendão do *fibularis tertius* é visualizado como um conjunto de fibras ecóicas com arranjo paralelo e uniforme. Quando se acompanham as fibras do tendão em direção distal, é possível identificar fibras musculares do extensor digital longo e a origem do músculo *tibialis cranialis* imediatamente após a fossa extensora da tíbia, que é coberta por cartilagem (CAUVIN, E. R. J. et al., 1996) (Figura 19).

2.4.5. Avaliação ultrassonográfica da face caudal do joelho

Embora pouco utilizadas na prática (FLYNN, K. A. e WHITCOMB, M. B., 2002), existem técnicas para avaliação da face caudal do joelho. São realizadas com transdutores convexos com frequências baixas (3,5 MHz) para obter profundidade de 6-14 cm. Em pôneis e cavalos pouco musculosos o exame pode ser executado com transdutores de profundidade média (5-7,5 MHz) (CAUVIN, E. R. J. et al., 1996).

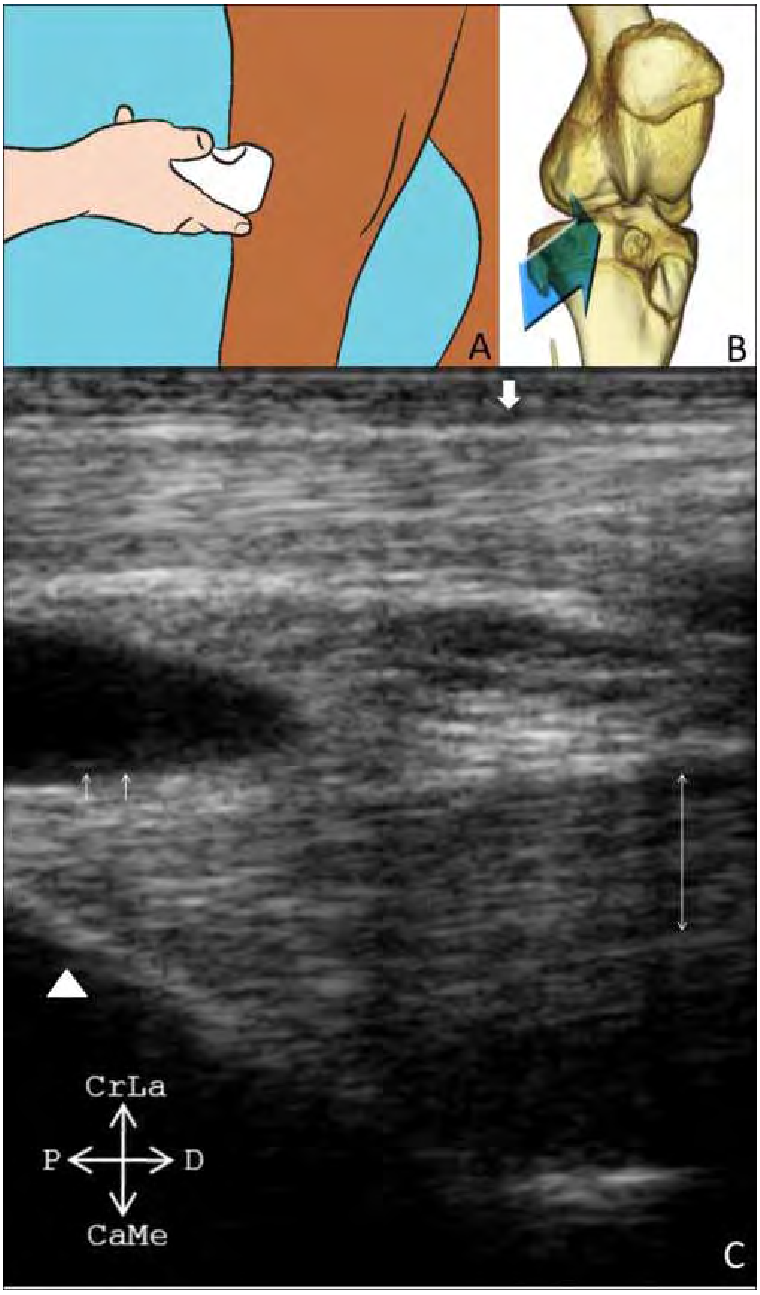

FIGURA 19. Desenho topográfico de posicionamento do transdutor (A) e de incidência do feixe de ultrassom (B) para realizar imagens ultrassonográficas em corte longitudinal do tendão comum para os músculos extensor digital longo e *fibularis tertius* (FT) na região da sua origem no fêmur. (C) Visualiza-se o FT (seta de duas cabeças) e a margem do fêmur (cabeça de seta). A imagem hipoecóica à esquerda (setas pequenas) corresponde ao recesso sinovial lateral da articulação femoropatelar lateral e é comum de ser observada em cavalos hígidos. A seta sólida superior assinala uma porção do ligamento patelar lateral. CrLa=craniolateral; CaMe=caudomedial; P=proximal; D=distal. Imagem obtida com aparelho MyLab 30, Esaote™ e transdutor linear multifrequência a 8 MHz, 2 focos e 5 cm de profundidade. **Imagens: Arquivo pessoal.**

Longitudinalmente, coloca-se o transdutor 2-3 cm proximal à dobra formada com o joelho em flexão. Assim, é possível visualizar as margens condrais dos côndilos medial e lateral, que se observam como linhas anecóicas, com a cápsula articular adjacente formando uma superfície refletora. É possível identificar o ligamento cruzado caudal e a sua inserção, axial à tíbia. Continuando em sentido lateral e girando levemente o transdutor no sentido horário para o membro direito e anti-horário para o esquerdo, identifica-se o ligamento femoromeniscal do menisco lateral, profundo às massas musculares do extensor digital superficial e poplíteo (CAUVIN, E. R. J. et al., 1996). A visualização do ligamento tibial caudal é impraticável (HOEGAERTS, M. e SAUNDERS, J. H., 2004).

2.5. Considerações gerais ás técnicas para avaliação ultrassonográfica da articulação do joelho

Por causa da complexidade e disposição espacial das estruturas anatômicas do joelho, cada uma destas deve ser avaliada individualmente (CAUVIN, E. R. J. et al., 1996).

Nas abordagens medial, cranial e lateral, todas as estruturas descritas são identificadas consistentemente quanto ao formato, posição e ecogenicidade o que coincide com estudos prévios (CAUVIN, E. R. J. et al., 1996; DENOIX, J. M. et al., 1996; DYSON, S. J., 2002; HOEGAERTS, M. e SAUNDERS, J. H., 2004). O ultrassom é a ferramenta diagnóstica de escolha no diagnóstico de lesões de ligamentos patelares (DYSON, S. J., 2002) e é útil para identificação de irregularidades nas margens ósseas da tróclea, no coxim gorduroso e nos ligamentos colaterais (DENOIX, J. M. et al., 1996; COUDRY, V. e DENOIX, J. M., 2005).

O desvio dos padrões ultrassonográficos pode ser indicativo da presença de doenças (CAUVIN, E. R. J. et al., 1996). Entretanto, estudos conduzidos pelo autor (dados não publicados) indicam que algumas alterações ultrassonográficas são frequentes em animais sob treinamento intenso, mesmo sem apresentar claudicação ou queda no rendimento atlético. Exemplo disto é a distensão do recesso poplíteo em cavalos de prova de apartação, sem significado clínico aparente.

Cranialmente, o ligamento LCxCr observa-se anecóico pela dificuldade para orientar o feixe perpendicular às fibras. Isto é particularmente evidente na falta de transdutor setorial. Além disso, o animal nem sempre tolera a flexão por tempo suficiente para realização do exame (HOEGAERTS, M. e SAUNDERS, J. H., 2004). A possibilidade de formação de imagens do LCxCr utilizando estas técnicas não está em questão, mas sim o seu valor diagnóstico no sentido de sensibilidade e especificidade (BARR, E. D. et al., 2006). Provavelmente a maior dificuldade para o avaliador esteja relacionada com a baixa repetibilidade das imagens e com o fato de existir técnicas como a radiografia e cintilografia que possuem sensibilidade de 100% para diagnóstico de alterações relacionadas com lesões nos ligamentos cruzados, embora sejam pouco específicas (26% de especificidade para radiografia e 14% para cintilografia). A cintilografia, como a radiografia, oferece informação com relação às desmopatias nas enteses, lesões ósseas e osteoartrite secundária (WALMSLEY, J. P., 2005), porém é pouco disponível. Assim, em casos de suspeita de ruptura de LCxCr a opção diagnóstica de escolha continua sendo artroscopia (BARR, E. D. et al., 2006). Para o exame do LCxCa as dificuldades na formação da imagem são as mesmas que as encontradas para examinar o ligamento cruzado cranial.

O ultrassom é útil na identificação de lesões nos meniscos e permite avaliar maior área destas estruturas quando comparado com a artroscopia (BARR, E. D. et al., 2006). Porém, dependendo do equipamento utilizado, lesões discretas nos meniscos podem passar despercebidas. Além do mais, a hipoecogenicidade nos meniscos não permite distinguir a natureza da lesão, como ruptura, fibroplasia ou edema (DE BUSSCHER, V. et al., 2006). Em todo caso, a diferenciação ultrassonográfica entre lesão aguda e lesão crônica ativa e inviável.

Utilizando abordagem caudal, foram descritas imagens ultrassonográficas da porção caudal dos meniscos (CAUVIN, E. R. J. et al., 1996; HOEGAERTS, M. e SAUNDERS, J. H., 2004). Contudo, independentemente do equipamento utilizado, as técnicas para a realização dessas imagens são ineficientes em virtude da dificuldade para obtê-las, seja pela grande quantidade de tecido muscular na face caudal do joelho ou pela dificuldade para posicionar o transdutor (FLYNN, K. A. e WHITCOMB, M. B., 2002; HOEGAERTS, M. e SAUNDERS, J. H., 2004). Mesmo assim parecem ser poucos os relatos que descrevem lesões na porção caudal do joelho (STICK, J. A. et al., 1992; HANCE, S. R. et al., 1993; JANICEK, J. C. e WILSON, D. A., 2007).

Anestesia intra-articular faz parte das técnicas incluídas no exame clínico e representa a infiltração de pelo menos 30 e até 60 mL de líquido, que pode criar artefatos na ultrassonografia. Assim, após administração de anestésico é possível observar pontos hiperecóicos em suspensão com aparência igual àqueles observados quando existem detritos de fibrina em suspensão dentro da cavidade articular. Em vista dos efeitos sobre as imagens ultrassonográficas da inoculação de anestésicos locais, recomenda-se que o exame ultrassonográfico seja realizado antes de qualquer um destes procedimentos, ou após absorção das substâncias.

2.6. Técnica radiográfica

Para formar uma imagem tridimensional básica do joelho equino, são necessários dois posicionamentos radiográficos: a lateromedial e caudocranial (BUSONI, V., 2007). Outras projeções podem ser utilizadas incluindo a caudo 60° lateral-craniomedial oblíqua; a lateromedial flexionada; a cranioproximal-craniodistal oblíqua (skyline); e a caudo 30° lateral-craniomedial oblíqua. A maioria de aparelhos portáteis de raios-X possui potencia suficiente para realização de radiografias lateromediais. Porém, para as exposições caudocraniais em cavalos adultos, utilizam-se valores na ordem dos 90 kV e 20 mAs (WEAVER, M. e BARAKZAI, S., 2010). Embora seja ideal a utilização de dispositivos para segurar o chassi à distância, o posicionamento correto com a utilização destas ferramentas é geralmente difícil. Assim, o chassi e mantido por um técnico, que utiliza luvas revestidas de chumbo. Além disso, uma boa colimação do feixe primário reduz a exposição do técnico à radiação (WEAVER, M. e BARAKZAI, S., 2010; BUTLER, J. et al., 2011).

Não é necessária preparação específica do paciente para levar a cabo radiografias do joelho. Porém, em alguns casos o animal deve ser sedado principalmente para as projeções laterais e oblíquas, nas quais o chassi é colocado em contato com a virilha do animal, o que nem sempre é bem tolerado. Assim mesmo, para as radiografias caudocraniais, o emissor de raios-X e o técnico radiologista, encontram-se posicionados atrás do membro, pelo que o risco de lesão ao operador ou dano ao dispositivo é alto (WEAVER, M. e BARAKZAI, S., 2010; BUTLER, J. et al., 2011). Entretanto, uma maneira simples de evitar a sedação e diminuir o risco de lesão ao operador e dano ao aparelho é a flexão e abdução do membro contralateral. Com isto, evita-se o movimento reflexo do animal que é provocado pelo contato do chassi, e por outro lado, melhora-se o posicionamento deste na projeção lateral, principalmente em animais muito musculosos e relativamente baixos, por exemplo, animais da raça QM.

2.6.1. Radiografia lateromedial

A radiografia lateromedial se obtém com o cavalo em apoio (BUSONI, V., 2007). Esta projeção é utilizada para avaliação das cristas medial, lateral e sulco da tróclea do fêmur, entese do ligamento cruzado cranial na tíbia e a crista tibial (WEAVER, M. e BARAKZAI, S., 2010).

Para posicionar o chassi, o membro do cavalo a ser avaliado deve ser colocado caudal ao membro contralateral (WEAVER, M. e BARAKZAI, S., 2010). O membro contralateral pode ser levantado ou abduzido para colocar o chassi o mais alto possível e assim evitar o corte da patela. O feixe deve estar centrado ao nível da articulação femorotibial e a colimação deve incluir o fêmur distal e a tíbia proximal (WEAVER, M. e BARAKZAI, S., 2010; BUTLER, J. et al., 2011).

2.6.2. Radiografia caudocranial

Este posicionamento permite a visualização das eminências intercondilares da tíbia, a fossa intercondilar do fêmur, as superfícies de apoio dos côndilos medial e lateral do fêmur e tíbia e as enteses dos ligamentos colaterais femorotibiais medial e lateral (MAULET, B. E. B. et al., 2005; WEAVER, M. e BARAKZAI, S., 2010; BUTLER, J. et al., 2011). Pode ser utilizado um filtro de alumínio para atenuar os raios laterais e mediais e evitar superexposição das margens lateral e medial da articulação, especialmente quando há suspeita de alterações periarticulares (BUTLER, J. et al., 2011).

O membro do cavalo a ser avaliado é posicionado esticado e em apoio, levemente caudal ao membro contralateral. Assim, o chassi é colocado na superfície cranial do joelho, enquanto que o feixe é direcionado com uma angulação de 10 a 20 graus proximodistal, considerando que o ponto de saída será a porção cranioproximal da tíbia. É importante tentar não apoiar o chassi no flanco sem prévio aviso ao animal, bem como tomar precaução para evitar o contato com o prepúcio no caso de animais machos (WEAVER, M. e BARAKZAI, S., 2010; BUTLER, J. et al., 2011).

2.6.3. Radiografia caudal 60° lateral-craniomedial oblíqua

Esta radiografia evidencia as mesmas estruturas que uma imagem obtida lateromedialmente, com a particularidade de ser mais fácil de obter em animais que são receosos ou muito sensíveis à colocação do chassi na região inguinal (WEAVER, M. e BARAKZAI, S., 2010; BUTLER, J. et al., 2011).

O chassi e posicionado no aspecto craniomedial do joelho tão alto quanto possível e com o membro em apoio. O feixe inclina-se aproximadamente 10° proximodistalmente o que facilita o alinhamento. As mesmas considerações descritas para as radiografias lateromediais devem ser tomadas com relação à colimação, para evitar exposição desnecessária do pessoal à radiação (WEAVER, M. e BARAKZAI, S., 2010; BUTLER, J. et al., 2011).

2.6.4. Radiografia lateromedial flexionada

Esta radiografia permite melhor observação das superfícies proximais das cristas da tróclea femoral, devido ao deslocamento distal da patela com relação ao fêmur. Também melhora a visualização da tíbia proximal ao nível das eminências intercondilares e o ápice da patela (WEAVER, M. e BARAKZAI, S., 2010; BUTLER, J. et al., 2011).

O membro é flexionado enquanto se posiciona o chassi na face medial. As porções distal do fêmur e proximal da tíbia devem ser incluídas. Deste modo, o feixe orienta-se horizontal e centrado à articulação femorotibial (WEAVER, M. e BARAKZAI, S., 2010; BUTLER, J. et al., 2011).

2.6.5. Radiografia cranioproximal-craniodistal oblíqua (skyline)

Esta projeção é utilizada quando existe suspeita de fratura da patela que não foi confirmada com outras radiografias. Também permite avaliar as cristas medial e lateral bem como o sulco da tróclea do fêmur (WEAVER, M. e BARAKZAI, S., 2010; BUTLER, J. et al., 2011).

Com o membro flexionado e o eixo da tíbia paralelo ao chão, coloca-se o chassi com a face sensível dirigida para cima e em contato com a crista tibial. Assim feito, o feixe direciona-se proximodistalmente com angulação lateromedial de aproximadamente 10 graus. A colimação deve incluir a face cranial da patela (WEAVER, M. e BARAKZAI, S., 2010).

2.6.6. Radiografia caudo 30° lateral-craniomedial oblíqua

Utiliza-se para isolamento do côndilo medial do fêmur e cristal lateral da tróclea femoral. É especialmente útil para confirmação da presença de cistos ósseos subcondrais no côndilo medial (CONTINO, E. K. et al., 2012).

O chassi é posicionado no aspecto craniomedial do joelho tão alto quanto possível e com o membro em apoio. O feixe inclina-se aproximadamente 10 graus proximodistalmente o que facilita o alinhamento perpendicular ao chassi. As mesmas considerações descritas para as radiografias lateromediais devem ser tomadas com relação à colimação, para evitar exposição desnecessária do pessoal à radiação.

2.7. Comentários finais

Algumas causas de claudicação do joelho incluem trauma direto (DYSON, S. J., 1994; SINGER, E. R. et al., 2008), trauma por diferença nas forças de tensão (FOWLIE, J. G. et al., 2012) e doenças do desenvolvimento (JACKMAN, B. R., 2001). São exemplos das anteriores: fraturas; ruptura ou inflamação de ligamentos patelares, colaterais, cruzados ou meniscais; lesões nos meniscos; osteocondrose; e os cistos subcondrais (JEFFCOTT, L. B. e KOLD, S. E., 1982a; BUKOWIECKI, C. F. et al., 1988; DENOIX, J. M. et al., 1996; WALMSLEY, J. P. et al., 2003; WALMSLEY, J. P., 2005; BUTLER, J. et al., 2011).

O diagnóstico de lesões no joelho requer exame físico, analgesia intra-articular e exames por imagem (WALMSLEY, J. P., 2005). A radiografia é essencial para a visualização de alterações ósseas (JEFFCOTT, L. B. e KOLD, S. E., 1982b; JEFFCOTT, L. B., 1984; WALMSLEY, J. P., 2005; BUTLER, J. et al., 2011). Porém, exceto em casos de luxação, fratura ou ruptura de ligamento colateral medial, a radiografia oferece pouca informação relacionada a problemas agudos e de tecidos moles (DENOIX, J. M. et al., 1996; BARR, E. D. et al., 2006).

Por outro lado, a ultrassonografia permite a visualização dos ligamentos patelares, colaterais femorotibiais, meniscos, ligamentos meniscais, cápsula articular femoropatelar e femorotibial, ligamentos cruzados, tendão comum para os músculos extensor digital longo e *fibularis tertius*, tendão poplíteo, superfícies da tróclea e côndilos femorais (PENNINCK, D. G. et al., 1990; CAUVIN, E. R. J. et al., 1996; DENOIX, J. M. et al., 1996; DYSON, S. J., 2002; FLYNN, K. A. e WHITCOMB, M. B., 2002; COUDRY, V. e DENOIX, J. M., 2005; DE BUSSCHER, V. et al., 2006; MARTINELLI, M. J. e RANTANEN, N. W., 2009). Contudo, muitos clínicos veterinários especialistas em equinos continuam utilizando unicamente radiografias, em vista de que o ultrassom é altamente dependente do treinamento do operador (BARRETT, M. F. et al., 2012). Porém, o ultrassom proporciona informações significativas que são imprescindíveis antes da realização de artroscopia, a mesma que é considerada como padrão ouro tanto para diagnóstico como para terapia do joelho (BARRETT, M. F. et al., 2012).

A radiografia e o ultrassom são elementos corolários a um exame físico acurado e sistemático (WALMSLEY, J. P., 2005). A radiografia é o alicerce do diagnóstico de alterações relacionadas ao esqueleto apendicular dos equinos incluindo o joelho (JEFFCOTT, L. B. e KOLD, S. E., 1982b; JEFFCOTT, L. B. e KOLD, S. E., 1982a; DENOIX, J. M. et al., 1996). O exame ultrassonográfico por sua vez, deve ser indicado sempre como procedimento de triagem quando há suspeita de claudicação do joelho para avaliação dos tecidos moles. Considera-se que por ser uma técnica não invasiva, de baixo custo, e proporcionar informações relevantes com relação ao status das estruturas do joelho dos equinos, a ultrassonografia deve ser incluída no dia a dia, não somente dos hospitais de referência, mas do Médico Veterinário no campo. Porém, existem seis condições para a sua realização: conhecimento anatômico, conhecimento do comportamento do ultrassom nos tecidos, conhecimento da aparência ultrassonográfica normal dos tecidos, bom equipamento, preparação do paciente e treinamento.

CAPÍTULO 2

Trabalho para ser enviado a revista científica *Equine Veterinary Journal*

ULTRASONOGRAPHIC AND RADIOGRAPHIC ABNORMALITIES IN THE STIFLE JOINT OF CUTTING QUARTER HORSES

Reasons for performing study: Cutting is one of the most popular uses of Quarter Horses. Although injuries related with the femorotibial and femoropatelar joints are common in this athletic activity, there is limited information regarding ultrasonographic and radiographic findings in horses under strenuous training or competition.

Objective: to investigate the most common ultrasonographic and radiographic abnormalities in Cutting Quarter Horses**.**

Study design: cross-sectional study.

Methods: Ultrasonographic examination, radiographs and lameness assessment of the stifles were performed in 3-year-old and more than 4-year-old Quarter Horses. Abnormalities were categorized and compared between groups.

Results: Ultrasonographic abnormalities were identified in 100% of the horses. Synovitis was the most common finding (89.3%), followed by rarefaction of the medial meniscus (64.3%) and desmitis of the medial femorotibial collateral ligament (33.9%). Abnormalities were present in radiographs in 59.5% of horses. Significant differences were found in the number of radiographic abnormalities between groups as well as in the number of lame horses before and after flexion test. Association between type of meniscal lesions and degree of lameness was not found.

Conclusions: There is a high proportion of ultrasonographic and radiographic changes in the stifles of cutting Quarter Horses, disregarding age. However, the number of radiographic abnormalities is higher among younger horses. A significant number of cutting horses performs while tolerating some degree of musculoskeletal pain.

Potential relevance: Practitioners should expect a high proportion of ultrasonographic and radiographic changes in the stifles of cutting Quarter Horses. They also should be aware of the importance of performing lameness examination and imaging tests in animals with no apparent lameness for trainers or owners.

Key words: Quarter Horse; cutting; ultrasonography; radiographs; lameness; stifle.

Introduction

In training and sporting events of cutting, horse and rider isolate and keep separated a cow from the herd, using for this quick stops and high torque turns. The characteristics of this stock-horse event also include high speed working and sudden changes of direction [1]. These repetitive pivot movements and abrupt stops cause stress in the hindlimb, and may lead to injuries at the tarsus, femorotibial and femoropatelar joints [2; 3].

Radiographs and ultrasonography are basic tools for detection of stifle abnormalities [4; 5]. Radiographic repositories have been created in order to aid in the detection and prevention of current or future orthopaedic diseases [2]. Such repositories have been specially useful at Thoroughbred yearling sales [6] and had become popular among owners and trainers of Quarter Horses intended for cutting, since were added to the National cutting Horse Association (NCHA) sales in 2005 [2]. There is one work describing the prevalence of radiographic chances in the tarsi, carpi, fetlocks and stifles of yearlings and 2-year-old Quarter Horses intended for cutting. This previous research pointed out the tarsi and stifles as the most common sites for abnormalities among this group of horses [2]. On the other hand, data on the ultrasonographic changes in the stifles of Quarter Horses, training or sporting in cutting has not been published. Thus, this study aims to describe common ultrasonographic and radiographic abnormalities at the stifles of cutting Quarter Horses.

Materials and Methods

Animals

Fifty-six cutting Quarter Horses from two ranches, with mean weight of 443 ± 30 kg, and mean height to the withers of 1.46 meters ± 4 centimetres were included. Animals were divided into two groups, corresponding to training group (TG): 33 horses aging between 28 and 47 months (mean 37 ± 6 months) and training for the futurity; and competition group (CG): 23 horses aging between 48 and 148 months (mean 76 ± 25 months) that had competed at least once in an official event and effectively competing at the moment of evaluation. Exclusion criteria: horses with lameness or drop of performance perceived by the trainer/owner; history of lameness from any musculoskeletal structure within two months prior to evaluation; history of infiltration in the stifle at any moment in the horses' lifetime; animals who had been infiltrated with corticosteroids in any other musculoskeletal structure within two months prior to evaluation. The research was approved by the Committee of Ethics and Animal Welfare of the School of Veterinary Medicine and Animal Science, UNESP - Univ. Estatual Paulista under the protocol number 75/2010.

Lameness evaluation

Animals were evaluated at walk and trot in a straight-line solid surface of approximately 50 meters-length. Lameness at trot was documented before and after flexion test specific for the stifle, and scored from 0 to 5, were 0 is a sound animal and 5 is inability to weight bearing [7].

Ultrasonographic evaluation

Ultrasonographic examination was performed in the stifles of the 56 animals using a systematic approach [8]. The area was clipped, washed and a quantity of coupling gel was applied. Scanning in transversal and longitudinal planes was performed with a linear 6-10 MHz transducer and a convex 2-8 MHz transducer (MyLab 30Vet, ESAOTE, Geneve, Italy). Evaluated anatomical structures included medial (MCL) and lateral (LCL) femorotibial collateral ligaments; Medial (MPL), intermediate (IPL) and lateral (LPL) patellar ligaments; medial (MFT) and lateral (LFT) femorotibial joints; femoropatellar joint (FP); chondral surfaces of the medial (MTR) and lateral (LTR) trochlear ridges as well as the trochlear groove (TrG); common tendon for the long digital extensor and *fibularis [peroneous] tertius* muscles (FT); popliteal tendon (PT); proximal (PB) and distal (DB) infrapatelar bursae; and the medial (MM) and lateral (LM) menisci. The MFT was evaluated taking in account the subextensor recess and the outpouching under the origin of the PT [9], which was named as popliteal recess.

Tendons and ligaments were evaluated as normal or affected, following the criteria by Genovese et al. [10]. In order to categorize the meniscal lesions, the classification by De Busscher et al. was used [11]. When more than one lesion was visualised into the meniscus, it was included in the most complex category. Protrusions were described separately.

Radiographic evaluation

Caudocranial (CC) and lateromedial (lateral) digital radiographs of both limbs of 37 horses (i.e., 26 TG and 11 CG) were performed. When images of the medial femoral condyle or lateral trochlear ridge were inconclusive caudal 30° lateral-craniomedial oblique views (CaLCrMO) were obtained.

Radiographs were evaluated by two authors. Any discrepancy was resolved by consensus. Abnormalities were categorized following the criteria by Contino et al. [2]. CC radiographs were mainly used to evaluate the MFC and the medial surface of the proximal tibia. Lateral views were used to visualize the margins of the MTR, LTR, TrG and surfaces of the patella.

Statistical analysis

Frequency of ultrasonographic and radiographic abnormalities, as well as presence and degree of lameness were determined by limb and by animal. Then, Qui-square or Fisher exact tests were used to compare: (1) proportion of ultrasonographic and radiographic abnormalities between groups; (2) proportion of lame horses before and after flexion test; (3) distribution of the scores of lameness between groups before and after flexion test; and (4) presence and degree of lameness of the overall number of horses before and after the flexion test. Regarding the menisci, association between type of meniscal lesion and degree of lameness was investigated by group and by the total number of animals, considering the number of evaluated limbs (n=112). All analysis were performed with PROC FREQ (SAS Institute, 2011), and significance was stipulated at 0.05.

Results

Ultrasonographic examinations of 112 hindlimbs were performed. Additionally, 203 radiographs from the stifles of 37 horses were evaluated. Data on (1) lameness before and after flexion test, (2) ultrasonographic and (3) radiographic abnormalities are summarized in tables 1, 2 and 3, respectively. *(P)* values are only discussed when significant differences were found. Results are presented either by animal or by limb.

Lameness evaluation

Forced flexion of the stifle was performed in 45 of the 56 horses. Before flexion, 14.3% (8/56 horses, three form TG and five from CG) showed spontaneous grade 1 lameness. After flexion, 57.8% (26/45) presented grade 1 lameness, which was bilateral in three of the animals (one of them had grade 2 lameness). One horse from the TG had grade 3 lameness after forced flexion. Statistical differences between groups regarding the number of lame horses before or after the flexion test were not found. However, the number of lame horses was significantly higher after flexion test between animals of the TG ($P=0.04$). This significant difference was also observed between the limbs of the TG ($P=0.01$), the limbs of the CG ($P<0.01$), the total number of lame horses ($P<0.01$) and the total number of limbs, before and after flexion test ($P<0.01$).

Ultrasonographic evaluation

Ultrasonographic abnormalities were found in the overall number of horses. Synovitis of the popliteal recess of the LFT was observed in 71.4% (40/56) of horses, which was bilateral in 45% (18/40). Three of these horses also presented synovitis of the subextensor recess, which was bilateral in one animal. When considering the number of affected limbs, synovitis of the LFT was recorded in 51.8% (58/112) of the stifles.

The MFT was affected in 39.3% (22/56) of horses. The most common findings were free-floating echoic dots as well as fibrin trabeculae adhered to the synovial lining and loculations in the medial recess.

The FP had ultrasonographic signs of synovitis in 69.6% (39/56) of horses. Lateral and medial effusion was observed in 92.3% (36/39) and 20.51% (8/39) of horses, respectively. The latter, was observed as a prominent sac, full of liquid and in contact with the medial recess of the MFT. Severe bilateral effusion was identified cranially in two horses belonging to the CG, characterized by the presence of fibrin detritus adjacent to the trochlear groove.

As a whole, synovitis in at least one joint compartment (i.e., MFT, LFT and FP) was found in 89.3% (50/56) of horses and 50% were bilaterally affected.

Regarding the MM, changes were identified in 64.3% (36/56) of horses, from which, 26.8% had bilateral changes i.e., 10 from TG and five from CG. Forty-one per cent (23/56) had type one lesion; 28.6% (16/56) type 2; 7.1% (4/56) type 3 and 1.8% (1/56) type 4.

Ten of fifty-six horses (17.9%) had changes recorded in the LM and two of these were bilaterally affected. Five horses (8.9%) had type 1 lesion; two (3.6%) type 2; one (1.8%) type 3; and two (3.6%) type 4. Protrusion was present in one MM of the TG and one LM of the CG.

When evaluated by limb, the TG had a higher number of abnormalities in the MCL than the CG (P=0.03). Lesions in the MCL were found in 33.9% (19/56) of horses, including hypoechogenicity (desmitis) and peripheral oedema. Ultrasonographic changes compatible with mild desmopathy were present in the LCL in 12.5% (7/56) of horses.

Rarefaction of the chondral surface (two TG horses) and subchondral defect no visualized in radiographs (two CG horses) were recorded in 7.1% of the animals at the LTR. Likewise, rarefaction of subchondral bone of the MTR was present in 8.9% (5/56) of the TG. The same change was identified in the trochlear groove of two TG and two CG horses.

Hypoechoic and hyperechoic regions consistent with desmitis and ligament scars were present in the patellar ligaments. The MPL was affected in 33.9% (19/56), IPL in 37.5% (21/56) and LPL in 12.5% (7/56) of horses.

Other changes such as proximal and distal infrapatellar bursitis, sclerosis of the patella surface and oedema of the patellar fat pad were also observed.

Radiographic evaluation

Twenty-two of 37 evaluated horses (59.5%) had at least one radiographic change recorded. The overall number of changes was significantly higher in the TG than in the CG, when compared by animal (P=0.02) and by limb (P<0.01).

Changes at the MFC were identified in 43.2% (16/37) of horses and seven (18.9%) of these presented changes in both limbs. Grade 1 changes were present in 37.8% (14/37), grade 3 in 5.4% (2/37) and grade 4 in 5.4%.

Radiographic changes which were exclusively identified in the TG included lucency and flattening at the LTR in 8.1% (3/37); irregularity of the TrG in the same percentage of animals; patellar osteophytes in 5.4% (2/37); patellar lucency in 10.8% (4/37). Other changes included roughening of the lateral and medial surfaces of the tibial condyles and rarefaction of the contact surface of the tibia with the medial meniscus. Tibial osteophytes were identified in both groups.

Discussion

Lameness evaluation

All of the animals were reported by the trainer/owner as working without evident lameness or drop of performance. Even so, some degree of lameness was identified by the veterinarians either before or after forced flexion, the same that was identified by the trainer after the flexion test. It is important to consider that cutting horses execute sort, high-speed runs, making difficult the identification of subtle lameness for an untrained eye. Additionally, this horses work on soft, sandy ground, which may hide gait abnormalities and so, lameness only will be evident when horses are evaluated on a solid surface [1].

As in previous studies [1; 12; 13] the percentage of horses with lameness identified after forced flexion (56.5%), together with the high number of ultrasonographic and radiographic abnormalities, indicate that a number of cutting horses are willing to perform suffering from some degree of pain, without apparent detriment on their athletic ability. This highlights the need for communication and team work between veterinarians and trainers to carry out not only therapeutic but preventive work within the ranches.

Flexion test was unviable in 11 TG horses which had as the main changes: tibial osteophytes, flattening of the LTR, changes on the MFC, different types of meniscal lesions and mild desmitis of the MPL. It was not possible to establish if the reluctance to forced flexion was caused by pain secondary to the abnormalities or by the horses' temperament (all of the non-flexed animals belonged to the younger -TG- group), since diagnostic analgesia was not performed.

Ultrasonographic evaluation

Synovitis of the LFT was the most common ultrasonographic finding. Images of what could be considered the popliteal recess were previously published [11], but no specific reference to this compartment has been made hitherto. The term synovitis of the LFT may also include synovial proliferation of the subextensor recess, which seems not to be a common finding among cutting horses i.e., it was present in only 7.5% (3/40) of affected animals. In contrast, distension of the popliteal recess was recorded in 100% (40/40) of cutting horses with LFT changes. Although the presence of liquid into the LFT has been described as abnormal [8; 14], the results from this work pointed out the distension of the popliteal recess as a common and expected finding in cutting horses.

Synovitis of the FP affected 69.6% of horses. Previous studies with a number of breeds and equestrian modalities identified FP synovitis in 17.6% of the evaluated horses [15]. In this sense, lack of data on ultrasonographic findings in cutting horses makes the comparison of results difficult. Liquid adjacent to the LTR was previously described as normal [15]. Thus, synovitis was considered only in the presence of prominent swelling and distension of the lateral compartment of the FP, ensheating the FT and reaching the extensor fossa, distally. Medially, the increased synovia, fibrin trabeculae and hyperechoic dots suggest additional attrition between the medial joint surfaces of the trochlea and patella. Surprisingly, the two horses presenting fibrin detritus adjacent to the groove and bilateral severe effusion of the FP had no spontaneous gait abnormalities, and only one of them presented grade 1 lameness after flexion.

The percentage of synovitis of the MFT herein (39.3%) was lower than in a previous study, which reported this pathological entity in 54% of patients with meniscal lesions [11]. In the present study however, animals without evident clinical manifestations were used. Since the presence of liquid is normal in the MFT recess [8], synovitis was considered in the presence of capsule thickening, hyperechoic trabeculae, or well-consolidated dots, suggesting fibrin or mineral clusters within the medial recess [11; 16-18].

In general, results from this study showed the stifle effusion as a common finding in cutting Quarter Horses. Laboratorial analysis would be important in order to determine the nature of this synovia [11], in an attempt to establish an association between the composition of the liquid and the ultrasonographic findings.

The percentage of meniscal changes (64.3% of the total horses) was higher than expected for a group of animals without decreased performance. A retrospective study in animals with lameness referable to the femorotibial joint showed meniscal lesions in 86.5% of horses [11]. However, no articles have been published describing ultrasonographic changes in the menisci of horses with any evident gait abnormality. The frequencies of types of lesions were different in cutting horses than in a previous study with animals suspicious of meniscal injury [11]. Type 1 lesion was previously described in 23.2% (16/69) of affected menisci, type 2 in 27.5%, type 3 in 7.5%, type 4 in 10.15% and type 5 in 29%. In the present study, type 1 lesion was recorded in 55.4% (36/65) of affected menisci, type 2 in 29.2%, type 3 in 10.8% and type 4 in 4.6%. Type five injuries were not recorded herein. Type 1 lesions were two-fold higher than in a previous study [11] and type 2 had the same proportion. Few inferences arise in this respect: the increased number of type 1 ultrasonographic lesions in cutting horses may be a consequence of repetitive stress caused by the quick stops and high-torque turns [1; 3], mainly affecting the tarsus and the stifle and so predisposing to meniscal degeneration. Other hypothesis derives from the fact that a deleterious effect in the menisci would be expected as a consequence of a chronic inflammatory process (i.e., synovitis), which was identified in the stifle of almost every horse in this study. On the other hand, it was not possible to elucidate if the central hypoechogenicity of the menisci should be classified as a true lesion in young animals since, in the practice, authors had observed this phenomenon in musculoskeletal-immature horses from other breeds, used for a variety of equestrian modalities. Finally, association between type of meniscal lesion and degree of lameness before or after forced flexion was not identified, which coincides with a previous study [11]. Further studies should be conducted in order to investigate the clinical meaning of these findings in specific athletic modalities.

As in previous reports [11; 19], most of the meniscal changes were observed in the MM (83%) as compared with the LM (18,5%), which is explained by the higher load imposed to the MM, making it more susceptible to compression and tearing [20].

Ultrasonographic assessment is essential in a suspicion of meniscal damage, because of its high sensitivity and specificity (80% and 87,5%, respectively) [21]. The system created by De Busscher et al. [11] is an adaptation of a method described in human medicine using magnetic resonance [22], which allows to classify the type of meniscal abnormalities. No other studies had been published using this classification. However, diffuse echogenicity caused by fibrosis without mineralization into the meniscus seems not to be explicitly contemplated in the system. Thus, this diffuse echogenicity, when present as a single entity, was classified as a type 1 lesion.

A significantly higher number of injuries were detected in the MCL of the TG when compared with the CG. A previous work pointed out an increased number of lesions between younger Quarter Horses in the stock horse competitions [1]. Nevertheless, this significant difference was not identified in the ultrasonographic examination of any other anatomical structure of the stifle. Anecdotally, one of the horses identified as suffering from mild desmitis of the MCL, was presented to the Teaching Hospital of the Univ. Stadual Paulista six months after the ultrasonographic assessment. In this occasion, the horse had grade 3 lameness with origin in the stifle. Ultrasonographic reassessment revealed swelling, hypoechogenicity and loss of the oval format of the MCL. Lameness immediately dropped from grade 3 to grade 1 after periligamentar injection of lidocaine. Unfortunately, this lesion impeded the horse to participate in the futurity. Studies should be conducted to identify the economic losses caused by horses that are not able to participate in the futurities due to subtle injury over the training period. The ultrasonographic evaluation had potential to identify risks of injury and so may be an aid to orient the type of exercises and intensity of the training. In cutting horses, special attention must be paid to the tarsus and stifle [3].

At the trochlea, ultrasound was described as the technique of choice to identify discrete defects, no detected in radiographs [23]. In this study, rarefaction, chondral and subchondral defects were identified. Ultrasonography may be used as a primary tool to identify changes such as LTR chondral defects, even before the use of oblique radiographic views, so following the principles of ALARA (as low as reasonably achievable) by decreasing radiation exposure to patients and medical personnel [24; 25].

Hypoechoic and hyperechoic areas compatible with desmopathy were recorded in the patellar ligaments. Previous studies stated that injury in these structures may go unnoticed, due to the absence of clinical signs [5]. Overall, most of the changes were identified in the IPL when compared with the MPL and LPL [5; 17].

Radiographic evaluation

The TG had more changes than the CG ($P \leq 0{,}05$), which reinforces previous anecdotal statements [1], in the sense that younger animals present more injuries than older horses.

The prevalence of radiographic findings in yearlings and two-year-old Quarter horses intended for cutting was previously described [2], making evident changes in the stifles in 44.5% of radiographed horses. In the present study, changes in the stifle were present in 59.5% of horses, which may be explained because we evaluated older animals, under intense training or already competing. Still, the data herein support anecdotal information that stifle is predisposed to injury in cutting horses, ranking only behind tarsal lesions [3; 26]. The difference in the percentage of radiographic lesions when compared with the results from Contino et al. [2] may be caused by the high number of tibial osteophytes, patellar lucency, groove defects and rarefaction of the medial an lateral surfaces of the tibial condyles. Presumably, these changes are exacerbated by training and repetitive stress and so, are less common among untrained horses.

The percentage of changes in the MFC was as high (43.2%) as in a previous report (41.4%) [2]. This similarity is expected since most of the horses in the present study had not previous radiographs and congenital abnormalities might be unnoticed in the absence of clinical signs. In this study, subchondral cysts were identified in two horses without evidence of lameness of drop of performance. Grade 1 lameness was identified in one limb of one of these horses, but only after forced flexion. Cysts may be an incidental finding and may or may be not related to lameness [1]. In this sense, an study in Thoroughbreds pointed out a negative impact of the presence of cyst in sales but no in athletic performance [27]. No similar studies have been published in cutting Quarter Horses.

These ultrasonographic and radiographic findings make evident the importance of preventive veterinary care for both futurity prospects and competition horses. Correct scheduling, including rest periods and variation in intensity and type of exercises may help to decrease the risk of injury [1] and so the losses caused by a horse that has been thoroughly trained, but exhibits suboptimal performance. Understanding and identification of risk factors is critical for prevention of common injuries. Although at high level competition complete elimination of injury is unlikely, is the veterinarian's task to identify the preventable risk factors in order to diminish or, if possible, eliminate them [28].

The veterinarian should be aware of the changes present in the stock horse and western modalities, and to be familiar with the activity and risk factor inherent to each competition group. From the very beginning of their athletic lives, a high number of ultrasonographic and radiographic changes might be expected in the stifles of cutting Quarter Horses. Further studies should be conducted to investigate the influence of these changes in the long-term performance.

Conclusions

There is no evidence of difference in the proportion of horses with subtle lameness between young-adults and adult horses, both under intensive training for cutting. However, many horses compete in the futurity suffering from injuries or lameness, which is not always evident for the trainer/owner.

A high number of ultrasonographic and radiographic changes are expected in cutting horses.

The most common ultrasonographic finding in the stifle of cutting horses is synovitis followed by some type of meniscal abnormality.

There is no evidence of association between the type of meniscal lesions and lameness degree, as well as between the presence of meniscal abnormalities and presence of lameness.

In horses with similar training schedules, the presence of radiographic changes is higher among younger horses.

There is a need for preventive veterinary care in order to detect subtle abnormalities that may be potential causes of lameness.

Acknowledgements

The authors thank the CAPES PEC/PG (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior) for providing the PhD scholarship for the first author. We also would like gratefully acknowledge Haroldo Pessoa de Araújo Sobrinho and Francisco Carlos Taboga for their help and generosity.

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TABELAS DO CAPÍTULO 2

Table 1. Lameness before and after forced flexion of the stifles of cutting Quarter Horses in training for futurities (TG, 28 to 47 months old) and in competition (CG, 48 months and older).

Lameness	Training (TG)			Competition (CG)		
	Right limb (%)	Left limb (%)	Horses (%)	Right limb (%)	Left limb (%)	Horses (%)
Before flexion	n=33	n=33	n=33	n=23	n=23	n=23
Grade 0	31 (93.9)	32 (97)	30 (90.9)	22 (95.7)	19 (82.6)	18 (78.3)
Grade 1	2 (6.1)	1 (3)	3 (9.1)	1 (4.3)	4 (17.4)	5 (21.7)
Grade 2	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Grade 3	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Grade 4	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Grade 5	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Total	2 (6.1)	1 (3)	3 (9.1) **ac**	1 (4.3)	4 (17.4)	5 (21.7) **ac**
After flexion	n=22	n=22	n=22	n=23	n=23	n=23
Grade 0	16 (72.7)	16 (72.7)	10 (45.5)	15 (65.2)	14 (60.9)	9 (39.1)
Grade 1	5 (22.8)	6 (27.3)	11 (50)	8 (34.8)	8 (34.8)	13 (56.5)
Grade 2	0 (0	0 (0)	0	0 (0)	1 (4.3)	1 (4.3)
Grade 3	1 (4.5)	0 (0)	1 (4.5)	0 (0)	0 (0)	0 (0)
Grade 4	0 (0	0 (0)	0 (0	0 (0)	0 (0)	0 (0)
Grade 5	0 (0	0 (0)	0 (0	0 (0)	0 (0)	0 (0)
Total	6 (27.3)	6 (27.3)	12 (54.5) **ad**	8 (34.8)	9 (39.1)	14 (60.9) **ac**

Different low case letters in the same row (a-b) differ in P-value (< 0.05).
Different low case letters in the same column (c-d) differ in P-value (< 0.05).

Table 2. Ultrasonographic abnormalities in the stifles of cutting Quarter Horses in training for futurities (TG, 28 to 47 months old) and in competition (CG, 48 months and older).

	Training (TG)			**Competition (CG)**		
	Right limb (%)	Left limb (%)	Horses (%)	Right limb (%)	Left limb (%)	Horses (%)
Stifles changes	n=33	n=33	n=33	n=23	n=23	n=23
No	2 (6.1)	0 (0)	0 (0)	2 (8.7)	0 (0)	0 (0)
Yes	31 (93.9)	33 (100)	33 (100)	21 (91.3)	23 (100)	23 (100)
MCL	n=33	n=33	n=33	n=23	n=23	n=23
No	23 (69.7)	23 (69.7)	19 (57.6)	19 (82.6)	21 (91.3)	18 (78.3)
Yes	10 (30.3)	10 (30.3)	14 (42.4) **a**	4 (17.4)	2 (8.7)	5 (21.7) **b**
LCL	n=33	n=33	n=33	n=23	n=23	n=23
No	29 (87.9)	26 (78.8)	23 (69.7)	21 (91.3)	21 (91.3)	19 (82.6)
Yes	4 (12.1)	7 (21.2)	10 (30.3)	2 (8.7)	2 (8.7)	4 (17.4)
Medial meniscus	n=33	n=33	n=33	n=23	n=23	n=23
Normal	19 (57.6)	19 (57.6)	10 (30.3)	12 (52.2)	9 (39.1)	2 (8.7)
Type 1	6 (18.2)	10 (30.3)	12 (36.4)	4 (17.4)	10 (43.5)	11 (47.8)
Type 2	6 (18.2)	2 (6.1)	8 (24.2)	5 (21.7)	4 (17.4)	8 (34.8)
Type 3	2 (6.1)	2 (6.1)	3 (9.1)	1 (4.3)	0 (0)	1 (4.3)
Type 4	0 (0)	0 (0)	0 (0)	1 (4.3)	0 (0)	1 (4.3)
Type 5	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Lateral meniscus	n=32	n=33	n=33	n=23	n=23	n=23
Normal	29 (90.7)	28 (84.8)	27 (81.8)	20 (87)	22 (95.7)	19 (82.6)
Type 1	1 (3.1)	1 (3)	1 (3)	3 (13)	1 (4.3)	4 (17.4)
Type 2	0 (0)	2 (6.1)	2 (6.1)	0 (0)	0 (0)	0 (0)
Type 3	1 (3.1)	1 (3)	1 (3)	0 (0)	0 (0)	0 (0)
Type 4	1 (3.1)	1 (3)	2 (6.1)	0 (0)	0 (0)	0 (0)
Type 5	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
MFT (synovitis)	n=33	n=33	n=33	n=23	n=23	n=23
No	24 (72.7)	26 (78.8)	21 (63.6)	20 (87)	17 (73.9)	13 (56.5)
Yes	9 (27.3)	7 (21.2)	12 (36.4)	3 (13)	6 (26.1)	10 (43.5)
LFT (synovitis)	n=33	n=33	n=33	n=23	n=23	n=23
No	14 (42.4)	15 (45.5)	8 (24.2)	15 (65.2)	10 (43.5)	8 (34.8)
Yes	19 (57.6)	18 (54.5)	25 (75.8)	8 (34.8)	13 (56.5)	15 (65.2)
FP (synovitis)	n=33	n=33	n=33	n=23	n=23	n=23
No	19 (57.6)	17 (51.5)	10 (30.3)	13 (56.5)	11 (47.8)	7 (30.43)
Yes	14 (42.4)	16 (48.5)	23 (69.7)	10 (43.5)	12 (52.2)	16 (69.6)

Different low case letters in the same row (a-b) differ in P-value (< 0.05).
MCL = medial femorotibial collateral ligament; LCL = lateral femorotibial collateral ligament; MFT = medial femorotibial joint; LFT = lateral femorotibial joint; FP = femoropatellar joint.

Table 2. *Continuation.* Ultrasonographic abnormalities in the stifles of cutting Quarter Horses in training for futurities (TG, 28 to 47 months old) and in competition (CG, 48 months and older).

	Training (TG)			Competition (CG)		
	Right limb (%)	Left limb (%)	Horses (%)	Right limb (%)	Left limb (%)	Horses (%)
Lateral trochlear ridge	n=33	n=33	n=33	n=23	n=23	n=23
No	32 (96.7)	31 (93.9)	31 (93.9)	22 (95.7)	22 (95.7)	21 (91.3)
Yes	1 (3.3)	2 (6.1)	2 (6.1)	1 (4.3)	1 (4.3)	2 (8.7)
Medial trochlear ridge	n=33	n=33	n=33	n=23	n=23	n=23
No	28 (84.8)	32 (96.7)	28 (84.8)	23 (100)	23 (100)	23 (100)
Yes	5 (15.2)	1 (3.3)	5 (15.2)	0 (0)	0 (0)	0 (0)
Trochlear groove	n=33	n=33	n=33	n=23	n=23	n=23
No	32 (96.7)	32 (96.7)	31 (93.9)	22 (95.7)	22 (95.7)	21 (91.3)
Yes	1 (3.3)	1 (3.3)	2 (6.1)	1 (4.3)	1 (4.3)	2 (8.7)
Medial patellar ligament	n=33	n=33	n=33	n=23	n=23	n=23
No	24 (72.7)	30 (90.9)	22 (66.7)	16 (69.6)	20 (87)	15 (65.2)
Yes	9 (27.3)	3 (9.1)	11 (33.3)	7 (30.4)	3 (13)	8 (34.8)
Intermediate patellar ligament	n=33	n=33	n=33	n=23	n=23	n=23
No	23 (69.7)	24 (72.7)	20 (60.6)	18 (78.3)	19 (82.6)	15 (65.2)
Yes	10 (30.3)	9 (27.3)	13 (39.4)	5 (21.5)	4 (17.4)	8 (34.8)
Lateral patellar ligament	n=33	n=33	n=33	n=23	n=23	n=23
No	30 (90.1)	31 (93.4)	30 (90.1)	19 (82.6)	23 (100)	19 (82.6)
Yes	3 (9.1)	2 (6.1)	3 (9.1)	4 (17.4)	0 (0)	4 (17.4)
Fibularis tertius	n=33	n=33	n=33	n=23	n=23	n=23
No	33 (100)	33 (100)	33 (100)	20 (86.9)	23 (100)	20 (87)
Yes	0 (0)	0 (0)	0 (0)	3 (13)	0 (0)	3 (13)
Popliteal tendon	n=33	n=33	n=33	n=23	n=23	n=23
No	33 (100)	33 (100)	33 (100)	23 (100)	23 (100)	23 (100)
Yes	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Patella	n=33	n=33	n=33	n=23	n=23	n=23
No	31 (93.4)	32 (96.7)	31 (93.4)	22 (95.7)	23 (100)	22 (95.7)
Yes	2 (6.1)	1 (3)	2 (6.1)	1 (4.3)	0 (0)	1 (4.3)
Fat pad	n=33	n=33	n=33	n=23	n=23	n=23
No	31 (93.4)	33 (100)	31 (93.4)	21 (91.3)	21 (91.3)	19 (82.6)
Yes	2 (6.1)	0 (0)	2 (6.1)	2 (8.3)	2 (8.3)	4 (17.4)
Proximal infrapatellar bursa	n=33	n=33	n=33	n=23	n=23	n=23
No	32 (96.7)	31 (93.4)	31 (93.4)	23 (100)	23 (100)	23 (100)
Yes	1 (3)	2 (6.1)	2 (6.1)	0 (0)	0 (0)	0 (0)
Distal infrapatellar bursa	n=33	n=33	n=33	n=23	n=23	n=23
No	33 (100)	32 (96.7)	32 (96.7)	22 (95.7)	22 (95.7)	21 (91.3)
Yes	0 (0)	1 (3)	1 (3)	1 (4.3)	1 (4.3)	2 (8.3)

Different low case letters in the same row (a-b) differ in P-value (< 0.05).

Table 3. Radiographic abnormalities in the stifles of cutting Quarter Horses in training for futurities (TG, 28 to 47 months old) and in competition (CG, 48 months and older).

	Training (TG)			Competition (CG)		
	Right limb (%)	Left limb (%)	Horses (%)	Right limb (%)	Left limb (%)	Horses (%)
Stifle changes	n = 26	n = 26	n = 26	n = 11	n = 11	n = 11
No	9 (34.6)	9 (34.6)	7 (26.9)	8 (72.7)	9 (81.8)	8 (72.7)
Yes	17 (65.4)	17 (65.4)	19 (73.1) **a**	3 (27.3)	2 (18.2)	3 (27.3) **b**
Medial femoral condyle	n = 26	n = 26	n = 26	n = 11	n = 11	n = 11
Grau 0	14 (53.8)	17 (65.4)	10 (38.5)	10 (90.9)	10 (90.9)	9 (81.8)
Grau 1	9 (34.6)	7 (26.9)	12 (46.1)	1 (9.1)	1 (9.1)	2 (18.2)
Grau 2	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Grau 3	2 (7.7)	1 (3.8)	2 (7.7)	0 (0)	0 (0)	0 (0)
Grau 4	1 (3.8)	1 (3.8)	2 (7.7)	0 (0)	0 (0)	0 (0)
Lateral trochlear ridge	n = 26	n = 26	n = 26	n = 11	n = 11	n = 11
Normal	26 (100)	23 (88.5)	23 (88.5)	11 (100)	11 (100)	11 (100)
Flattened	0 (0)	2 (7.7)	2 (7.7)	0 (0)	0 (0)	0 (0)
Defect	0 (0)	1 (3.8)	1 (3.8)	0 (0)	0 (0)	0 (0)
Fragment	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Medial trochlear ridge	n = 26	n = 26	n = 26	n = 11	n = 11	n = 11
Normal	26 (100)	26 (100)	26 (100)	11 (100)	11 (100)	11 (100)
Flattened	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Defect	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Fragment	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Groove defect	n = 26	n = 26	n = 26	n = 11	n = 11	n = 11
No	24 (92.3)	25 (96.1)	23 (88.5)	11 (100)	11 (100)	11 (100)
Yes	2 (7.7)	1 (3.8)	3 (11.5)	0 (0)	0 (0)	0 (0)
Patellar lucency	n = 26	n = 26	n = 26	n = 11	n = 11	n = 11
No	23 (88.5)	25 (96.1)	22 (84.6)	11 (100)	11 (100)	11 (100)
Yes	3 (11.5)	1 (3.8)	4 (15.4)	0 (0)	0 (0)	0 (0)
Patellar fragment	n = 26	n = 26	n = 26	n = 11	n = 11	n = 11
No	26 (100)	26 (100)	26 (100)	11 (100)	11 (100)	11 (100)
Yes	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Patellar osteophytes	n = 26	n = 26	n = 26	n = 11	n = 11	n = 11
No	24 (92.3)	25 (96.2)	24 (92.3)	11 (100)	11 (100)	11 (100)
Yes	2 (7.7)	1 (3.8)	2 (7.7)	0 (0)	0 (0)	0 (0)
Tibial osteophyte	n = 26	n = 26	n = 26	n = 11	n = 11	n = 11
No	23 (88.5)	22 (84.6)	21 (80.8)	10 (90.9)	10 (90.9)	10 (90.9)
Yes	3 (11.5)	4 (15.4)	5 (19.2)	1 (9.1)	1 (9.1)	1 (9.1)
Other changes	n = 26	n = 26	n = 26	n = 11	n = 11	n = 11
No	24 (92.3)	23 (88.5)	22 (84.6)	11 (100)	11 (100)	11 (100)
Yes	2 (7.7)	3 (11.5)	4 (15.4)	0 (0)	0 (0)	0 (0)

Different low case letters in the same row (a-b) differ in P-value (< 0.05).

CAPÍTULO 3

Artigo a ser enviado para a revista científica *BMC Veterinary Research*

ULTRASONOGRAPHIC ABNORMALITIES IN HORSES WITH LAMENESS REFERABLE TO THE STIFLE

Abstract

Background: Ultrasonography and radiography are primary tools for diagnosis of abnormalities at the femorotibial (FTJ) and femoropatellar (FPJ) joints in horses. Nevertheless, ultrasound is still underused in field. The aims of this report were to describe ultrasonographic findings in 17 horses with lameness localized at the stifle joint, to classify the type of meniscal injuries, and to investigate the association between the type of meniscal lesions and degree of lameness. Lameness at trot was graded on a scale 0-5 (0=sound; 5=inability to weight bearing). All of the 17 horses examined were lame with scores ranging from 1 to 4. **Results:** The average time from lameness onset until presentation for evaluation was 80 days and more than half of the animals had grade 3 lameness. The most affected structures were the FPJ (synovitis, 76% of horses), medial FTJ (synovitis, 76%), medial menisci (MM) (65%), and intermediate patellar ligament (65%). Ultrasonographic abnormalities including MM tears, synovitis, and patellar desmopathy were detected in three cases without radiographic findings and positive response to intra-articular analgesia. Two animals died, eight presented insidious lameness, five had decreased performance or were oriented to reproduction, and only one horse fully recovered. Association between type of meniscal lesion and degree of lameness was not observed. **Conclusions:** Ultrasonographic examination of the FPJ and FTJ should be a routine procedure in cases of lameness, trauma and/or synovitis. The ultrasonographic findings at the menisci must be interpreted cautiously and under the light of clinical examination including palpation, flexion and intra-articular anesthesia. Using radiography as a single method for imaging diagnosis of the stifle induces bias in the detection of soft tissue abnormalities representing primary causes of lameness such as sprain or rupture of the collateral femorotibial ligaments, patellar desmitis, meniscal tearing and protrusion and early stages of osteochondrosis of the femoral trochlear ridges. In general, stifle lesions are multifactorial and negligence in the use of imaging diagnostic methods may cause delay in treatment and probably worsen the prognosis from returning to full use.

Key words: meniscus, horse, ultrasound, stifle.

Background

The femorotibial (FTJ) and femoropatellar (FPJ) joints are susceptible to injury causing lameness in horses [1-3]. Diagnostic methods for injuries at these joints include physical examination and intra-articular analgesia [4, 5], radiographs [6, 7], ultrasonography [8-11], scintigraphy [12-14] and arthroscopy [1]. Computed tomography [15] and magnetic resonance [16, 17] allow gathering accurate data on the stifle status, but they are not usually available [18]. Although radiography and ultrasonography are basic tools for diagnosis of injuries in the stifle [19, 20], the latter is underused in practice because it is highly operator-dependent [1]. Nonetheless, ultrasonography gives valuable information about stifle abnormalities and is considered as a logical step for imaging this region [18] before computed tomography, magnetic resonance or arthroscopy, which require general anesthesia and are expensive [1, 12, 21]. Several publications describe ultrasonographic protocols for the assessment of the stifle [1, 8, 9, 12, 19, 20, 22-25]. Nevertheless, there is only one study classifying the type of meniscal lesions using ultrasound as well as looking for an association of these injuries with the degree of lameness [26]. Thus, the aim of this paper was to describe the ultrasonographic findings at the stifle of lame horses; to classify the type of meniscal lesions using a known scale; and to investigate possible association between type of meniscal lesion and degree of lameness.

Methods

Ultrasonographic examination was performed in 17 horses with lameness referable to the stifle. Eight horses were presented at the Large Animal Surgery Department of Univ. Estadual Paulista and nine were attended in the field. The inclusion criteria were: lameness located at the stifle by physical examination (including flexion of the stifle without flexing the tarsus), intra-articular analgesia or joint effusion, and/or radiographic findings. Animals suffering from septic arthritis were excluded. Lameness was scored from 0 to 5 (0=sound to 5=inability to weight bearing) using the scale of the American Association of Equine Practitioners [27].

Ultrasonographic examination

B-mode ultrasonographic examination was performed with the scanner devices model MyLab 70 and Mylab 30, ESAOTE and linear array multifrequency probe 4-12 MHz and 6-10 MHz, respectively. The stifle region was clipped and cleaned with soap and water and acoustic gel was applied. Examination was conducted as in previous reports [8, 9, 11, 23] in order to get image and assess the medial (MCL) and lateral (LCL) collateral ligaments, medial (MPL), intermediate (IPL) and lateral (LPL) patellar ligaments, synovial compartments of the medial (meFTJ) and lateral (laFTJ) femorotibial joints, and femoropatellar joint (FPJ) including the medial (meTR) and lateral (laTR) trochlear ridges of the femur; the common tendon of origin of the long digital extensor and *fibularis [peroneus] tertius* (FT) muscles, the popliteal tendon (PT), and proximal and distal infrapatellar bursa, as well as the medial (MM) and lateral (LM) menisci. The lateral femorotibial synovial compartment between the PT and the LCL was arbitrarily named as popliteal recess (PR) which was also evaluated.

Integrity of ligaments and tendons was assessed using the 0-4 scale by Genovese et al. [28]. In order to classify meniscal lesions, the system referred by De Busscher et al. [26] was used. When more than one abnormality was identified into the same meniscus it was considered regarding the most complex injury. Protrusions were reported independently [26]. The score attributed to each meniscal lesion was analyzed using the Proc Freq (SAS Institute, 2008). Comparison between type of meniscal lesion and degree of lameness was performed using the Fisher exact test considering each meniscus as an independent sample and the significance level was stated at $P \leq 0.05$.

Clinical outcome

Outcome was obtained by reassessment or by telephone interview. Data regarding patient status were classified as good, moderate or poor. A good outcome was considered when the animal attained the previous work level. A moderate outcome, when lameness stopped but the horse had a lower performance than before presentation or were used for reproduction. Animals were classified in this group considering physical and economical potential. So animals were included if: (1) they presented no spontaneous lameness after treatment and rest, (2) the response after trying to return to full work in their respective disciplines was unsatisfactory and (3) they worked in a lower level without lameness and so might be used for pleasure or reproduction. A bad outcome was considered when insidious lameness was present, the animal died, or was humanely destroyed.

Results and discussion

Clinical and radiographic findings of the 17 cases are summarized in Table 1. The group was formed by nine males, five females and three geldings from 2-7 years old (mean 6.5).

The average time from lameness onset until ultrasonographic assessment was 80 days. Lameness from 1/5 to 4/5 was found in all the horses. Twelve of them remained the same degree and five worsened after forced flexion. Intra-articular analgesia was performed in seven animals (41%) where clinical evidence was inconclusive after forced flexion and palpation.

Radiographic findings

Abnormalities were observed in eight of 11 radiographed horses (Table 1), including radiolucency or bone proliferation of the intercondylar eminence (n=3), radiolucency of the subchondral margins of the lateral trochlear ridge (n=2), and osteophytes at the condyles and patella. Radiography predominantly allows evaluation of osseous structures such as subchondral margins, cancellous bone, enthesis, and sites of attachment of the joint capsule and synovial lining [5]. However, when considering damage to the articular cartilage, rupture of cruciate ligaments and meniscal injury as primary diseases, radiographic findings are unspecific [12]. In the cases herein, for instance, radiolucency of cancellous bone of the medial femoral condyle was observed in one animal suffering from bilateral rupture of *fibularis tertius* as the primary injury. Hence, following this logic, radiographic changes such as remodeling of the tibial intercondylar eminence and periarticular osteophytes (cases 2, 3, 6 and 8 in our study) may be considered as non-specific evidence of intra-articular disease [12]. Moreover, irregularity of the subchondral bone of the medial femoral condyle was not considered specific of primary cartilaginous injury (specificity for detection using radiographs of 22%), since this sign was also observed in horses suffering from primary lesion of the meniscus (specificity of 19%) [12].

Ultrasonographic examination

Stifle injury was identified in the 17 horses, and abnormalities were found in 24 of 26 evaluated limbs. Figure 1 shows the frequency of ultrasonographic changes identified in at least one anatomical structure per animal. Although evaluated, the LCL, PT, LPL and the meTR were not included in Figure 1, since changes were not observed in these structures. A description of lesions in the infrapatellar bursa is included in the text below.

Femorotibial and femoropatelar joints

Synovitis was present in 16 (94%) horses. The meFTJ was affected in 13 cases (76%), the laFTJ in eight (47%) and the FPJ in 13 (76%). Altogether, 14 horses (82%) had synovitis at the femorotibial joint, proportion similar to other studies which reported this finding in 85% [21] and 69% of horses with meniscal abnormalities [26]. The latter study also stated positive correlation between FTJ effusion and lesion of the respective ipsilateral meniscus. We were not able to identify such association, which is probably explainable by the low number of cases available.

As in previous studies [26, 29, 30], we observed hyperechoic dots and fibrin loculations, apparently attached to the synovial lining without acoustic shadowing, indicative of synovitis of the meFTJ (Figure 2). These hyperechoic dots have been already described as fibrin, or detritus of cartilage and meniscus [31].

Fluid in the subextensor recess of the laFTJ has been described as abnormal [11, 32]. In this study, mild to severe synovitis was detected (Figure 3), along with echoic trabeculae surrounding the FT muscle, from its origin to the extensor groove (Figure 4). Distension of the popliteal recess caused by synovitis was also observed (Figure 5).

Synovitis of the FPJ was found either medially or laterally. In animals without evident swelling, ultrasonography allowed to visualize synovial distension and to identify contact between the mediodistal femoropatellar joint margin and the medial recess (Figure 6). This distension was also visualized cranially, between the MPL, IPL and LPL (Figure 7). Cranially, there were observed hyperechoic trabeculae (fibrin) within focal anechoic intra-articular areas adjacent to the trochlear groove (Figure 8). We also visualized irregularity of the chondral and subchondral margins in the lateral trochlear ridge (Figure 9), as well as bone fragments forming acoustic shadows between the IPL and LPL consistent with degenerative fragmentation of the lateral trochlear groove, that was confirmed through arthroscopy.

Menisci

Eight horses (47%) presented injury in at least one MM and three (18%) had lesion in both lateral and medial menisci. Thus, abnormalities of the MM were observed in 11 horses (65%) including type 1-5 lesions, protrusion and peripheral liquid as a result of femorotibial synovitis (Figure 10, A-D). Regarding the LM, Type 2 and 5 lesions and hyperechoic dots indicative of fibrosis were visualized (Figure 10. E).

Regarding limbs, eighteen menisci of the 26 evaluated stifles presented some abnormality. Type 1 lesion was found in three horses (MM injured=3. 17%); type 2 in six horses (MM=5 and LM=1. 33%); type 3 in two horses (MM=2. 11%); type 4 in five horses (MM=4 and LM=1. 28%); and type 5 in two horses (MM=1 and LM=1. 11%). Mild protrusion was identified in only one animal with type 2 lesion (Figure 11).

As in previous studies, the presence of lesions was higher in the MM than in the LM [19, 21, 26]. This might be explained by the greater load on the MM which makes it more likely to suffer damage caused by compressive and pivotal forces [33]. Type 2 was the most frequent lesion in the menisci, followed by type 4, 1 and 3, respectively. Type 5 lesions were previously described as the most frequent abnormalities in the LM [26] and were identified in our series in one MM and one LM. It is important to notice that the type 5 lesion in the MM herein was observed with the animal in a rest position. When in a weight-bearing position, the meniscus was imaged as having a type 2 lesion which highlights the fact that meniscal injuries may be either underestimated (since the weight on the menisci can compress a fissure and so hide an abnormality [30]) or overestimated (because some hypoechoic lines can be observed in sound menisci or occur by equivocal positioning of the transducer [26]). These linear lesion-like artifacts mentioned above have also been identified in our practice. It is important to point out that one meniscus can also have different type of injuries at different planes so comprehensive scanning is critical for classification.

Ultrasound is essential for the diagnosis of meniscal injury and has evidenced sensitivity and specificity of 80% and 87.5%, respectively [12]. The classification of lesion by De Busscher et al. [26] used in this report is an adaptation of the system used in humans with magnetic resonance [34] and allowed grouping efficiently and didactically the type of meniscal abnormalities identified with B-mode ultrasonography. However, we found some difficulties in classifying diffuse hyperechoic dots or areas presumptively caused by fibrosis and rarefaction of the meniscal margins, since these abnormalities do not seem to be contemplated by the system. Thus, when present as a single entity, diffuse hyperechoic dots and irregularity of the margins were classified as type 1 lesions.

The ultrasonographic approach for the diagnosis of meniscal injury has known limitations. The presence of hypoechoic lines, which has been previously considered as tears, does not allow identifying the nature of the lesion [26], that is, if this is a real tear, fibroplasia or edema. So, ultrasonographic differentiation between an acute and a chronic-active lesion is difficult. In talking about our study, an important limitation is the lack of post-mortem data and the fact that arthroscopy was performed in only one case, which impeded assessment of the gross and histological characteristics of the abnormal menisci.

As in a previous study [26], association between type of meniscal lesion and degree of lameness was not found. Clinical and telephonic follow-up showed that only one of the 11 horses with damage in the menisci completely recovered and returned to its previous performance level after eight months of rest and conservative treatment. Even though, it is important to mention that this animal had as a main abnormality a mild, type 1, meniscal lesion. According to Flynn and Whitcomb, for instance [21], only two of 11 animals with meniscal lesion returned to work, one of them after 15 months in rest. Nonetheless, a series of cases describing results after arthroscopic treatment, showed that 47% of horses returned to full athletic activities [35].

Patellar ligaments

Ultrasound is the technique of choice in diagnosing patellar ligament abnormalities. In chronic cases, radiographic signs may be observed at the enthesis [20]. Injury may be associated with trauma [20] or, in the case of the MPL, upward fixation of the patella [24]. The IPL was the most affected among the patellar ligaments (11 horses, 65%), coinciding with previous reports [20, 29]. Hypoechoic and hyperechoic areas were easily identified in cross sectional and longitudinal images as well as patellar entesophytes which were confirmed in radiographs. Proximal and distal infrapatellar bursitis and fibrosis were also observed (Figure 12). Six animals (35%) had MPL abnormalities including hypoechoic areas indicative of desmopathy at different stages and entesophytes at the tibial insertion. In a case of upward fixation of the patella, abnormal cranial displacement and positioning over the medial trochlear groove, together with unspecific signs such as subcutaneous fibrosis and swelling were visualized. LPL abnormalities were not identified.

Medial and lateral femorotibial collateral ligaments

Four animals (24%) had mild desmopathy of the MCL, characterized by hypoechoic and anechoic areas, circumscribed or diffuse at cross section indicative of desmitis grade 2 and 3 (Figure 13) and elevation of the MCL caused by femorotibial synovitis (Figure 14). Hypoechogenicity and increase of the area may be also observed in cases of hemorrhage and presence of granulation tissue [23]. Different than other reports describing cases of chronic desmitis, [36] entesophytes were not observed in this series. However, periosteal proliferation of the medial tibial condyle along with edema surrounding the MCL were identified. All horse presenting MCL lesion had some degree of ipsilateral meniscal abnormality, which is expected as they are attached to each other. Lateral collateral ligament injury is uncommon [23], and similarly to other reports, was not observed.

Other structures

One horse was referred to the hospital to investigate radiographic and ultrasonographic abnormalities secondary to bilateral avulsion of the FT muscle. Loss of continuity and thickening of the FT were identified caused by distension after trauma. At the proximal tibia, swelling and a hypoechoic area surrounding the FT were observed in longitudinal scans between the origin of the *tibialis cranialis* muscle and the long digital extensor. Cross-sectional and longitudinal scans of the contralateral (right) limb at the level of the extensor groove, allowed identifying a hemorrhagic region and one of the disrupted ends of the FT (Figure 15). In other animals, osteophytes on the cranial and abaxial lateral margins of the patella and heterogeneous echogenicity of the infrapatellar fat pad were observed.

It is important to recognize the value of the ultrasonographic examination to evaluate the stifle. Three horses with positive response to intra-articular analgesia (case 5, 13 and 15) had ultrasonographic abnormalities without radiographic signs. Case 5 had type 2 lesions of the MM and fibrinous synovitis of the femorotibial (i.e., medial and subextensor recess) and femoropatellar (i.e., suprapatellar recess) joints. Case 13 showed loculated fibrinous synovitis at the FTJ and FPJ as well as desmitis of the MPL and IPL. Case 15 presented bilateral type 4 lesions of the MM, fibrinous synovitis of the FTJ and FPJ together with grade 2 IPL desmitis. Horses with soft tissue injury do not necessarily have bone abnormalities. However, in animals presenting bone abnormalities, soft tissues are often compromised. These abnormalities, when active, are detected by ultrasonography as hypoechoic areas related to an inflammatory process; or as hyperechoic well-consolidated dots or areas which may be caused by fibrosis or mineralization. Insertional desmopathy with low grade changes are only detected with ultrasound.

Despite of radiography is useful for diagnosis of appendicular musculoskeletal disorders in horses [6, 9, 36], detection of some lesions, mainly those in soft tissue, may be neglected with the sole use of this technique [9]. With the exception of luxation, fracture and, in any case, avulsion of the medial femorotibial collateral ligament, radiograph gives poor or no information regarding acute injury of tendons, joint capsule, subcutaneous tissue, fibrocartilage, menisci, and cartilage [9, 12]. Thus, when stifle lameness is suspected, ultrasonographic examination should be indicated in both reference institution and in the field as a routine procedure. Stifle lameness may be suspected based upon history of trauma, soft tissue swelling, lameness associated with positive flexion test and/or positive response to intra-articular analgesia [23].

Outcome

There is no data regarding one of the horses and eleven of them presented poor outcome, which was verified at least one year after diagnosis. Two horses died, one from complications related to bilateral FT rupture and other for musculoskeletal unrelated reasons. Five animals were retired due to osteoarthritis, another by recurrent upward fixation of the patella (i.e., owner refused desmotomy) and the last two by persistent lameness.

From the five horses with moderate outcome, three (2 males and 1 female) were used for reproductive purposes and the others were used for low-level walking. None of the animals used for reproductive purposes were actually lame except for one mare, which was retired from high-level competitions due to osteoarthritis. One of the males was 12 years old at the moment of retirement, and had been three-time pole racing champion at the open category of the AQHA Official Show, Brazil. Surgery was performed in the third male, which remains untrained. The outcome of all horses but the last, which was operated on for osteochondrosis dissecans, was verified at least one year from diagnosis. Since this horse presented no lameness at the moment of recheck, reassessment would be expected after beginning of training.

Only one animal returned to its previous athletic level, after conservative treatment and eight months in rest. Treatment included non-steroidal anti-inflammatory drugs, two months of stall rest, and six month on pasture-stall regimen. This animal suffered from a mild, type 1, meniscal lesion and a discrete osteophyte of the medial tibial condyle.

Despite far from the aims of this work, it is important to notice that at least five animals (29%) were referred only after 90 days of lameness and a variety of medical treatments. On the other hand, arthroscopy was performed in only one animal. Poor outcome seems to be the most logical picture in this series of cases due to the presence of signals of degenerative joint disease at presentation, the long period of time from the initial lameness outbreak until presentation for definitive diagnosis, and probably the kind of treatment, since all but one owner accepted arthroscopy. This procedure has proved to ameliorate the prognosis in cases of meniscal tears [35] which indeed, was present in 65% of the animals herein.

This series of cases supports the need of a universal system of ultrasonographic classification of meniscal lesions in horses. Such a system is didactic and easy to use for anyone familiar with the normal and abnormal aspect of the menisci, including natural variations and the presence of artifacts. Following a known protocol may contribute to make these data available for further studies and meta-analysis. Overall, the practitioners should consider a list of lesions that may be accurately detected with the use of ultrasonography including synovitis, distension or rupture of femorotibial collateral ligaments, patellar desmopathy, tear and protrusion of the menisci, and early stages of cartilage degeneration of the trochlear margins.

Conclusions

Ultrasonographic examination of the femorotibial and femoropatellar joints should be immediately indicated in cases of lameness, trauma and joint swelling from the stifle. The type of meniscal lesion should be assessed with caution due to its dynamic presentation. The ultrasonographic findings should be associated with clinical examination, including palpation, flexion and intra-articular analgesia. Ultrasonography is not merely a complementary tool but a cornerstone in primary imaging diagnosis when soft tissue injury is suspected. Negligence in the use of the variety of imaging diagnostic methods may cause delay in treatment and probably worsen the prognosis from returning to full activities.

Acknowledgements

The authors thank the CAPES PEC/PG (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior) for providing the PhD scholarship for the first author.

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TABELAS DO CAPÍTULO 3

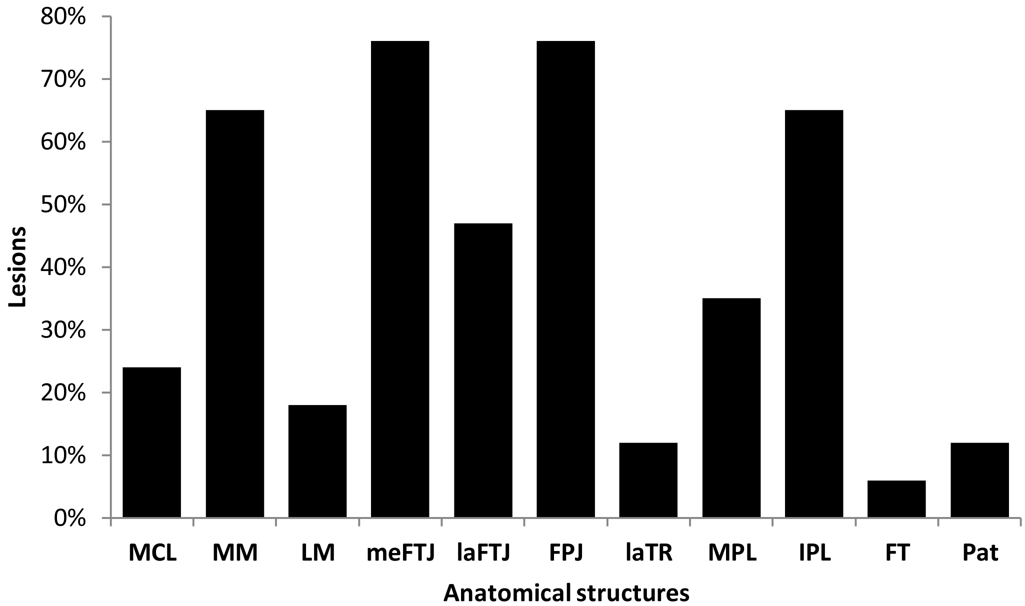
Table 1. Clinical and radiographic findings of 17 horses with stifle lameness.

Case	Breed	Sex	Age years/ months	Lameness (days)	Lameness before/after flexion test	Limb	Radiographic findings
1	ml	f	8/0	6	3/3	r	Ventral patellar periostitis
2	qh	m	5	20	2/2	l	LHL: remodeling of the medial; osteophyte of the medial tibial and femoral condyles RHL: bone proliferation cranial to the tibial eminence.
3	cr	m	12/0	21	3/3	b	LHL: radiolucency of the lateral trochlear ridge and medial femoral condyle RHL: rarefaction of the lateral trochlear ridge. Osteophyte of the lateral tibial condyle
4	qh	m	12/0	450	2/4	r	-
5	qh	m	3/0	20	3/3	l	NA
6	camp	g	3/0	10	3/3	r	Osteophyte of the medial tibial condyle
7	qh	g	8/0	30	3/4	l	Decreased opacity cranial to the tibial intercondylar eminence
8	ml	f	6/0	4	3/3	b	LHL: bone proliferation at the cranial surface of the patella .RHL: lateral osteophyte and apex entesophyte of the patella. Radiolucency of the tibial intercondylar eminence
9	ml	m	2/6	90	1/2	r	-
10	bh	f	7/0	210	1/2	l	-
11	mb	g	17/0	7	3/3	b	-
12	qh	m	3/5	0	0/1	b	-
13	mb	m	10/0	7	3/3	r	NA
14	qh	f	6/0	240	3/3	b	Osteochondrosis
15	qh	f	3/0	90	2/2	r	NA
16	qh	m	4/0	120	3/3	b	-
17	qh	m	2/0	ni	3/3	b	Bilateral osteochondrosis dissecans of the lateral trochlear ridge

ml = Mangalarga; qh=Quarter Horse; cr = Crioulo; camp = Campolina; bh = Brasileiro de Hipismo; mb = mixed-breed; f = female; m = male; g = gelding; r = right; l = left; b=bilateral; ni = no informed; RHL = right hindlimb; LHL = left hindlimb; NA = no abnormalities were present.

FIGURAS DO CAPÍTULO 3

Figure 1. Relative distribution of lesions between studied horses.



Medial (MCL) and lateral (LCL) femorotibial collateral ligaments; medial (MM) and lateral (LM) menisci; medial (meFTJ) and lateral (laFTJ) femorotibial joints; femoropatellar joint (FPJ); lateral trochlear ridge (laTR); medial (MPL) and intermediate (IPL) patellar ligaments; *fibularis [peroneus] tertius* muscle (FT); patella (Pat).

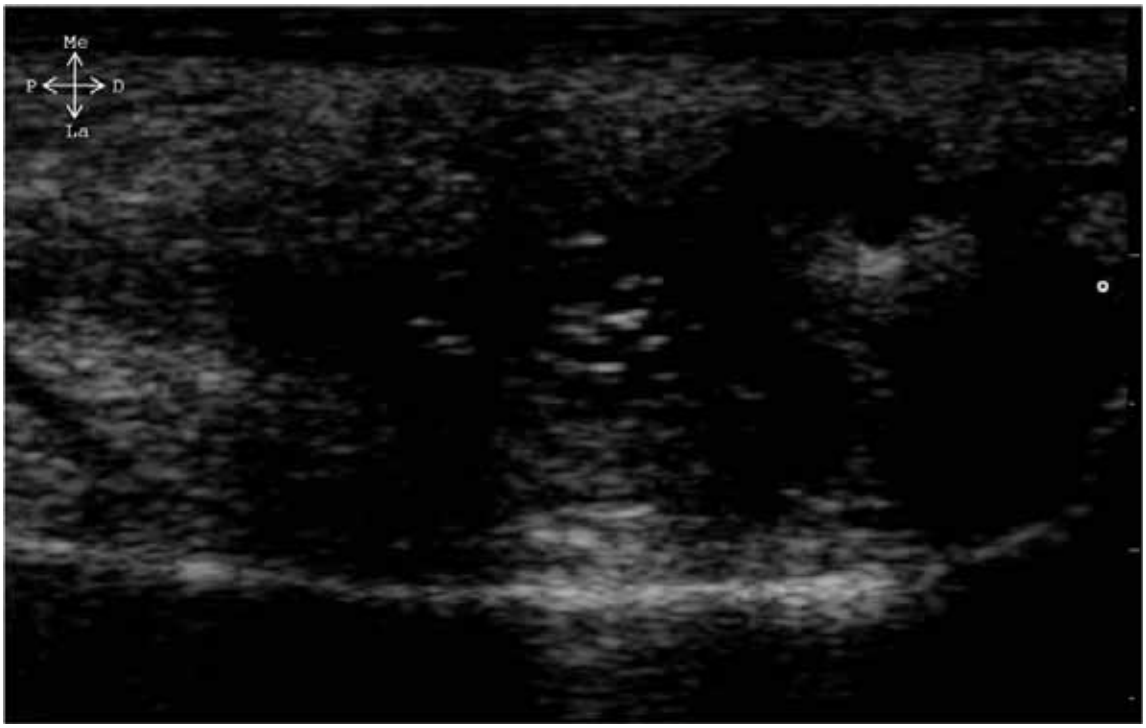


Figure 2. Longitudinal scan of the medial femorotibial recess. Notice the free-floating hyperechoic dots and synovial effusion. The synovial lining is heterogeneous. Although a quantity of fluid is expected in this compartment, swelling was observed during the physical examination. Me=medial; La=lateral; P=proximal; D=distal.

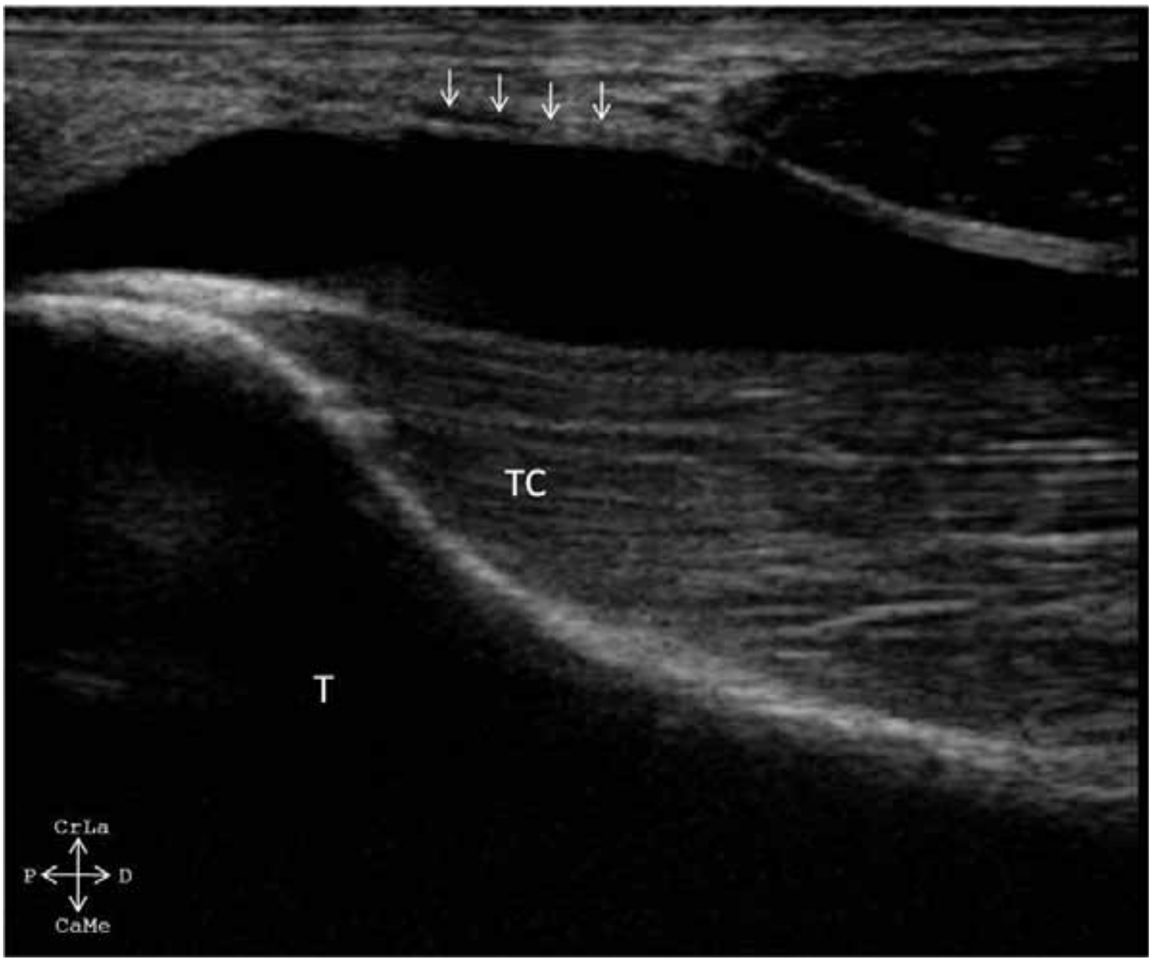


Figure 3. Craniolateral (CrLa) to caudomedial (CaMe) longitudinal scan, distal to the extensor fossa of the tibia of a Quarter horse used for cutting. Notice the anechoic well delimited area (arrows) consistent with synovial effusion of the subextensor recess of the lateral femorotibial joint. TC=origin of the *tibialis cranialis* muscle; T=tibia. P=proximal; D=distal.

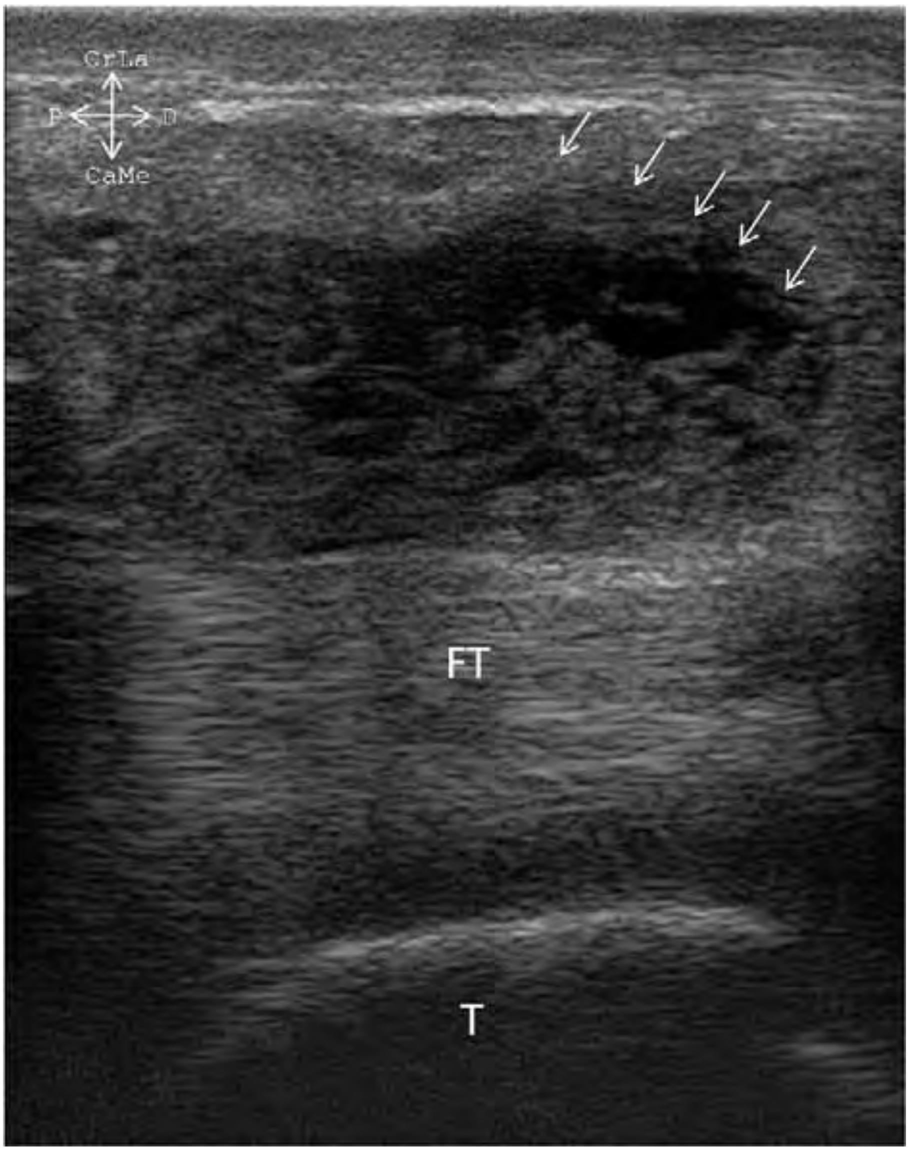


Figure 4. Craniolateral (CrLa) to caudomedial (CaMe) longitudinal scan of the tibial extensor fossa of a Quarter horse used for barrel racing. Notice the loculated synovial effusion (arrows) of the subextensor recess surrounding the long digital extensor and *fibularis tertius* (FT) muscles from its origin to approximately 5 centimeters distal to the tibial plateau. P=proximal; D=distal; T=tibia.

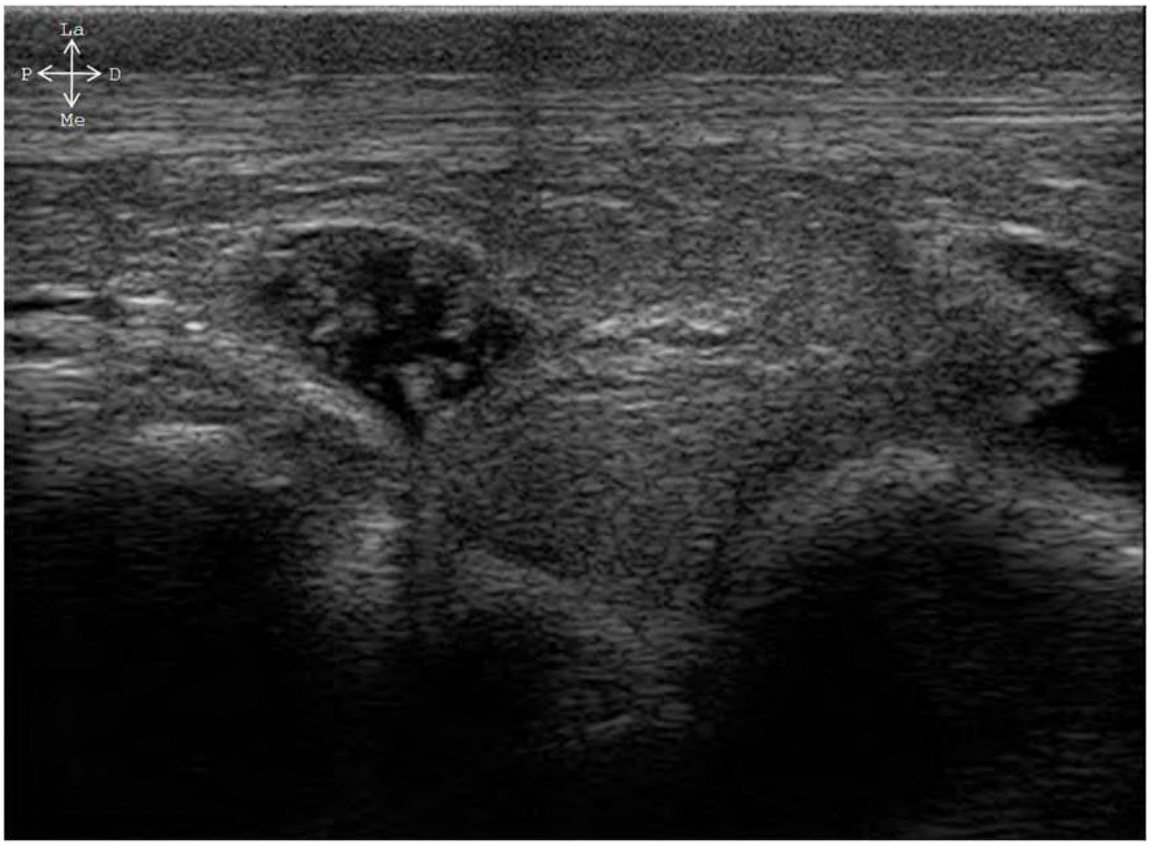


Figure 5. Longitudinal sonogram of the lateral aspect of the femorotibial joint of a horse with upward fixation of the patella. Synovitis and effusion of the popliteal recess of the lateral femorotibial joint was observed close to the lateral meniscus. La=lateral; Me=medial; P=proximal; D=distal.

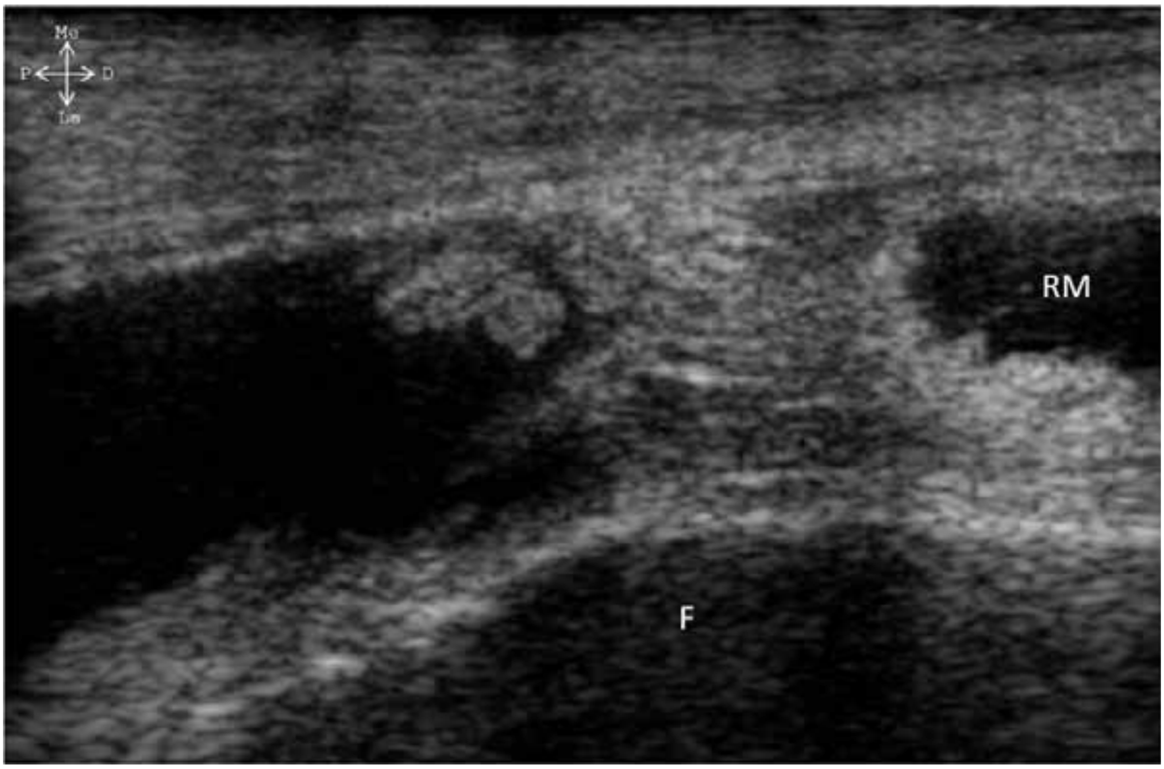


Figure 6. Longitudinal scan of the medial femur of a Mangalarga horse with synovitis of the femoropatellar joint (hypoechoic area to the left). The medial femorotibial recess (RM) is to the right. F=femur; La=lateral; Me=medial; P=proximal; D=distal.

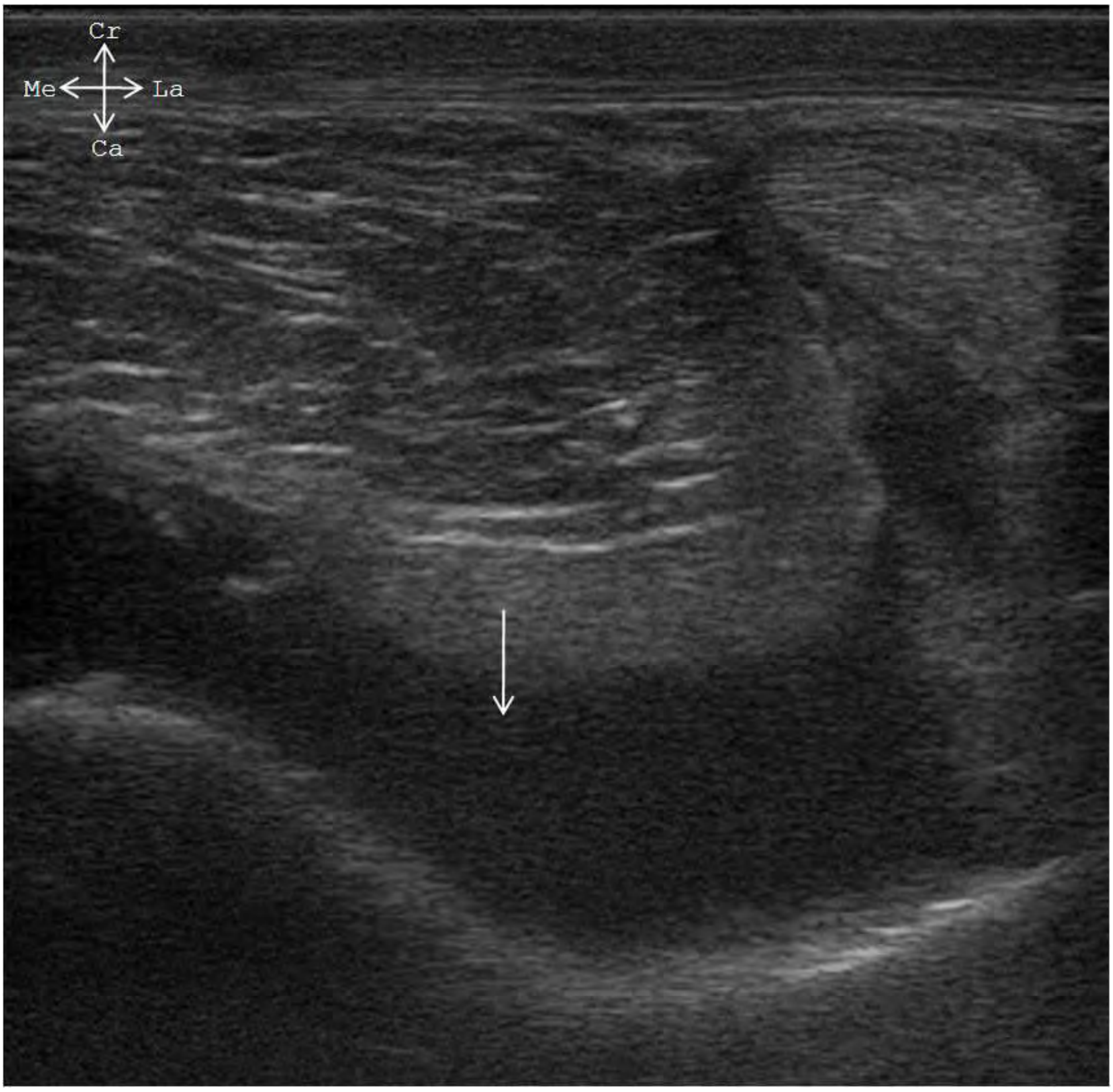


Figure 7. Transversal sonogram of the cranial aspect of the femoral trochlea of a Mangalarga mare. Femoropatellar effusion is evident (arrow). Isoechogenicity between the synovia and the cartilage impedes differentiation of the chondral margins. Cr=cranial; Ca=caudal; Me=medial; La=lateral.

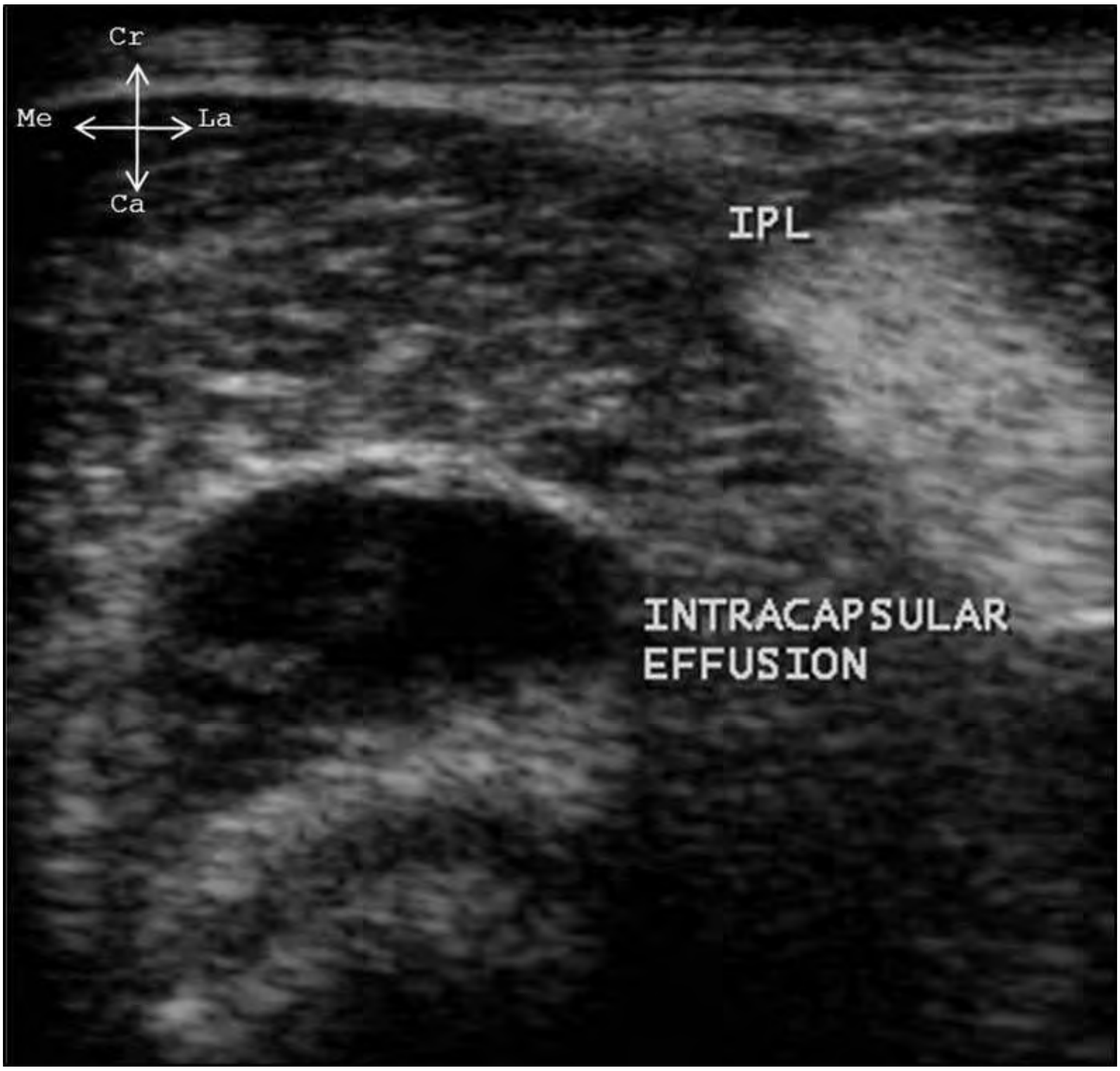


Figure 8. Transversal sonogram of the cranial aspect of the medial trochlear ridge of a Quarter mare used for cutting. IPL=intermediate patellar ligament. Notice the femoropatellar effusion and hyperechoic fragments into the synovial fluid. This mare was retired due to degenerative joint disease and used for reproductive purposes. Cr=cranial; Ca=caudal; Me=medial; La=lateral.

Figure 9. Longitudinal sonogram of the cranial aspect of the lateral trochlear ridge of a four-year-old Quarter stallion used for cutting. Integrity of the chondral and bone margins is lost (arrow). This image is consistent with osteochondrosis. Cr=cranial; Ca=caudal; P=proximal; D=distal.

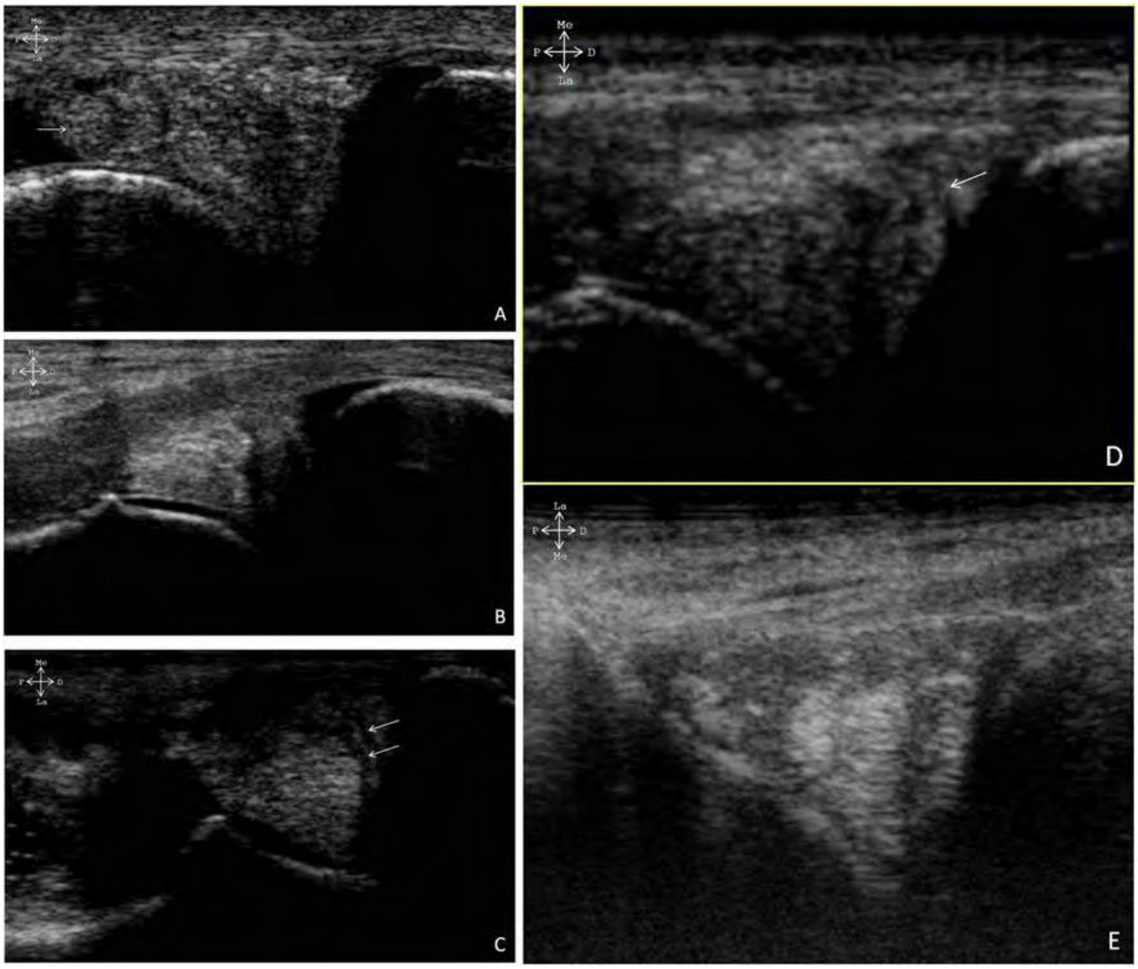
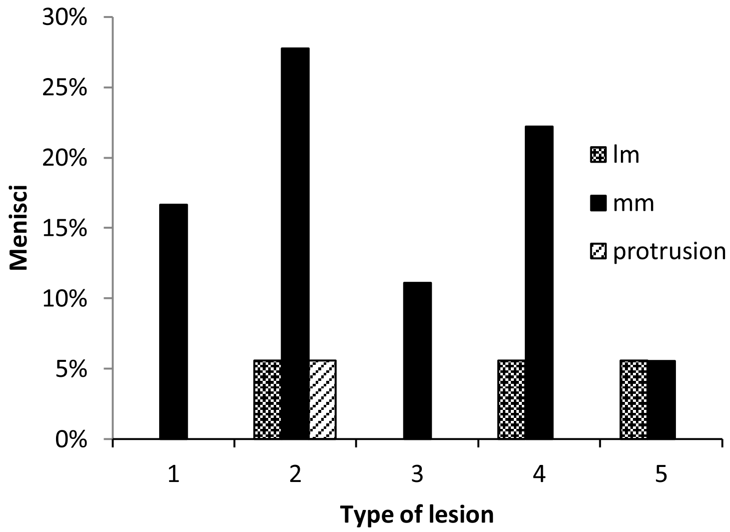


Figure 10. Longitudinal sonograms of the medial (MM) and lateral (LM) menisci. (A) MM of a Brasileiro de Hipismo mare used for jumping. There is central degeneration of the meniscus (type 1 lesion) and peripheral hyperechoic dots. The echoic mass dorsal to the MM (arrow) is a fibrin cloth into the medial femorotibial recess and should not be misdiagnosed as a meniscal fragment. (B) MM of a three-year-old Quarter Horse used for cutting. A horizontal hypoechoic line is evident (type 2 lesion) separating the menisci into proximal and distal fragments. (C) MM of a Quarter mare used for cutting. Anechoic oblique lines (arrows) consistent with type 3 lesion of the meniscus; incongruence between the dorsal margin of the meniscus and the surface of the medial condyle indicative of mild protrusion, is observed. (D) MM of a Campolina horse used for cavalcade. Notice the hypoechoic horizontal and oblique lines (arrow), and diffuse hypoechogenicity in the meniscus (type 4 lesion). (E) LM of a four-year old horse used for cutting. Diffuse echoic pattern and multiple hypoechoic lines crossing the meniscus are evident (type 5 lesion). Me=medial; La=lateral; P=proximal; D=distal.

Figure 11. Percentage of lesions by menisci, classified according to De Busscher et al (2006).



lm=lateral meniscus; mm=medial meniscus. Protrusion was found at the medial meniscus only.

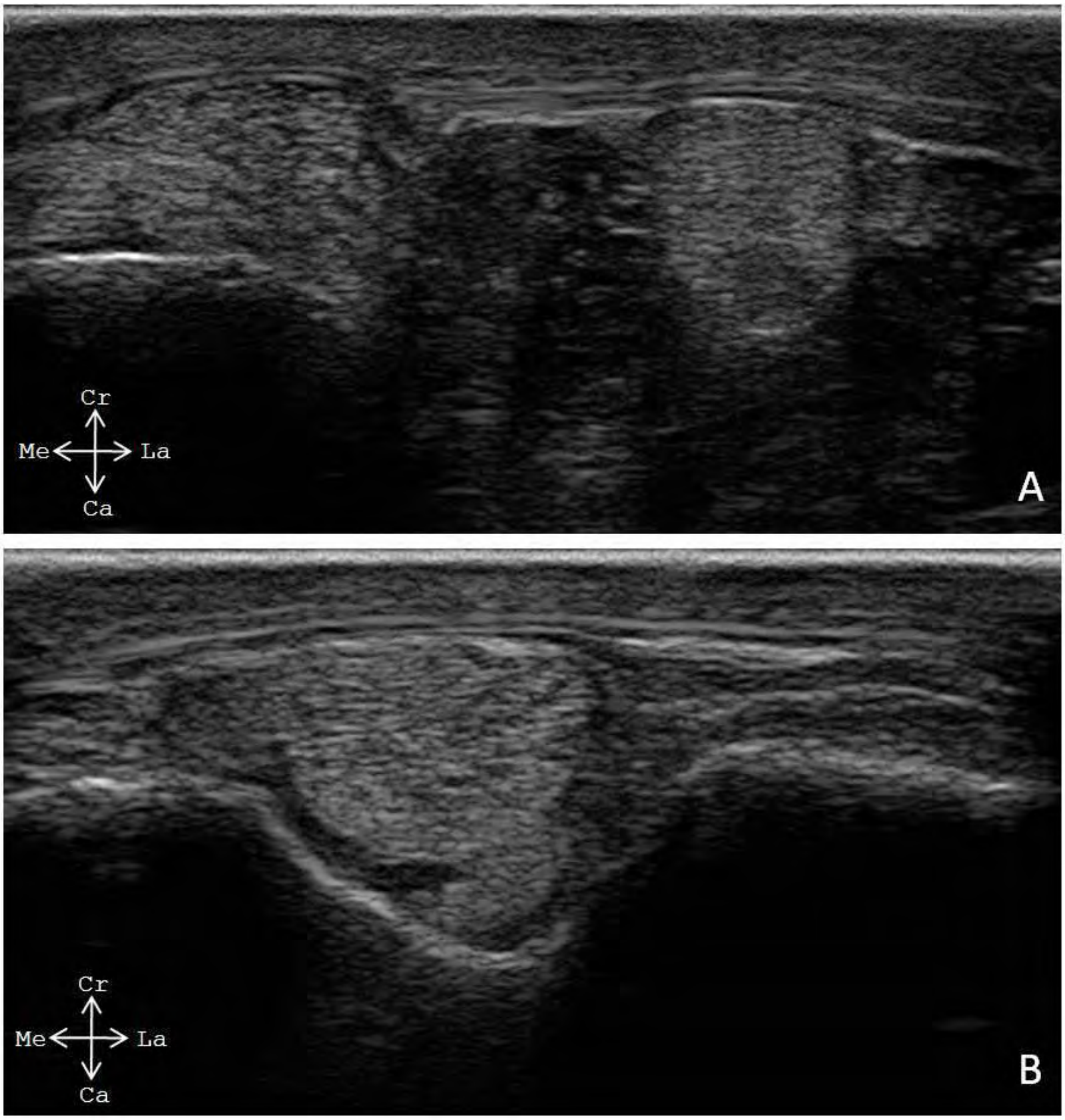


Figure 12. Transversal sonographic scans of the cranial aspect of the stifle of a Mangalarga mare used for pulling a buggy. This animal had severe atrophy of the gluteal musculature of the right hindlimb. (A) Diffuse hypoechogenicity and loss of the trabecular pattern of the medial patellar ligament (left) and a hypoechoic region of 8mm2 in a total area of 75mm2 (approximately 9%) at the caudal aspect of the intermediate patellar ligament (right). (B) Same stifle but distal to (A). Fibrosis of the distal infrapatellar bursa. Cr=cranial; Ca=caudal; Me=medial; La=lateral.

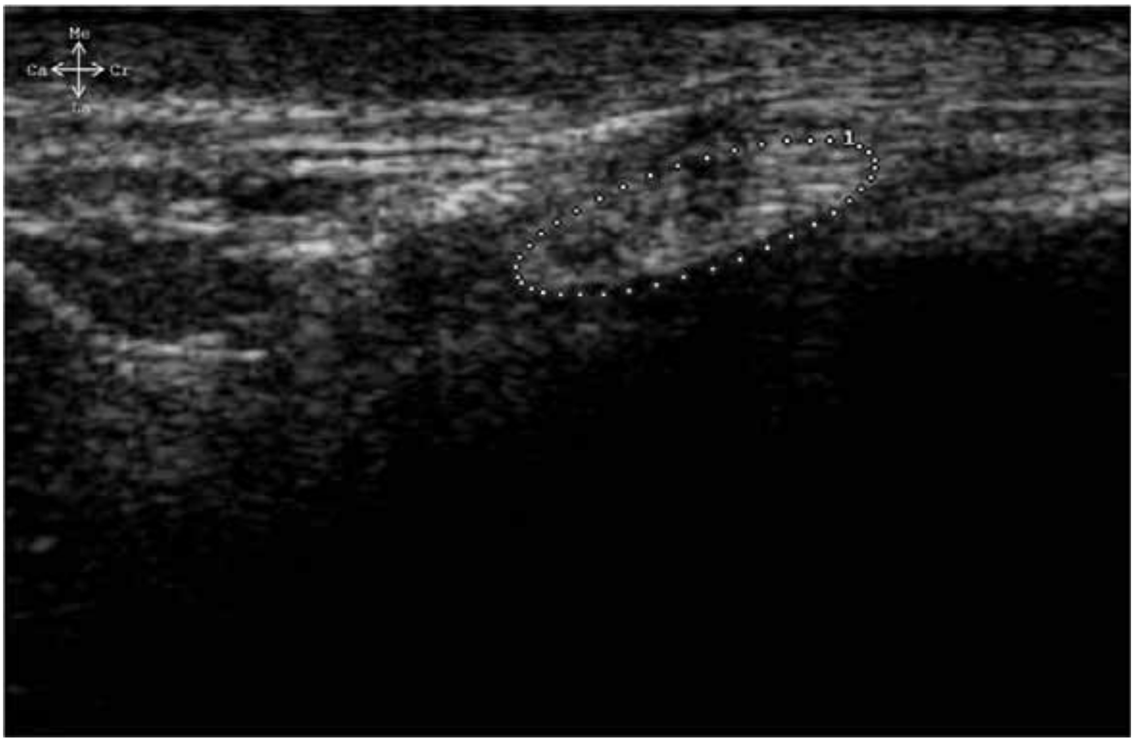


Figure 13. Transverse sonogram of the medial femorotibial collateral ligament (MCL) of a seven-year-old Brasileiro de Hipismo mare used for jumping, with history of low performance and chronic lameness. There is mild diffuse hypoechogenicity of the MCL consistent with desmopathy. This animal also suffered from type 1 meniscal lesion and fibrosis of the *vastus intermedius* muscle. Me=medial; La=lateral; Ca=caudal; Cr=cranial.

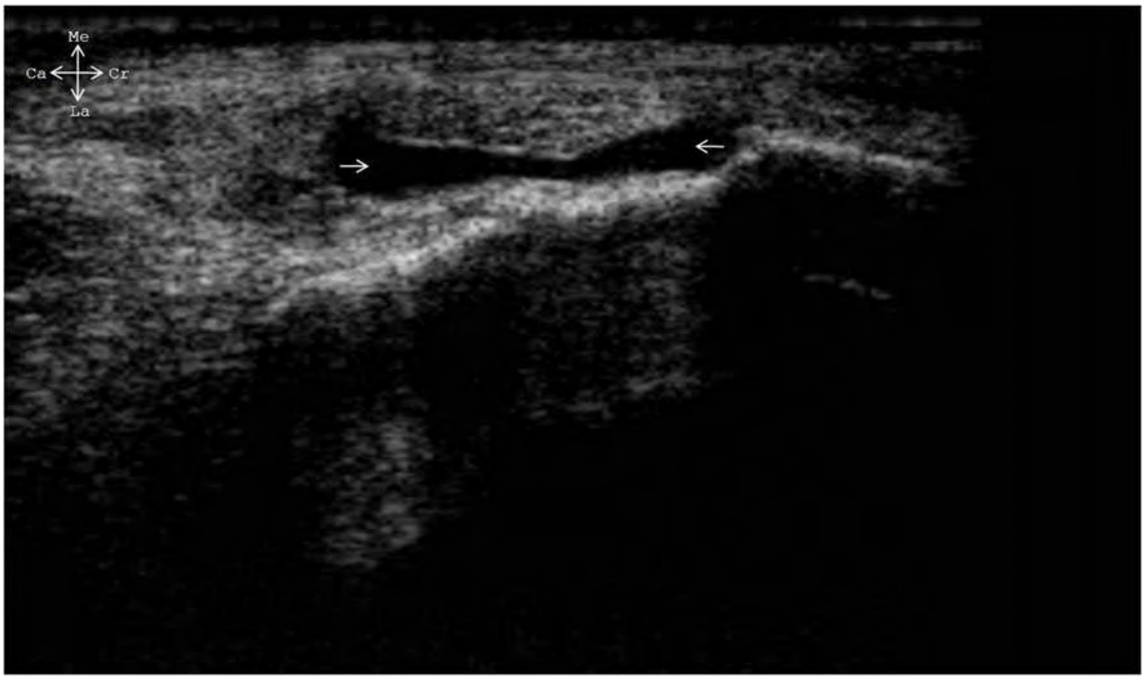


Figure 14. Transverse sonogram of the medial femorotibial collateral ligament (MCL) of a six-year-old Quarter mare used for cutting, with history of chronic lameness and stifle swelling. Notice the anechoic well defined area (arrows), representing femorotibial synovial effusion and separating the MCL from the tibia. Me=medial; La=lateral; Ca=caudal; Cr=cranial.

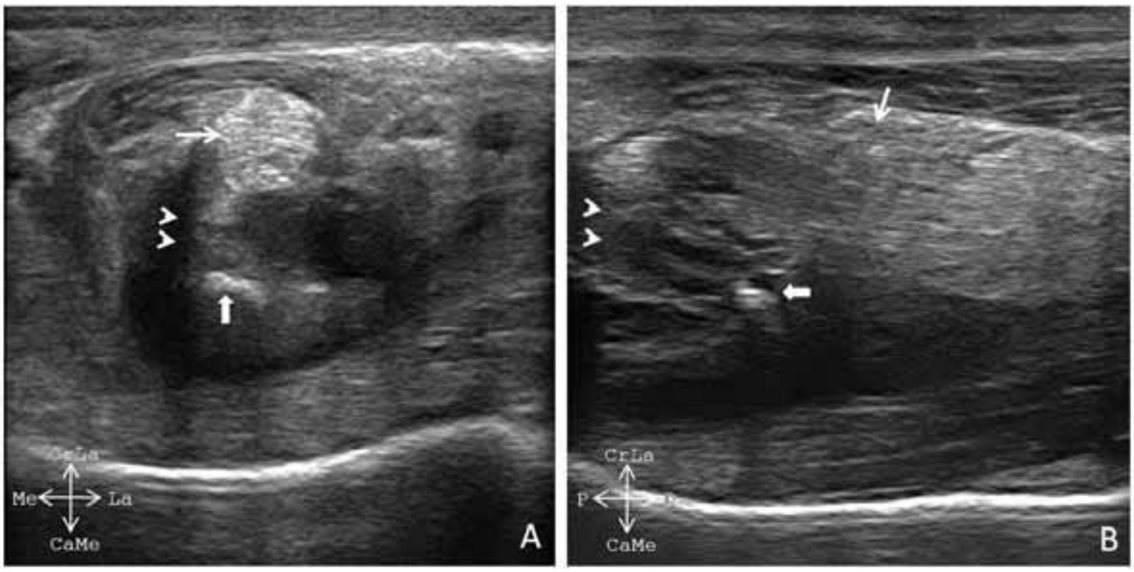


Figure 15. Transversal (A) and longitudinal (B) images of the proximal end of the tibia of a horse suffering from bilateral rupture of the *fibularis tertius* muscle (FT). The distal end of the ruptured *fibularis tertius* (arrows) and the hypoechoic loculated region (arrowheads) are consistent with edema and hemorrhage. The hyperechoic dots displaying acoustic shadow (solid arrows) indicate mineralization. CrLa=craniolateral; CaMe=caudomedial; Me=medial; La=lateral; P=proximal; D=distal.

CAPÍTULO 4

CONCLUSÕES GERAIS

Em cavalos Quarto de Milha na modalidade de apartação, não há evidencia de diferença na proporção de animais com claudicação moderada entre os indivíduos jovens-adultos e os adultos, ambos sob treinamento intensivo.

Porcentagem importante de animais estreia no Potro do Futuro apresentando lesões e claudicação não evidentes para o treinador e proprietário, mas sim para a avaliação veterinária.

Existe alta proporção de animais que apresentam anormalidades no joelho quando realizados exames ultrassonográficos e radiográficos.

Na modalidade de apartação, esperar a presença de alguma anormalidade ultrassonográfica na maioria ou em todos os animais, sendo comum a sinovite, seguida por algum grau de lesão nos meniscos, indicativo de degeneração provocada pelo tipo de atividade ou por sobre-esforço. Pesquisas precisam ser realizadas para elucidar esta questão.

Não há evidencias de associação entre o tipo de lesão ultrassonográfica nos meniscos e o grau de claudicação, bem como entre a presença de anormalidade ultrassonográfica nos meniscos e a presença de claudicação.

Em cavalos de apartação submetidos a treinamentos similares, a ocorrência de anormalidades radiográficas no joelho é maior entre os animais mais jovens.

Existe necessidade de acompanhamento veterinário e de consulta preventiva para detecção precoce de anormalidades que são potenciais causa de claudicação em animais de apartação. Especial cuidado deve ser dado ao joelho.

São necessárias pesquisas para elucidar a relação das anormalidades encontradas nos exames por imagem e o número de animais que potencialmente podem apresentar claudicação.

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EQUINE VETERINARY JOURNAL. AUTHOR GUIDELINES

1. GENERAL INTRODUCTION

Equine Veterinary Journal (*EVJ*) publishes evidence to improve clinical practice or expand scientific knowledge underpinning equine veterinary medicine. This unrivalled international scientific journal is published 6 times per year with around 128 pages per issue, containing articles with original and potentially important findings. Contributions are received from sources worldwide.

EVJ publishes original articles and reviews on all aspects of equine veterinary science. Categories include General Articles, Short Communications, Technical Notes, Case Reports and Retrospective Series and Review Articles (see section 3). They present new developments in research being carried out both by researchers in institutes and from clinicians in practice devoted to equine and/or comparative physiology, pathology, medicine or surgery.

Original Manuscripts first undergo an Internal Editorial Assessment to ensure that the manuscript content is of interest to our readership and the manuscript format conforms to our guidelines described below. If deemed appropriate, manuscripts are then assessed by at least two external peer reviewers and our review process is double-blinded (i.e. the reviewers are not identified to the authors and vice versa, see Marr, C.M. (2011) Masking the peer review process: better or worse? Equine vet J, 43, 249). Many manuscripts also undergo assessment by an individual with expertise in study design and data analysis. Our median time for completing the review process for original submission is less than 5 weeks. If accepted for publication, the copyright becomes the property of EVJ Ltd. Accepted manuscripts are pre-published and submitted for indexing within a few days of acceptance.

Please read the instructions below carefully for details on format and submission of manuscripts, the journal's requirements and standards as well as for information concerning the procedure after a manuscript has been accepted for publication in *EVJ*. Authors are encouraged to visit Wiley Blackwell Publishing's Author Services for further information on the preparation and submission of articles and figures.

2. ETHICAL GUIDELINES

2.1 Previous publications

Authors submitting an article do so with the understanding that the work and its essential substance have not been published in full before and is not being considered for publication elsewhere. If abstracts have been published, in print or online, full papers will be considered only if the published abstract was less than 1000 words and did not contain figures or tables. A copy of the abstract should be uploaded to ScholarOne at the time of submission as a supporting file (see Section 5.4). *EVJ* accepts material that has been presented orally at conferences that include web-based distribution of video or audio recording of presentations. Manuscripts are screened with software designed to detect plagiarism.

2.2 Authorship

EVJ adheres to the definition of authorship set up by The International Committee of Medical Journal Editors (ICMJE). According to the ICMJE, authorship criteria should be based on 1) substantial contributions to conception and design of, or acquisition of data or analysis and interpretation of data, 2) drafting the article or revising it critically for important intellectual content and 3) final approval of the version to be published. Authors should meet conditions 1, 2 and 3. Except in the case of complex large-scale or multi-centre research, the number of authors should normally not exceed six. Corresponding authors should ensure that all authors are entered as co-authors when uploading the manuscript to ScholarOne. A statement of each author's contribution to study design, data collection and study execution, data analysis and interpretation, and preparation of the manuscript will be published. Contributors who do not qualify as authors should be mentioned under **Acknowledgements**.

2.3. Use of animals in research

Manuscripts will be considered for publication only if the work detailed therein:

o Follows international, national, and/or institutional guidelines for humane animal treatment and complies with relevant legislation in the country in which the study was conducted; o For studies using client-owned animals, demonstrates a high standard (best practice) of veterinary care and involves informed client consent. Has been approved by the ethics review committee at the institution, hospital or practice at which the studies were conducted where such a committee exists; Where such a committee does not exist, authors are expected to have their research in a manner likely to be approved by ethics committees in most countries.

Submissions must be accompanied by a statement, in our submission form, certifying that legal and ethical requirements have been met with regards to the humane treatment of animals described in the study and specifying the ethical review committee that has overseen this process and/or the international, national and/or institutional guidelines followed. This information will be included in the Declarations Section. Please also include this information under the separate heading of "Ethical Considerations" in the “Title page” document (see Section 4.2)

When research animals are used, the methods section must clearly indicate that adequate measures were taken to monitor and address the welfare of the animals involved. Editors reserve the right to reject papers if there is doubt as to whether appropriate procedures have been used and also to insist that information is provided in the text as to the measures taken to protect the welfare of subjects and the outcome of procedures undertaken in respect to any pain or suffering caused.

2.4 Antimicrobial Stewardship Policy

Authors should consider WHO’s risk management strategies for the containment of antimicrobial resistance in humans due to non-human use of antimicrobials. In particular, where quinolones, extended spectrum beta-lactam antimicrobials (such as 3rd/4th generation cephalosporins) or macrolides not labelled for use in horses in the country from which the report originates have been used as part of the treatment regimen, or are being evaluated for potential use in equine patients, the reasons underlying these choices must be robustly defended. Information on Critically Important Antimicrobials for Human Medicine can be found here. Further discussion of EVJ’s Antimicrobial Stewardship Policy can be found in Bowen, Equine Veterinary Journal, Volume 45, Issue 2.

2.5 Competing Interests

Authors are required to disclose any competing interests. These include financial interests (for example patent, ownership, stock ownership, consultancies, speaker’s fee). Employment with commercial companies whose products are used in the study should be stated and intention to apply for a patent by an individual or company in any way related to the authors must be declared. If the author does not include a competing interest statement in the submission form, then the following statement will be included by default: “The authors have declared no competing interests”. Please also include this information under the separate heading of "competing interests" in the “Title page” document (see Section 4.2)

2.6 Source of Funding

Authors are required to specify the source of institutional, private and corporate financial support for their research when submitting a paper. Please include this information under the separate heading of "Source of Funding" in the ”Title page” document (see Section 4.2)

2.7 Permissions

If all or parts of previously published illustrations are used, permission must be obtained from the copyright holder concerned. It is the author’s responsibility to obtain these in writing and provide copies to the Publishers. If material is used that has been published elsewhere or is given as a personal communication, it is the author’s responsibility to obtain permission from the publisher and author.

2.8 Appeal of Decision

Authors who wish to appeal the Editors’ decision on their submitted manuscript may do so by e-mailing Editor@evj.co.uk with a detailed explanation of their rationale for appealing the decision.

2.9 Complaints procedure

Details of the procedure for complaints can be found here.

2.10 Copyright Assignment

The submission of the manuscript by the authors means that the authors agree to sign a Copyright Transfer Agreement form when the manuscript is accepted for publication. When the manuscript is accepted for publication, the copyright becomes the property of EVJ Ltd.

If your paper is accepted, the author identified as the formal corresponding author for the paper will receive an email prompting them to login into Author Services; where via the Wiley Author Licensing Service (WALS) they will be able to complete the license agreement on behalf of all authors on the paper.

For authors signing the copyright transfer agreement: If the OnlineOpen option is not selected the corresponding author will be presented with the copyright transfer agreement (CTA) to sign. Please ensure you read the details of the copyright agreement thoroughly and carefully to ensure that you are able to fully comply with the terms. The terms and conditions can be previewed in the sample associated with the copyright FAQs below:

CTA terms and conditions
http://authorservices.wiley.com/bauthor/faqs_copyright.asp. In section 8 you will find samples of the CTA. EVJ uses CTA-A.

For authors choosing OnlineOpen: If the OnlineOpen option is selected the corresponding author will have a choice of the following Creative Commons License Open Access Agreements (OAA):

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3. MANUSCRIPT TYPES ACCEPTED

3.1 General Articles

General Articles should describe experimental or clinical research studies, including systematic reviews, and normally be around 4000 words including figure legends, table legends and references, with up to 3 tables and 6 figures (this includes figures labelled a, b, c etc).

A summary, no more than 300 words in total, should be provided with the following headings.

• **Reasons for performing study**: i.e. why the work was undertaken in the first place, the background behind the decision to choose this subject to study.
• **Objectives**: The statement that is being tested, and is testable by the methods (below); or the original aims of the study and the deliverables.
• **Study Design**: concise statement of the study design.
• **Methods**: Brief description of materials and methods and methods of testing hypotheses.
• **Results**: Brief highlights of the results obtained.
• **Conclusions**: Conclusions drawn from results.
• **Potential relevance**: The potential relevance/significance of the results to clinical application and/or the need for further research or work.

The introduction should be limited to 500 words and the remainder of the manuscript should be presented in the following sections; Materials and Methods, Results, and Discussion, with subheadings, including data analysis, as appropriate. Manuscripts exceeding 5000 words will not be sent to external peer reviewers. Additional tables, figures, video material or text describing further details of methods or results can be submitted as Supporting Information Items (see below). All quantitative results should be analysed by appropriate statistical methods.

To minimise publication bias, *EVJ* encourages authors to publish negative results, providing authors can demonstrate that the study had adequate power to detect differences.

Pairs or larger sets of papers that must be reviewed, and subsequently read, together are not accepted and articles must describe discrete studies, using references where appropriate to make methods sections concise. If appropriate, authors can upload in press articles with identifying details removed, for reviewers' use.

Prior to submission of reports of epidemiological studies, authors are recommended to consult:

Von Elm, E, Altman, D.G., Egger, M., Pocock, S.J. Gotzsche, P.C., Vandenbroueke, J.P. and STROBE Initiative (2007). Strengthening the reporting of observational studies in epidemiology (STROBE) statement: Guidelines for reporting observational studies. Br. Med J. 335, 806-808.

Boden, L.A. and Parkin, T.D.H. (2008) Current guidelines on good reporting of analytical observational studies in epidemiology. Equine vet J, 40, 84-86.http://onlinelibrary.wiley.com/doi/10.2746 /042516408X255927/ abstract. Woolhouse, M.E.J., Fèvre, E.M., Handel, I., Heller, J., Tildesley, M.J., Parkin, T. and Reid, S.W.J. (2011). Guide to Good Practice for Quantitative Veterinary Epidemiology. http://www.qve-goodpracticeguide.org.uk/

Prior to submission of reports of **clinical studies**, authors are recommended to consult:

Altman, D.G. (1996) Better reporting of randomised controlled trials: the CONSORT statement. Br. med. J. 313, 570-571.

Boden, L.A. (2011) Clinical studies utilising ordinal data: pitfalls in the analysis and interpretation of clinical grading systems. Equine vet J, 43, 383-387. http://onlinelibrary.wiley.com/doi/10.1111/j.2042-3306.2011.00414.x/abstract

Additionally, although no substitute for obtaining advice on study design and data analysis at the time of planning their work, prior to submission authors should as far as possible address and include the points in the following checklist:

Clinical research studies should:

• Address a clearly defined clinical question.

• Describe a study, based on naturally occurring disease that provides strong clinical evidence to define outcomes relating to specific therapeutic or diagnostic interventions, and/or to refine prognostic indicators.

• Provide objective and unambiguous case definition criteria that are rigorously applied and explicitly reported.

• Evaluate clearly defined clinical outcomes that are rigorously applied and explicitly reported.

• Utilise and explicitly report the details of appropriate controls, randomisation and blinding as appropriate or feasible.

• Include a pre-study estimate of the power of the study to resolve a clinically useful difference (or other appropriate estimation of numbers required). For therapy or prevention trials, this power value should be 80% or more*.

• Include a flow chart of subjects through each stage of eligibility, stating numbers agreeing to participate, randomisation, receiving the intervention in question, completing the study protocol and analysed for the study outcome. Retrospective studies should also include a flow chart to account for the numbers of animals eligible, recruited and used n the analysis.

• Use appropriate analytical methods and report effect size estimates and the confidence intervals of any results*.

*See Rossdale, P.D., Jeffcott, L.B. and Holmes, M.A. (2003) Clinical evidence: an avenue to evidence-based medicine. Equine vet. J. 35, 634-635.http://onlinelibrary.wiley.com/doi/10.2746/042516403775696267/abstract

3.2 Case Reports and Retrospective Case Series

Larger retrospective case series should conform to the guidelines for General Articles. Smaller Case series (target word count 4000 words for up to 5-20 cases and up to 4 figures and 2 tables; 3000 words for 1-5 cases and up to 3 figures and 2 tables) with a short structured summary in our usual format (see Section 3.1), will normally be considered if their content addresses **at least one of of** the following aims:

o Evidence of an hitherto unrecognised syndrome or disease o Evidence of emerging diseases in a previously unaffected country or region

o Unreported or unusual side effects or adverse reactions involving medications, surgical or other veterinary procedures

o Findings that shed new light on the possible pathogenesis of a disease

o Findings that document a new diagnostic technique or novel application of existing technology

Reports of novel treatments or invasive diagnostic techniques are unlikely to be considered unless a sufficiently large number of patients have been included to have reasonable insight in effectiveness and possible harms. Likewise, descriptions of congenital anomalies that have been well characterised in other species are unlikely to be of interest, except where the report fulfils other aims listed above. Authors should note that fewer than 10% of case reports are accepted.

Prospective studies conforming to the guidelines for format for general articles will be included in "General Articles" section of the Journal.

3.3 Brief Reports

Two types of Brief Reports are considered:

3.3a Technical notes

A technical note is a short article giving a brief description of a specific development, technique or procedure, or it may describe a modification of an existing technique, procedure or device applicable to equine veterinary medicine (clinically or in a research setting). The technique, procedure or device described should have practical value and should contribute to clinical diagnosis or management, or be applicable in research. It could also present a software tool, or an experimental or computational method. The article must describe a demonstrable advance on what is currently available. The main criteria for publication will be the novelty of concepts involved, the validity of the technique and its potential for clinical or research applications. The method needs to have been well tested in terms of accuracy, repeatability and safety, and ideally, but not necessarily, used in a way that proves its value.

Technical notes should be around 2000 words in length and should contain no more than 2 figures and 2 tables, with a short structured summary in our usual format (see Section 3.1).

3.3 b Short Communications

Equine Veterinary Journal encourages authors to aim to complete substantial studies in order to produce a manuscript appropriate for a general article and the publishing of preliminary results is discouraged. Short Communications can be submitted to describe focused experimental or clinical research studies. This format is designed for publication of concise pieces of robust research that may not have enough volume of data for a standard manuscript but is still of stubstantial impact to the research and/or clinical community. To justify publication in the short communication format, the studies must be novel and well designed with appropriate data analysis. Studies reporting negative results (i.e. null hyopthesis cannot be rejected) will also be considered.

Short communications should be around 2000 words in length and should contain no more than 2 figures and 2 tables, with a short structured summary in our usual format (see Section 3.1).

3.4 Review Articles

Review Articles are encouraged, however a preliminary discussion with the Editor regarding subject and length of the article is advisable before submission.

4. MANUSCRIPT FORMAT AND STRUCTURE (A checklist is available at Section 7).

4.1. Manuscript File Format

Manuscripts should be submitted as Word documents (.doc, .docx) (not write-protected) in double spacing on A4 page size, the pages should be numbered and there should be line numbers continuously throughout the document. Division of the paper should be indicated clearly by major headings, subheadings and sub-subheadings.

The text file must contain the manuscript including summary, text, references, tables and figure legends but no embedded figures. Figure tags should be included in the file. A word count for the entire text including figure and table legends and references (but not including

Supplementary Information) should be provided. Figures should be provided in separate files (see below for details of format).

4.2 Authors' Identity and Declarations

Authors' names, institutes, affiliations and declarations should not be included within the manuscript in order to facilitate double blinded review. To ensure the integrity of this process, authors must create a separate Word document and upload this separately as the "Title page" document. This document should contain Ethical Considerations, Competing Interests, Sources of Funding, and Acknowledgements. Also list here any other details included in the Materials and Methods which might allow the reviewer to identify the authors or host institute, for example, hospital or ethical review committee names or reference to "in press" publications. In their place, within the main manuscript, state "detail to be provided on acceptance" and highlight this. All such details must be included in the "Title Page" document, with reference to the appropriate line number. With all revisions, authors must re-insert such details into a clean, unblinded version of their manuscript that should be uploaded in addition to the blinded version with revisions marked. Copies of "in press" publications, with relevant identifying details removed, should be uploaded as "supporting information" for reviewers' use.

4.3 Keywords for publication

Authors can provide up to six keywords for publication immediately below the title which may help readers find the article in various search engines. These should reflect the manuscript content and should usually include the word "horse" or similar but not "Equine" or "Veterinary" as these terms will be included in most Search Engines as being within the journal's title. Keywords for publication should be listed within the "Title Page" document immediately below the authors' names and addresses. Note during the submission process, you will also be required to choose ScholarOne keywords which facilitate the review process – see Section 5.6.

4.4 Language

The language of publication is English. Authors for whom English is a second language should consider having their manuscript professionally edited before submission to make sure the English is of high quality. A list of independent suppliers of editing services can be found: athttp://authorservices.wiley.com/bauthor/english_language.asp. All services are paid for and arranged by the author, and use of one of these services does not guarantee acceptance or preference for publication.

EVJ provides Chinese translations of article summaries online within the Supporting Information section. Authors are welcome to include foreign language translations of their summary as supplementary items for online only publication. Such items should be entitled "Supplementary Item: Summary in French" etc. If appropriate, start numbering any additional supplementary items from 1.

4.5 Abbreviations, Symbols and Nomenclature

Doses and measurements should be given in metric (SI) units with /kg bwt added where appropriate. Abbreviations are discouraged. Where possible, only abbreviations that are in common use within the relevant field or discipline should be used. Authors must avoid inventing their own abbreviations for anatomical terms and techniques. Where they are unavoidable, abbreviations must be explained in both the summary and main text. Spelling should conform to the Oxford English Dictionary, medical terminology to Dorlands Medical Dictionary and units, symbols and abbreviations should conform to the International System of Units defined by Baron, D.N. (Ed) (1994) "Units, Symbols, and Abbreviations: A Guide for Medical and Scientific Editors and Authors, 5th edn." Royal Society of Medicine Press, London.

4.6 Drug names and Manufacturers' details

The generic name of drugs, equipment or other materials should be given in the text, with product name in brackets. Recommended International Non-Proprietary Names (rINNs) should be used. The manufacturer's details (company name, town/city, state/county and country) should then be listed under a "Manufacturers' details" heading at the end of the article before the Reference section. References to this list should be indicated as superscript letters throughout the text. Brand or trade names should not be used in the title and must be in brackets throughout the text.

4.7 Optimising Your Article for Search Engines: Many students and researchers looking for information online will use search engines such as Google, Yahoo or similar. By optimising your article for search engines, you will increase the chance of someone finding it. This in turn will make it more likely to be viewed and/or cited in another work. We have compiled these guidelinesto enable you to maximise the web-friendliness of the most public part of your article.

4.8 References

It is the responsibility of the authors to ensure that all reference details are accurate. References are indicated throughout the text as numbers within square brackets [1, 2, 3 etc] and the final list of references must correspond with the order in which they appear in the main body of the text.

The format in the reference list is as follows: author(s) name(s) and initials, year of publication in parentheses, full title of article, journal title (in italics) as abbreviated in the World List of Scientific Periodicals, volume number (in bold) and page numbers: e.g. Foster, B.W., Codd, J. and Smith, R. (1992) Effect of stress on ulcers in foals. Equine vet. J. 35, 43-52.

References to book articles should be set out as follows: author(s) name(s) and initials, date of publication in parentheses, title of chapter or article, full title of book, edition, name(s) of editor(s) if relevant, publisher, place of publication and pages referred to: e.g. Robin, C. (1991) Calcium in plants eaten by horses. In: Dietary Calcium, 2nd edn., Ed: J. Chalk, Blackwells Scientific, London. pp 195-201.

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4.9 Table and Figure Legends

Legends should be short as appropriate but should be intelligible to the reader without reference to the text. Legends should also cite the reference used if appropriate. A list of figure legends should be placed at the end of the text (before references).

4.10 Tables

Tables should be referenced in the appropriate place in the text but should be placed at the end of the text (before references) accompanied by appropriate legends. Duplication of data in tables, figures and text should be avoided.

4.11 Figures

Illustrations should be provided when necessary to clarify the text. Figures should be referred to in the text. Authors are encouraged to use colour in graphs, line drawing and other figures.

Photomicrographs must state magnification, preferably with a scale bar, and staining technique. Line drawings should be original diagrams on clean white paper or board and must be submitted in digital form (see below). Symbols and lines should be standard and not drawn by hand. Do not include a “chart title” when creating graphs and charts.

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4.12 Supplementary Information

Supplementary information, such as data sets or additional figures, tables or video files, that will not be published in the print edition of the journal, but which will be viewable via the online edition, can be submitted as Supplementary Information Items. Where questionnaires have been used for data collection these should be included as supplementary items. There is no limit to the number of supplementary items that can be included. Supplementary Information Items are subject to the peer review process. Supporting Information Items is material that is not essential to the article but provides greater depth and background. It is not copyedited. The items should be referred to in the main body of the text of the article where appropriate. The availability of Supplementary Information should be indicated in the main manuscript by a list, to appear after the References, headed " Supplementary Information Items" and providing titles of figures, tables, etc. See section 5.4 for details of how to upload these files.

Supplementary Information items must be prepared as follows:

1. Submit each item as a separate file.
2. Text: Word only; A4, page set up portrait, double spaced, top and bottom margins 3.17 cm; left and right margins 2.54 cm, continuous line numbers, page numbers, font – Arial size 10.
3. Figures: GIF, TIFF, EPS, PNG, JPEG, BMP, PDF; labelling Arial font, with size selected as appropriate for figure.
4. Tables: Word only; A4, Page set up can be as portrait or landscape as appropriate; top and bottom margins 3.17 cm; left and right margins 2.54 cm, continuous line numbers, page numbers, font – Arial, size as appropriate.
5. Movies: MPEG, AVI or quicktime movie format. Any labelling should be should be in Arial font, with size selected as appropriate. Several small clips are preferable to one large movie.
6. The maximum size of each individual supplementary item is 5Mb.

5. SUBMISSION OF MANUSCRIPTS

5.1 ScholarOne Manuscripts

EVJ uses ScholarOne Manuscripts for online manuscript submission and peer review found athttp://mc.manuscriptcentral.com/evj. Full instructions and support are available on the site and a user ID and password can be obtained on the first visit. If you require assistance then click the Get Help Now link that appears at the top right of every ScholarOne Manuscripts page.

5.2 Covering Letter

Submissions should include a covering letter stating that the paper is original, has not been submitted or published elsewhere, and has the approval of all authors.

5.3 Submission Fee

There is a submission fee of £62.00 for every paper that goes through the peer review process. When a manuscript has been submitted to EVJ via ScholarOne, the corresponding author will receive an email with a submission fee form attached. The form can be filled in with credit card details and returned to the Editorial Office. If you prefer to pay by another method contact our Editorial Office (jane@evj.co.uk). This fee is waived if, during the Internal Editorial Assessment, it is decided the paper will not undergo further peer review.

5.4 Submission Process

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3. Figure files under the file designation “Figure”
4. Tables under the file designation “Table”

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6. Supporting information (e.g. copies of previously published abstracts or “in press” publications, with identifying information removed for use by editors and reviewers) under the file designation “Supporting Information”

5.5 Suggesting a Reviewer

In order to facilitate the review process, EVJ is prepared to receive suggestions from authors as to appropriate peer reviewers (with e-mail addresses) but these will be followed at the editor’s discretion.

5.6 ScholarOne Keywords

During the submission process, you will be required to select keywords describing the discipline(s) and body system/disease(s) that most closely relate to the content of your article from prescribed lists. These are used by the Editorial Office to match your article with appropriate reviewers. Note: these words can also be used as the “Keywords for Publication”, but this is not obligatory – see Section 4.3.

5.7 Suspension of Submission Mid-way in the Submission Process

You may suspend a submission at any phase before clicking the "Submit" button and save it to submit later. The manuscript can then be located under "Unsubmitted Manuscripts" and you can click on "Continue Submission" to continue your submission when you choose to.

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After submission you will receive an e-mail to confirm receipt of your manuscript. If you do not receive the confirmation e-mail after 24 hours, please check your e-mail address carefully in the system. If the e-mail address is correct, please contact your IT department. The error may be caused by spam filtering software on your e-mail server. Also, the e-mails should be received if the IT department adds our e-mail server (uranus.scholarone.com) to their whitelist.

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Revised manuscripts must be uploaded within 12 weeks of authors being notified that they are invited to submit a revision. Locate your manuscript under "Revised Manuscripts in Draft" and click on "Continue Submission" to submit your revised manuscript. If, however, it is not possible to resubmit within this time frame, please contact the Editorial Office (jane@evj.co.uk). It is essential that you provide a point-by-point response to each of the reviewers’ comments.

All revised articles must be uploaded with:

(i) a main document containing a version which does not have a title page and is blinded and in which all changes are highlighted using 'track changes' mode. This version will be sent out to reviewers.

(ii) a document containing a version which includes the title page and in which blinding details have been re-inserted and changes are not marked. This should be uploaded with the file designation 'Clean Non-Blinded Copy'. If your manuscript is accepted, this version will be uploaded to our Accepted Articles section.

6. AFTER ACCEPTANCE

6.1 Pre-publishing of Accepted Articles

EVJ pre-publishes accepted articles online within a few days of acceptance. This service is not available with editorial material or for some supplements. Accepted articles are given a Digital Object Identifier (DOI), which allows the article to be cited and tracked before it is allocated to an issue. After print publication, the DOI remains valid and can continue to be used to cite and access the article. Information about accepted articles is submitted to indexing services as soon as they appear online.

BMC – Veterinary Research. Instructions for authors

Research articles

Criteria

Research articles should report on original primary research, but may report on systematic reviews of published research provided they adhere to the appropriate reporting guidelines which are detailed in our Editorial Policies. Please note that non-commissioned pooled analyses of selected published research will not be considered.

Submission process

Manuscripts must be submitted by one of the authors of the manuscript, and should not be submitted by anyone on their behalf. The submitting author takes responsibility for the article during submission and peer review.

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See below for examples of word processor and graphics file formats that can be accepted for the main manuscript document by the online submission system. Additional files of any type, such asmovies, animations, or original data files, can also be submitted as part of the manuscript.

During submission you will be asked to provide a cover letter. Use this to explain why your manuscript should be published in the journal, to elaborate on any issues relating to our editorial policies in the 'About *BMC Veterinary Research*' page, and to declare any potential competing interests. You will be also asked to provide the contact details (including email addresses) of potential peer reviewers for your manuscript. These should be experts in their field, who will be able to provide an objective assessment of the manuscript. Any suggested peer reviewers should not have published with any of the authors of the manuscript within the past five years, should not be current collaborators, and should not be members of the same research institution. Suggested reviewers will be considered alongside potential reviewers recommended by the Editorial team, Editorial Advisors, Section Editors and Associate Editors.

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- Portable document format (PDF)
- TeX/LaTeX (use BioMed Central's TeX template)
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Publishing Datasets

Through a special arrangement with LabArchives, LLC, authors submitting manuscripts to BMC Veterinary Research can obtain a complimentary subscription to LabArchives with an allotment of 100MB of storage. LabArchives is an Electronic Laboratory Notebook which will enable scientists to share and publish data files in situ; you can then link your paper to these data. Data files linked to published articles are assigned digital object identifiers (DOIs) and will remain available in perpetuity. Use of LabArchives or similar data publishing services does not replace preexisting data deposition requirements, such as for nucleic acid sequences, protein sequences and atomic coordinates.

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Authors linking datasets to their publications should include an Availability of supporting data section in their manuscript and cite the dataset in their reference list.

Preparing main manuscript text

General guidelines of the journal's style and language are given below.

Overview of manuscript sections for Research articles

Manuscripts for Research articles submitted to *BMC Veterinary Research* should be divided into the following sections (in this order):

- Title page
- Abstract
- Keywords
- Background
- Results and discussion
- Conclusions
- Methods (can also be placed after Background)
- Availability of supporting data
- List of abbreviations used (if any)
- Competing interests
- Authors' contributions
- Authors' information
- Acknowledgements
- Endnotes
- References
- Illustrations and figures (if any)
- Tables and captions
- Preparing additional files

The **Accession Numbers** of any nucleic acid sequences, protein sequences or atomic coordinates cited in the manuscript should be provided, in square brackets and include the corresponding database name; for example, [EMBL:AB026295, EMBL:AC137000, DDBJ:AE000812, GenBank:U49845, PDB:1BFM, Swiss-Prot:Q96KQ7, PIR:S66116].

The databases for which we can provide direct links are: EMBL Nucleotide Sequence Database (EMBL), DNA Data Bank of Japan (DDBJ), GenBank at the NCBI (GenBank), Protein Data Bank (PDB), Protein Information Resource (PIR) and the Swiss-Prot Protein Database (Swiss-Prot).

You can download a template (Mac and Windows compatible; Microsoft Word 98/2000) for your article.

For reporting standards please see the information in the About section.

Title page

The title page should:

- provide the title of the article
- list the full names, institutional addresses and email addresses for all authors
- indicate the corresponding author

Please note:

- abbreviations within the title should be avoided

Abstract

The Abstract of the manuscript should not exceed 350 words and must be structured into separate sections: **Background,** the context and purpose of the study; **Results**, the main findings;**Conclusions**, brief summary and potential implications. Please minimize the use of abbreviations and do not cite references in the abstract.

Keywords

Three to ten keywords representing the main content of the article.

Background

The Background section should be written in a way that is accessible to researchers without specialist knowledge in that area and must clearly state - and, if helpful, illustrate - the background to the research and its aims. The section should end with a brief statement of what is being reported in the article.

Results and discussion

The Results and discussion may be combined into a single section or presented separately. The Results and discussion sections may also be broken into subsections with short, informative headings.

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This should state clearly the main conclusions of the research and give a clear explanation of their importance and relevance. Summary illustrations may be included.

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The methods section should include the design of the study, the type of materials involved, a clear description of all comparisons, and the type of analysis used, to enable replication.

For studies involving human participants a statement detailing ethical approval and consent should be included in the methods section. For further details of the journal's editorial policies and ethical guidelines see 'About this journal'.

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BMC Veterinary Research encourages authors to deposit the data set(s) supporting the results reported in submitted manuscripts in a publicly-accessible data repository, when it is not possible to publish them as additional files. This section should only be included when supporting data are available and must include the name of the repository and the permanent identifier or accession number and persistent hyperlink(s) for the data set(s). The following format is required:

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List of abbreviations

If abbreviations are used in the text they should be defined in the text at first use, and a list of abbreviations can be provided, which should precede the competing interests and authors' contributions.

Competing interests

A competing interest exists when your interpretation of data or presentation of information may be influenced by your personal or financial relationship with other people or organizations. Authors must disclose any financial competing interests; they should also reveal any non-financial competing interests that may cause them embarrassment were they to become public after the publication of the manuscript.

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We suggest the following kind of format (please use initials to refer to each author's contribution): AB carried out the molecular genetic studies, participated in the sequence alignment and drafted the manuscript. JY carried out the immunoassays. MT participated in the sequence alignment. ES participated in the design of the study and performed the statistical analysis. FG conceived of the study, and participated in its design and coordination and helped to draft the manuscript. All authors read and approved the final manuscript.

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Acknowledgements

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Endnotes should be designated within the text using a superscript lowercase letter and all notes (along with their corresponding letter) should be included in the Endnotes section. Please format this section in a paragraph rather than a list.

References

All references, including URLs, must be numbered consecutively, in square brackets, in the order in which they are cited in the text, followed by any in tables or legends. Each reference must have an individual reference number. Please avoid excessive referencing. If automatic numbering systems are used, the reference numbers must be finalized and the bibliography must be fully formatted before submission.

Only articles, datasets and abstracts that have been published or are in press, or are available through public e-print/preprint servers, may be cited; unpublished abstracts, unpublished data and personal communications should not be included in the reference list, but may be included in the text and referred to as "unpublished observations" or "personal communications" giving the names of the involved researchers. Obtaining permission to quote personal communications and unpublished data from the cited colleagues is the responsibility of the author. Footnotes are not allowed, but endnotes are permitted. Journal abbreviations follow Index Medicus/MEDLINE. Citations in the reference list should include all named authors, up to the first 30 before adding '*et al.*'..

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Koonin EV, Altschul SF, Bork P: **BRCA1 protein products: functional motifs.** *Nat Genet* 1996,**13:**266-267.

Article within a journal supplement

Orengo CA, Bray JE, Hubbard T, LoConte L, Sillitoe I: **Analysis and assessment of ab initio three-dimensional prediction, secondary structure, and contacts prediction.** *Proteins* 1999,**43**(Suppl 3):149-170.

In press article

Kharitonov SA, Barnes PJ: **Clinical aspects of exhaled nitric oxide.** *Eur Respir J*, in press.

Published abstract

Zvaifler NJ, Burger JA, Marinova-Mutafchieva L, Taylor P, Maini RN: **Mesenchymal cells, stromal derived factor-1 and rheumatoid arthritis [abstract].** *Arthritis Rheum* 1999, **42:**s250.

Article within conference proceedings

Jones X: **Zeolites and synthetic mechanisms.** In *Proceedings of the First National Conference on Porous Sieves: 27-30 June 1996; Baltimore.* Edited by Smith Y. Stoneham: Butterworth-Heinemann; 1996:16-27.

Book chapter, or article within a book

Schnepf E: **From prey via endosymbiont to plastids: comparative studies in dinoflagellates.** In *Origins of Plastids. Volume 2.* 2nd edition. Edited by Lewin RA. New York: Chapman and Hall; 1993:53-76.

Whole issue of journal

Ponder B, Johnston S, Chodosh L (Eds): **Innovative oncology.** In *Breast Cancer Res* 1998, **10:**1-72.

Whole conference proceedings

Smith Y (Ed): *Proceedings of the First National Conference on Porous Sieves: 27-30 June 1996; Baltimore.* Stoneham: Butterworth-Heinemann; 1996.

Complete book

Margulis L: *Origin of Eukaryotic Cells.* New Haven: Yale University Press; 1970.

Monograph or book in a series

Hunninghake GW, Gadek JE: **The alveolar macrophage.** In *Cultured Human Cells and Tissues.*Edited by Harris TJR. New York: Academic Press; 1995:54-56. [Stoner G (Series Editor): *Methods and Perspectives in Cell Biology,* vol 1.]

Book with institutional author

Advisory Committee on Genetic Modification: *Annual Report.* London; 1999.

PhD thesis

Kohavi R: **Wrappers for performance enhancement and oblivious decision graphs.** *PhD thesis.* Stanford University, Computer Science Department; 1995.

Link / URL

The Mouse Tumor Biology Database [http://tumor.informatics.jax.org/mtbwi/index.do]

Link / URL with author(s)

Neylon C: Open Research Computation: an ordinary journal with extraordinary aims. [http://blogs.openaccesscentral.com/blogs/bmcblog/entry/open_research_computation_an_ordinary]

Dataset with persistent identifier

Zheng, L-Y; Guo, X-S; He, B; Sun, L-J; Peng, Y; Dong, S-S; Liu, T-F; Jiang, S; Ramachandran, S; Liu, C-M; Jing, H-C (2011): **Genome data from sweet and grain sorghum (Sorghum bicolor)**.*GigaScience*. http://dx.doi.org/10.5524/100012.

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Abbreviations should be used as sparingly as possible. They should be defined when first used and a list of abbreviations can be provided following the main manuscript text.

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