NATHALIA DOS SANTOS ROSSE

AVALIAÇÃO DE ÍNDICES DOPPLERFLUXOMÉTRICOS RENAIS EM EQUINOS

Dissertação apresentada à Universidade Federal de Viçosa, como parte das exigências do Programa de Pós-Graduação em Medicina Veterinária, para obtenção do título de *Magister Scientiae*.

Orientadora: Emily Correna Carlo Reis

Coorientadores: Bruna W. de Freitas
Fabrício Luciani Valente
José Dantas Ribeiro Filho
Marcel F. Bastos Avanza

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APROVADA: 21 de outubro de 2022.

Assentimento:

Nathalia dos Santos Rosse
Autora

Emily Correna Carlo Reis
Orientadora

Dedico esta dissertação à Nilza de Mattos Rosse (*in memoriam*), minha tartaruguinha e estrela protetora, minha pessoa favorita no mundo.

“A saudade é o amor que fica.”

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RESUMO

ROSSE, Nathalia dos Santos, M.Sc., Universidade Federal de Viçosa, outubro do 2022. **Avaliação de índices dopplerfluxométricos renais em equinos.** Orientadora: Emily Correna Carlo Reis. Coorientadores: Bruna Waddington de Freitas, Fabrício Luciani Valente, José Dantas Ribeiro Filho e Marcel Ferreira Bastos Avanza.

Os rins são órgãos vitais responsáveis por diversas funções no organismo. Dentre tais funções, destacam-se a filtração do sangue para eliminação de produtos do metabolismo e de compostos potencialmente tóxicos, a manutenção do equilíbrio hidroeletrolítico e ácido base, a regulação do pH sanguíneo e a produção hormonal. A circulação sanguínea renal é de extrema importância para que as funções citadas sejam bem-sucedidas. Apesar de exames laboratoriais, como hemograma, bioquímico e urinálise, já serem parte da rotina veterinária, as alterações encontradas em tais exames são tardias quando relacionadas à função renal. Para tanto, o exame ultrassonográfico pode ser empregado, visando assim a detecção precoce de alterações na morfologia e hemodinâmica renais. A partir do exame ultrassonográfico Doppler é possível avaliar a perfusão do órgão, bem como quantificar a resistência arterial ao fluxo sanguíneo. Os índices de resistividade e pulsatilidade renais são calculados a partir de dados obtidos pelo gráfico da onda de pulso arterial e refletem a resistência arterial. Em humanos e pequenos animais, essa prática já vem sendo empregada ao longo das últimas décadas. Em equinos, os relatos ainda são escassos. Sendo assim, buscou-se com este trabalho realizar uma revisão sistemática dos artigos científicos já publicados sobre índices Dopplerfluxométricos em equinos, bem como realizar dois estudos experimentais afim de determinar valores de normalidade para os índices de resistividade e pulsatilidade renais em animais hígidos, além de avaliar a influência sofrida por estes índices após um período de jejum hídrico e alimentar. O primeiro estudo foi realizado em 29 animais adultos e saudáveis, em que o exame ultrassonográfico foi realizado por via transabdominal e transretal para comparação dos resultados obtidos. No segundo estudo, quatro animais submetidos a 24 horas de jejum hídrico e alimentar foram avaliados por via transabdominal para verificar se o jejum seria capaz de causar mudanças nos índices de resistividade e pulsatilidade renais. Não foram encontradas diferenças significativas entre rim esquerdo e rim direito tampouco entre as vias transretal e transabdominal, sugerindo que a via pode ser eleita de acordo com a habilidade do operador. Também não foram encontradas diferenças significativas entre os tempos de avaliação no estudo com animais em jejum, acreditamos que devido ao curto período de restrição. Espera-se com este trabalho elucidar questões a respeito do exame ultrassonográfico Doppler renal em equinos, além de buscar determinar um valor de referência para os índices Dopplerfluxométricos para a espécie equina.

Palavras-chave: Equino. Rim. Ultrassonografia. Doppler.

ABSTRACT

ROSSE, Nathalia dos Santos, M.Sc., Universidade Federal de Viçosa, October, 2022. **Evaluation of equine renal Doppler indexes.** Adviser: Emily Correna Carlo Reis. Co-advisers: Bruna Waddington de Freitas, Fabrício Luciani Valente, José Dantas Ribeiro Filho and Marcel Ferreira Bastos Avanza.

The kidneys are vital organs responsible for various functions in the body. Among these functions, some are highlighted: blood filtration to eliminate metabolic products and potentially toxic compounds, maintenance of hydroelectrolytic and acid-base balance, regulation of blood pH and hormone production. Renal blood flow is extremely important for the aforementioned renal functions to be successful. Although laboratory tests such as hematological, biochemical and urinalysis tests are already part of the veterinary routine, when related to renal function the alterations represented in those tests can already be linked to an advanced stage of disease. Therefore, ultrasound examination can be performed aiming at the early detection of changes in renal morphology and hemodynamics. From the Doppler ultrasound examination, it is possible to assess organ perfusion, as well as quantify arterial resistance to blood flow. Renal resistivity and pulsatility indexes are calculated from data obtained from the arterial pulse waveforms and reflect arterial resistance. In humans and small animals, this procedure has already been used over the last few decades. In horses, scientific reports are still scarce. Therefore, this manuscript sought to carry out a systematic review of articles already published on Doppler indexes in horses, as well as to realize two experimental studies. The aims of the experimental studies were to determine a normal range of values for renal resistivity and pulsatility indexes in healthy animals, in addition to evaluating the influence of a period of fasting on those indices. The first study enrolled 29 healthy adult animals, in which the ultrasound examination was performed transabdominally and transrectally to compare the obtained renal resistivity and pulsatility indexes results. In the second study, four animals were submitted to 24 hours of water and food fasting. Renal transabdominal ultrasonography was performed to verify whether fasting would be able to cause changes in renal resistivity and pulsatility indexes. No significant differences were found between the left and right kidneys, nor between the transrectal and transabdominal approaches, suggesting that the approach can be chosen according to the operator`s skills. Also, no significant differences were found between the evaluation time-points in the study with fasted animals. We believe that the lack of significant difference between time-points may be due to the short fasting period. The purpose of the author by this manuscript is to elucidate questions about the renal Doppler ultrasound examination in horses and to stablish a normal range of value of the Doppler indexes for the species.

Keywords: Equine. Kidney. Ultrasonography. Doppler.

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1. CAPÍTULO 1 - INTRODUÇÃO GERAL

Os rins são órgãos vitais ao organismo, sendo responsáveis por diversas funções essenciais à manutenção da vida. Em diferentes afecções, a função renal pode ser comprometida, seja por componentes inflamatórios, agentes infecciosos, medicamentos potencialmente nefrotóxicos ou ainda, agentes tóxicos externos ou internos (Toribio, 2007). A detecção e diagnóstico dessas afecções nem sempre é simples, podendo-se utilizar, além de exames laboratoriais, os exames de imagem, como ultrassonografia renal. Através do exame ultrassonográfico pode-se avaliar, além da morfologia e arquitetura renais pelo modo bidimensional, também sua hemodinâmica e vascularização pelo modo Doppler colorido e através de índices gerados a partir de informações do modo Doppler espectral (Novellas et al., 2007). Em equinos, no entanto, a técnica e resultados esperados a partir da avaliação Doppler espectral dos rins não são bem elucidados. Sendo assim, esta dissertação objetiva apresentar uma breve revisão bibliográfica acerca do tema “índices dopplerfluxométricos renais em equinos”, desenvolver uma revisão sistemática a partir de artigos científicos publicados no assunto, além de apresentar os dados obtidos em dois experimentos realizados com equinos hígidos e sob privação hídrica e alimentar.

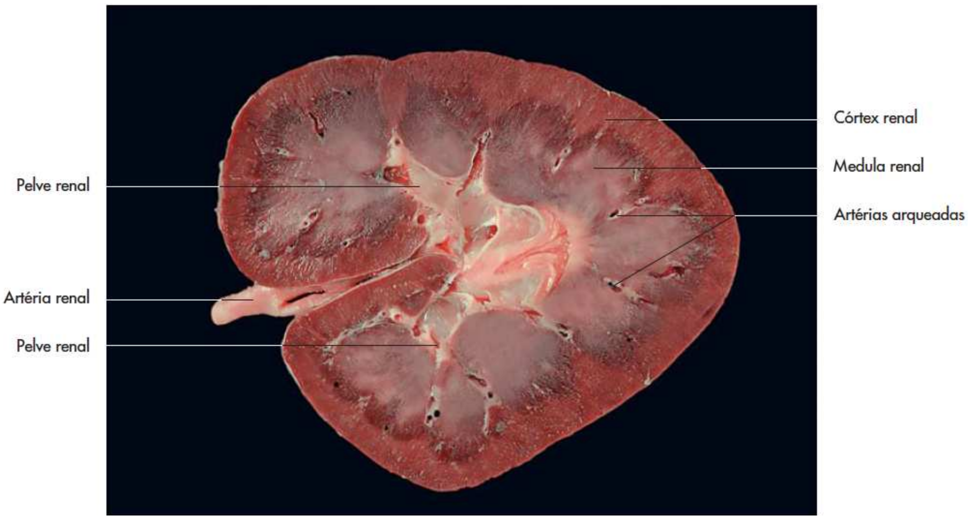
1.1. Anatomia renal

Os rins são órgãos de consistência firme e coloração marrom-avermelhada que se encontram envoltos em uma camada de gordura, estando localizados em posição retroperitoneal (Dyce et al., 2010). Nos equinos, o rim esquerdo está mais próximo ao plano mediano e apresenta formato de grão de feijão, estando situado ventral à última costela e aos primeiros dois ou três processos transversos lombares. Já o rim direito apresenta formato de coração e se situa ventral às últimas duas ou três costelas e ao primeiro processo transverso lombar, portanto, estando mais cranial quando comparado ao rim esquerdo em aproximadamente a metade de seu próprio comprimento (Brudas, 2012). O rim direito está em contato com o diafragma em sua superfície dorsal, com o fígado pelo seu polo cranial que toca na impressão renal do fígado e com o ceco e pâncreas ventralmente, a margem medial está relacionada à veia cava caudal e à glândula adrenal direita (Dyce et al., 2010). O rim esquerdo está localizado medial ao baço e ligado a ele por meio do ligamento nefroesplênico (Brudas, 2012).

O parênquima renal é constituído de uma porção medular interna, uma porção cortical externa (Figura 1.1), pelo hilo renal e é envolto por uma espessa cápsula fibrosa. O rim equino é classificado como unipiramidal, pois possui uma única massa medular que é envolvida por uma concha cortical contínua. A unidade funcional renal é chamada de néfron e é compreendida pelas seguintes porções: cápsula glomerular, túbulo contorcido proximal, alça de Henle (túbulo reto proximal ou ramo descendente, túbulo atenuado ou alça, túbulo reto distal ou ramo ascendente) e túbulo contorcido distal, que se junta ao túbulo coletor reto, que se unirá a vários iguais a este para formar um ducto papilar e desembocar na pelve renal. A cápsula glomerular faz contato com o glomérulo, um plexo de capilares sanguíneos formados pela arteríola glomerular aferente, dando origem ao corpúsculo renal (Konig e Liebich, 2016).

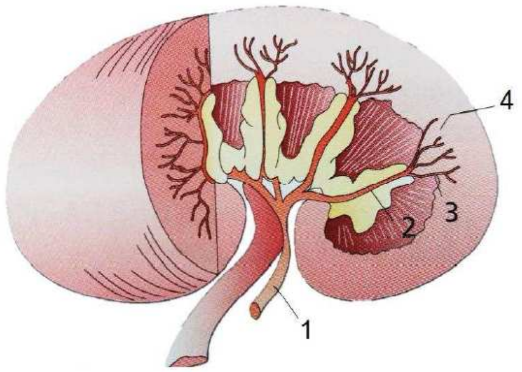
Quanto a vascularização, cada rim é irrigado pela artéria renal correspondente, que são ramos da artéria aorta abdominal. A artéria renal se divide em numerosas artérias interlobares, que atravessam a região medular e seguem em direção à região cortical. Na transição córtico-medular, as artérias se curvam nas bases das pirâmides e passam a ser chamadas então de arqueadas, e estas, por sua vez, darão origem às artérias interlobulares (Figura 1.2). As veias são, de modo geral, satélites, recebendo a mesma nomenclatura e desembocam na veia cada caudal (Dyce et al., 2010).

Figura 1.1 - Rim direito de um equino em secção equatorial.



Fonte: Konig e Liebich (2016).

Figura 1.2 - Figura esquemática de rim de cão e gato para demonstração da anatomia vascular. 1: artéria renal, 2: artéria interlobar, 3: artéria arqueada e 4: artéria interlobular.



Fonte: Adaptado de Carvalho (2009).

1.2. Importância dos rins na homeostase

Entre as funções dos rins, destacam-se a regulação do equilíbrio hidroeletrolítico e ácido base, manutenção do pH sanguíneo, excreção de produtos do metabolismo e/ou tóxicos ao organismo, regulação da pressão arterial, bem como produção, secreção e degradação hormonal (Scott II, 2018).

O rim, como uma estrutura anátomo-fisiológica complexa composta por um extenso sistema tubular e vascular, possibilita a filtração do sangue quando este permeia no parênquima renal. Nos túbulos, são excretados produtos indesejáveis provenientes dos processos fisiológicos do organismo ou que apresentam toxicidade ao mesmo e absorvidas substâncias importantes, como glicose, água, eletrólitos e proteínas de baixo peso molecular (Savage, 2008). Neste processo é formado o produto primário chamado de ultrafiltrado, que passará por reabsorção e excreção até a formação do produto final, a urina (Scott II, 2018).

As funções de manutenção do equilíbrio hidroeletrolítico e ácido base, pH sanguíneo e níveis séricos de diversos eletrólitos são resultantes do fluxo sanguíneo renal, da filtração glomerular, da reabsorção e secreção nos túbulos e dos efeitos do eixo renina – angiotensina – aldosterona. Em equinos, os rins recebem cerca de 20% do débito cardíaco e o fluxo sanguíneo renal em adultos varia entre 10 a 25 mL/kg/min (Toribio, 2007). Esta alta taxa de perfusão é essencial para que a filtração glomerular seja eficiente, assim como para que as necessidades metabólicas do próprio órgão sejam supridas (Scott II, 2018).

Além disso, os rins são importantes órgãos endócrinos que produzem renina, eritropoietina e a forma ativa de vitamina D; eles também desempenham um papel importante na degradação e excreção de uma série de outros hormônios, incluindo a gastrina e o paratormônio (Waldridge, 2010; Scott II, 2018).

Em suma, os rins são órgãos essenciais para a homeostase sanguínea, permitindo a estabilização dos componentes sanguíneos, retendo compostos importantes ao organismo e eliminando produtos tóxicos ou que se encontram em excesso (Thomassian, 2005).

1.3. Afecções renais

Apesar das enfermidades renais primárias serem relatadas como pouco frequentes nos equinos, existem diversos relatos de casos sobre as mais variadas doenças acometendo os rins de cavalos, além de revisões de literatura acerca do tema (Toribio, 2007; McLeland, 2015; Savage et al., 2019; DeNotta, 2022). Além de enfermidades primárias, os rins podem ser acometidos secundariamente a diversas outras doenças, intoxicações e diminuição de volume sanguíneo (Thomassian, 2005). Ainda, segundo Knottenbelt e Pascoe (1994), lesões renais são frequentemente encontradas no exame post-mortem em cavalos, apontando para um possível quadro de doenças renais subdiagnosticadas.

Os sinais clínicos e laboratoriais mais comumente encontrados nas doenças do trato urinário são relacionados à frequência, volume e características da urina, por exemplo: disúria, estrangúria, incontinência, hematúria, piúria, cristalúria, poliúria, anúria, oligúria, além de predispor o acúmulo de substâncias tóxicas no sangue (uremia) (Van Metre, 2014). As manifestações clínicas estão diretamente ligadas ao local de origem da lesão no trato urinário, bem como à sua extensão e consequente redução da função do órgão (Knottenbelt e Pascoe 1994).

Dentre as afecções renais que os equinos podem apresentar primaria ou secundariamente estão: glomerulonefrite, nefrite intersticial crônica, pielonefrite bacteriana, neoplasia, nefropatia tóxica, hipoplasia renal, cálculos, hidronefrose, trauma, abscesso renal, rins policísticos e insuficiência renal aguda ou crônica (Matthews e Toal, 1996).

A glomerulonefrite em equinos é mais comumente causada pela deposição de imunocomplexos nos glomérulos, geralmente em decorrência de doenças infecciosas como a Anemia Infecciosa Equina ou causadas pelo agente *Streptococcus equi* (McLeland, 2015). Nesta doença ocorre perda da barreira de filtração glomerular, levando à passagem de moléculas maiores, que normalmente não seriam filtradas, causando, por exemplo, proteinúria, achado laboratorial mais evidente na lesão glomerular (Wilson, 2007). Clinicamente, os equinos podem apresentar anorexia, edema, perda de peso e apatia (McLeland, 2015).

Lesões tubulares e de interstício estão habitualmente correlacionadas, isto se deve à íntima relação de função e anatomia destas regiões. A injúria túbulo-intersticial pode acontecer por agentes tóxicos ou infecciosos e isquemia, podendo ser grave o suficiente para evoluir em doença renal aguda ou, persistindo o agravo, em doença renal crônica. Os principais achados são azotemia, redução da concentração da urina e dificuldade na regulação eletrolítica, causando perda da homeostase (McLeland, 2015).

Quanto à pelve renal, equinos tratados com anti-inflamatórios não-esteroides podem apresentar lesão nesta região, isto porque essa classe medicamentosa possui efeitos tóxicos às células da pelve renal. Além disso, em animais já em desidratação ou hipovolemia, os efeitos desses medicamentos nos rins podem ser potencializados. Nestes casos, a perda da definição estrutural da pelve renal é possível de ser visualizada através do exame ultrassonográfico (McLeland, 2015).

A doença renal aguda (DRA) é de grande relevência em equinos, esse termo é empregado para descrever lesões renais recentes, sejam discretas ou graves, até mesmo sem sintomatologia clínica. Ela pode ser definida pela presença de um ou mais dos seguintes sinais: aumento da creatinina sérica em 0,3 mg/dL ou mais em 48 horas, aumento da creatinina sérica em uma vez e meia ou mais de seu nível basal em uma semana ou oligúria persistente por seis horas mesmo com o animal hidratado (Savage et al., 2019; Divers, 2022). A DRA em equinos é comumente vista em animais hospitalizados, estando geralmente ligada a causas hemodinâmicas ou nefrotóxicas. No entanto, em sua maioria, os casos não evoluem para insuficiência renal ou doença renal crônica (Savage et al., 2019).

Já a doença renal crônica (DRC) tem duração de pelo menos três meses, sendo considerada degenerativa e irreversível, causando diminuição da taxa de filtração glomerular (Olsen e van Galen, 2022). A perda de função progressiva leva a incapacidade de concentrar a urina, retenção de produtos nitrogenados e outros potencialmente tóxicos, perda da capacidade de regulação hormonal, além da inabilidade de manutenção da homeostase (Schott, 2007). A concentração sérica de creatinina pode ser utilizada para determinar o prognóstico, no entanto, como citado anteriormente, a DRC é considerada irreversível, sendo o tratamento apenas paliativo (Jocelyn, 2020).

1.4. Ultrassonografia na avaliação renal

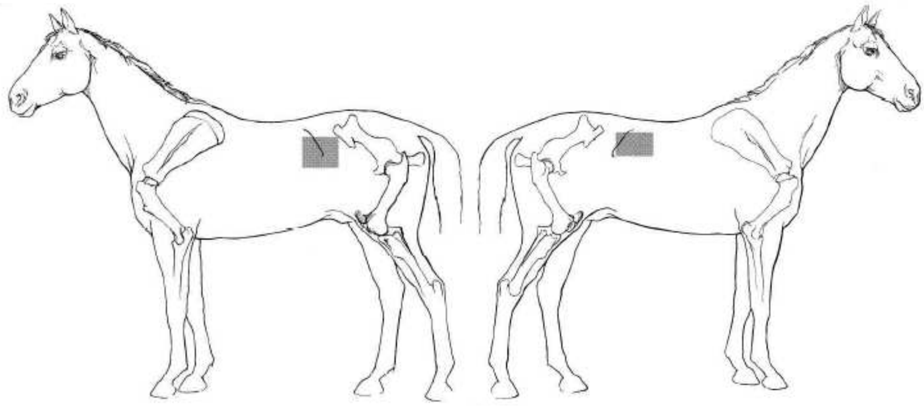
Além dos exames laboratoriais hematológicos e urinários, os exames de imagem têm se mostrado cada vez mais confiáveis na avaliação renal dos animais domésticos. Por se tratar de uma técnica não invasiva, a ultrassonografia se tornou essencial na medicina equina e, quando associada ao histórico e sinais clínicos do paciente, é capaz de gerar informações relevantes para auxiliar o veterinário na tomada de decisão (Kidd et al., 2014).

O exame ultrassonográfico auxilia no diagnóstico e no prognóstico das doenças renais em equinos, fornecendo informações dinâmicas sobre morfologia e vascularização do órgão (Rantanen, 1986; Macri et al., 2015). A avaliação da forma, ecotextura, tamanho e posicionamento renal são possíveis com o uso do modo B no aparelho de ultrassom, essas informações são cruciais para auxiliar um diagnóstico preciso do paciente (Rantanen, 1986). Já as informações a respeito da hemodinâmica e perfusão do órgão são fornecidas pelos modos Doppler colorido e pulsátil (Macri et al., 2015).

1.4.1. Técnicas do exame ultrassonográfico renal em equinos

Em equinos a avaliação ultrassonográfica dos rins pode ser feita por meio de duas técnicas, transcutânea abdominal ou transretal. Pela técnica transcutânea, o rim esquerdo é visto profundamente ao baço na fossa paralombar esquerda caudal ao 15 – 17º espaço intercostal, já o rim direito é visto caudal ao 14 – 17º espaço intercostal, próximo a parede abdominal (Reef, 1998). Na avaliação morfológica, o rim direito é mais oval no plano longitudinal que o rim esquerdo, o córtex renal se apresenta hipoecóico em relação aos órgãos adjacentes, a transição córticomedular é bem visível, sendo a região medular hipoecóica em relação a região cortical e o rim esquerdo costuma ter dimensões um pouco maiores que o rim direito (Kidd et al., 2014). As janelas acústicas para visualização dos rins estão representadas na Figura 1.3.

Figura 1.3 - Representação das janelas acústicas para visualização dos rins em um equino adulto.



Fonte: Adaptado de Reef (1998).

Segundo Schmidt (1989), os órgãos passíveis de avaliação ultrassonográfica pela técnica transretal são os mesmos que podem ser palpados pelo veterinário. Sendo assim, o polo caudal do rim esquerdo, que se encontra mais caudal em relação ao direito, normalmente é acessível e o rim direito pode ou não estar ao alcance da palpação, no entanto o mais comum é não ser palpável (Matthews e Toal, 1996; Reef, 1998). Tem-se descrito na literatura que imagens do rim direito obtidas pela janela transabdominal e do rim esquerdo pela janela transretal são de melhor detalhamento morfológico, e além disso, pela técnica transretal também seria possível avaliar artérias da região medular e da pelve renal, profundamente à região cortical (Reef, 1998).

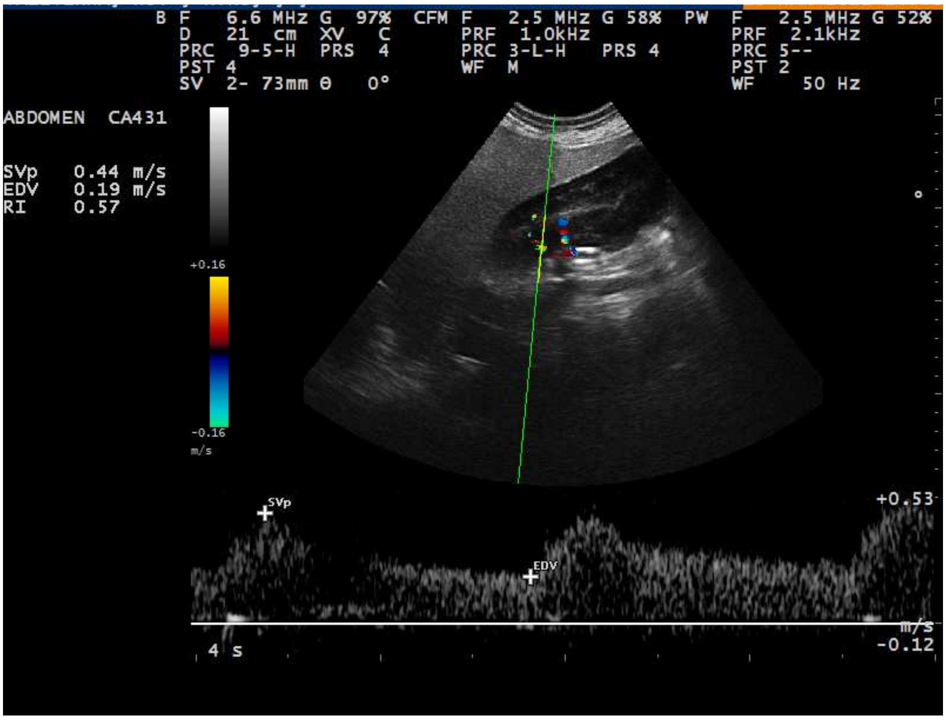
1.4.2. Modo Doppler colorido e pulsado

As funções Doppler colorido e pulsado ou espectral são um avanço muito importante para a medicina, pois possibilitam a avaliação da hemodinâmica do órgão, fornecendo imagens dinâmicas do fluxo sanguíneo, como sua direção, intensidade e velocidade. Dessa forma, geram informações qualitativas e quantitativas, como os índices de resistividade e pulsatilidade (Granata et al., 2014; Macri et al., 2015). Sendo assim, condições que alteram a perfusão do parênquima renal podem ser reconhecidas e quantificadas através da formação de ondas espectrais e geração dos índices supracitados (Petersen, 1997). O Doppler colorido relaciona a direção do fluxo sanguíneo ao transdutor utilizando as cores azul e vermelha como padrão, em que a cor azul representa a corrente sanguínea se distanciando do transdutor e a cor vermelha representa a aproximação (Kidd at al., 2014). O Doppler espectral é especialmente útil na determinação da velocidade e direção do fluxo arterial em relação ao tempo, formando uma imagem caracterizada por uma linha de base em que ondas formadas acima desta linha são representativas da corrente sanguínea se aproximando do transdutor e ondas abaixo desta linha são referentes a corrente se afastando do transdutor (Kidd et al., 2014).

1.4.3. Índices de Resistividade e Pulsatilidade e suas aplicações

Os índices de resistividade (IR) e pulsatilidade (IP) são calculados a partir de dados de velocidade da corrente sanguínea durante a sístole e a diástole obtidos através de ondas de pulso formadas no modo Doppler pulsado (Petersen, 1997), demostrada na Figura 1.4 abaixo. Estes índices representam, de forma indireta, a resistência vascular periférica do vaso avaliado, uma vez que a velocidade diastólica final é mais afetada pela resistência imposta pelo tecido à passagem no sangue (Novellas et al., 2007). IR e IP apresentam correlação negativa com a perfusão vascular do tecido irrigado pela artéria em questão, ou seja, quanto menores IR e IP maior será a perfusão vascular no tecido suprido por aquele vaso (Ginther, 2007). Os valores de IR e IP podem ser obtidos diretamente no aparelho de ultrassom, no entanto, as fórmulas para cálculo dos índices são: IR = ([pico de velocidade sistólica – velocidade diastólica final] / pico de velocidade sistólica); IP = ([pico de velocidade sistólica – velocidade diastólica final) / velocidade média) (Novellas et al., 2007).

Figura 1.4 - Traçado espectral da artéria arqueada de equino adulto. Em que SVp: pico da velocidade sistólica; EDV: velocidade diastólica final; RI: índice de resistividade.



Fonte própria.

Considerando a aplicação do IP e IR para a avaliação ultrassonográfica renal, deve-se ponderar que, por mais que a perfusão sanguínea destes órgãos seja elevada, os rins são altamente suscetíveis a hipóxia; isto porque a extração de oxigênio renal é muito baixa comparada ao aporte que os mesmos recebem e a distribuição do fluxo sanguíneo é desigual, sendo cerca de 80% para a cortical e 20% para medular (Toribio, 2007). Portanto, alterações na perfusão tecidual podem ser os primeiros sinais de alteração da função renal em afecções envolvendo estes órgãos e, sendo assim, a mensuração do IP e IR é utilizada como parâmetro para avaliar lesão renal (Platt, 1997).

A mensuração dos índices dopplerfluxométricos já são amplamente utilizados em humanos para investigar ou acompanhar a evolução de diversas afecções, além de auxiliar na determinação do prognóstico do paciente (Granata et al., 2014). Já foram publicados estudos acerca das alterações na perfusão renal (Harzmann e Weckermann, 1990), do efeito preditor dos índices para revascularização renal em casos de hipertensão renovascular (Santos et al., 2009), da avaliação da severidade da doença renal crônica (Petersen et al., 1997), bem como para o acompanhamento de transplantes (Tublin et al., 2013) e diversos outros tópicos inseridos na nefrologia, cardiologia e angiologia médicas.

Na Medicina Veterinária, os índices de pulsatilidade e resistividade já vem sendo empregados para avaliação renal em pequenos animais, como demostrado por diversos autores, por exemplo para avaliação de DRC em gatos (Matos et al., 2017), DRA em cães e gatos (Grauer, 2005), síndrome cardiorrenal em cães (Szczepankiewicz et al., 2021), hipertensão sistêmica em cães com hiperadrenocorticismo (Chen et al., 2016), além de estudos para determinação de valores de referência para espécie (Melo et al., 2006; Novellas et al., 2007; Moarabi et al., 2020). Em equinos, no entanto, os dados presentes na literatura ainda são escassos.

1.5. Objetivos e justificativa

Considerando a relevância dos índices dopplerfluxométricos nas espécies humana, canina e felina, com este trabalho objetiva-se avaliar a utilização dos índices de resistividade e pulsatilidade no exame renal em equinos. Para isso, realizou-se uma revisão sistemática acerca do índice de resistividade em equinos, buscando-se conhecer a metodologia deste exame e quais os resultados nos animais hígidos (parâmetros de normalidade) e nos doentes. Com base nos resultados da revisão, verificou-se a necessidade da execução de um estudo experimental para mensuração de índices dopplerfluxométricos em equinos saudáveis, buscando determinar uma faixa de normalidade e sua relação com a via de realização do exame, sendo estas as vias transcutânea e transretal. Além disso, considerando a influência da hidratação nos valores dos índices de resistividade e pulsatilidade em outras espécies, avaliou-se experimentalmente estes índices em equinos submetidos a um protocolo de desidratação e reidratação enteral por via intracecal.

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2. CAPÍTULO 2 - SYSTEMATIC REVIEW

Doppler renal resistivity index in horses: a systematic review.

Doppler renal resistivity index in horses: a systematic review.

Abstract

The renal resistivity index is a tool to evaluate the hemodynamics through arterial resistance. Considering low perfusion as one of the first signs of kidney injury, minimal alterations in renal blood flow could be especially important for the early detection of kidney damage. The aim of this systematic review was to retrieve published studies on equine renal resistivity index (RI) in order to develop a standardized method of renal ultrasound examination through a transabdominal approach as well as a reference range for renal RI value in horses. An electronic search in Science Direct, PubMed, Scopus and Web of Science databases was performed in February 2022 using the terms “RI” OR “resistivity index” OR “IP” OR “pulsatility index” AND (kidney OR renal) AND (equine OR horse) in titles, keywords and abstracts. The studies were screened based on inclusion criteria and variables of interest were extracted. To assess the methodological quality, the SYRCLES`s risk of bias tool was elected by the authors. From the search, a total of 134 studies were identified and five of them were considered eligible. The selected studies had all been conducted in healthy non-sedated horses thought transabdominal technique. One study did not present renal RI data for each kidney while the other four had. The upper limit of normality for renal RI was 0.58 ± 0.06 for the right kidney of untrained horses, which is lower than the value of 0.70 currently used for humans, cats and dogs. There were heterogenous outcomes among studies, where two out of the five demonstrated difference between RI value of left and right kidneys and one out of the five showed increased RI value in the elderly compared to foals and adult animals. It is necessary to determine a pattern to RI studies in horses to be conducted for the sake of good quality results and, finally, clinical application.

Keywords: equine, Doppler ultrasonography, resistive index, kidney

2.1. Introduction

The kidneys are vital organs as they are responsible for filtering blood so toxins and waste metabolic substances can be eliminated and also for homeostasis as they play a main role in the hydric, acid-base and electrolyte balance, help controlling blood pressure, having endocrine function as well[1]. Although renal diseases are reported as being uncommonly diagnosed in horses, there are several published reviews and case reports on different renal diseases[2-6] suggesting otherwise. A study on the prevalence of acute kidney injury (AKI) in hospitalized horses found that 14.8% of studied horses developed AKI[7]. Further, Knottenbelt e Pascoe[8] have noted that kidney injuries are frequently found in *post-mortem* examinations in horses and so, we are probably dealing with a scenario where kidney diseases are underdiagnosed.

Considering the difficulties on treatment of kidney disease, it is very important to stablish an early diagnosis due to its major impact on the body. There are several hematological and urinary exams available nowadays, but it is known that renal injuries up to 75% can be compensated by nephrons and possibly no relevant alterations would be seen in those exams[9]. In that sense, when kidney injuries are detected using these methods, effective treatment might be unattainable. In human medicine, ultrasonography is being widely used as a tool to enhance the early diagnosis of renal damage. It is an easy and non-invasive exam that provides information about the morphology, shape, length, echogenicity and even hemodynamics of the kidneys[10, 11].

On top of the well-known B mode, the Doppler ultrasound can detect alterations of reflected sound waves frequency when the object hit by the wave is in movement and, by an equation, the velocity of that object can be obtained[12]. The color Doppler shows the presence and direction of the object towards the probe, it is majorly represented by the blue and red colors, largely used for blood flow evaluation[13]. Furthermore, the pulsed Doppler is responsible for reading the sound pulses and generating a spectral wave that shows their number within the same frequency[12], being represented as velocity X time. Two indexes can be calculated from de information acquired from the spectral wave, the resistivity index (RI) and the pulsatility index (PI). These indexes stand for (1) RI ([peak systolic velocity – final diastolic velocity]/peak systolic velocity) and (2) PI ([peak systolic velocity – final diastolic velocity]/average velocity), according to Novellas et al.[14]. The RI and PI give information about perfusion of the organ and its vascular resistance[15,16].

Measurements of renal RI and PI are already being described in humans as a method to investigate and follow the progression of various diseases, not only related directly to the kidneys[17], as well as a valuable technique to evaluate the successfulness of renal transplantations[18]. In small animals, it has been reported that RI and PI would be advantageous to evaluate chronic renal disease in cats[19,20], acute renal disease in dogs and cats[21], cardiorenal syndrome in dogs[22] and a number of other conditions. In addition, there are numerous studies regarding the normal range for each species[14,23,24].

For horses, however, there are only few studies in respect to RI, consequently, limiting its clinical use. This review aimed to evaluate RI values in horses and analyze published studies concerning their methodology, appraise and compare their results and analyze their clinical utility. In addition, we were able to critically review possible limitations, difficulties and methodological flaws in the selected studies along with pointing achievable future directions on the subject.

2.2. Materials and methods

The present systematic review was conducted according to the premises of PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses)[25].

2.2.1. Search strategy

PubMed, Science Direct, Web of Science and Scopus were the databases chosen for this research. An extensive search was driven applying the following terms: RI OR “resistivity index” OR IP OR “pulsatility index” AND (kidney OR renal) AND (equine OR horse) in titles, keywords and abstracts. There were no limitations upon language, year or country of publication and the last search was performed on February 9, 2022.

2.2.2. Eligibility criteria

The studies screened in the searching were selected if they accomplished the following features: (1) horses were the animal model, (2) conducted in renal vessels,

(3) at least the RI was measured. Further, the studies were excluded if they were: (1) in another species other than equine, (2) not performed in the kidneys, (3) book chapters, (4) not mentioning RI or PI.

2.2.3. Data extraction

Extraction was carried out by the same people and the variables considered as being of interest were listed upon an Excel table. Extracted data were grouped according to articles information (authors, year and country of publication), experimental model (horses' breed, age, body weight and sex), methodology (animal grouping, sedated or non-sedated, athletes or not athletes, comparing or not left and right kidney, comparison between age groups, artery of interest), technical information (type of equipment and probe, frequency of the last, wall filter, sample volume) and the values obtained for RI.

2.2.4. Risk of Bias assessment

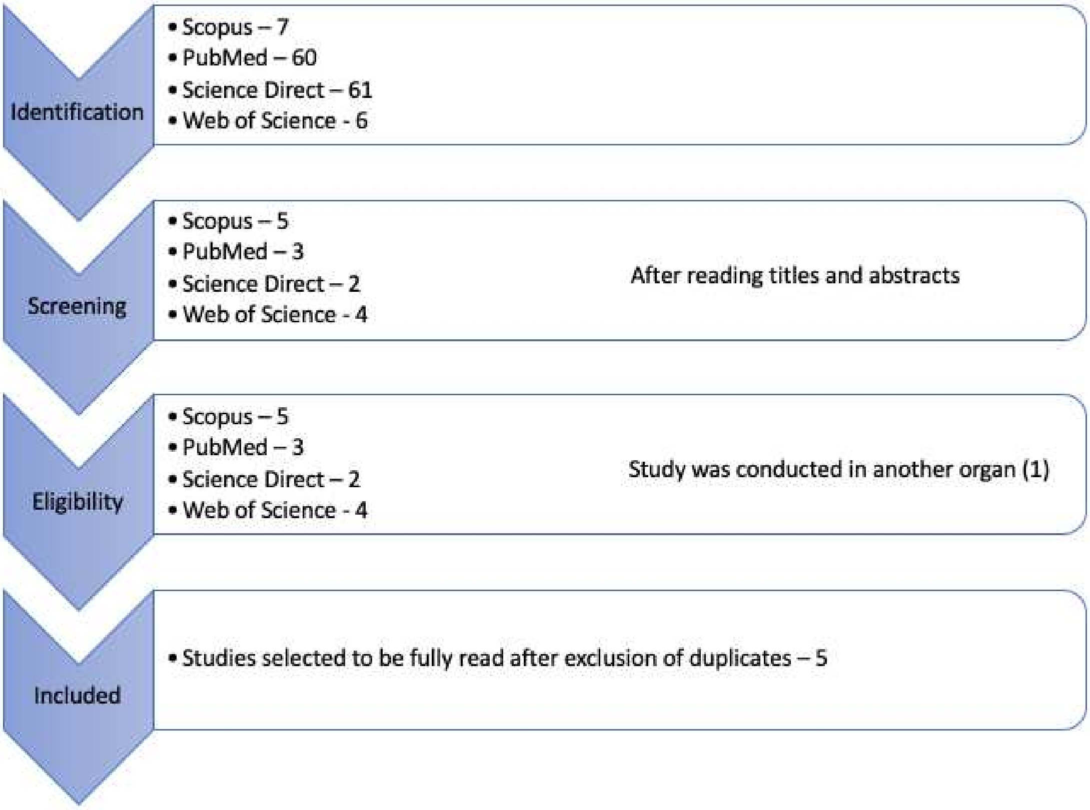
The quality assessment of the included studies was checked using the SYRCLE`s risk of bias tool for animal studies[26]. This method is based on ten signaling questions that can be adapted according to the reviewer and the papers being reviewed so the analyses can be more accurate.

2.3. Results

2.3.1. Selected studies

From the initial search, 134 studies were identified. The screening phase consisted of reading the titles and abstracts and evaluating if they match the previously cited inclusion criteria, stage at which 14 studies were selected. A great number of studies were not included because they were based on other experimental unit rather than horses or because they were not performed in kidneys. The selected records were downloaded and fully read. One study was not available online, so it was necessary to email the publisher claiming access and they promptly had a copy sent. Finally, after exclusion of duplicates and of one paper that did not address renal arteries[27], five studies were included in the present systematic review. All steps from identification to inclusion were taken by two examiners and there were no disagreements. It was not necessary to use a specific program or software to evaluate data due to the small number of eligible studies. The flow diagram on Figure 2.1 demonstrates how the process of selecting the articles was conducted.

Figure 2.1 - Flow diagram showing the sequency of how studies were selected to systematic review, according do PRISMA.



2.3.2. Study characteristics

Two out of the five eligible studies were published in the 2010s[11,28], two in the 2020s[29,30] and one is dated back in the 1990s[31]. From those, two were carried out in Poland[28,30], two in Italy[11,29] and one in Australia[31].

According to the findings of this systematic review, Hoffmann and colleagues[31] were the first research group to publish about RI measurement in horses. They considered a group of 11 healthy animals, being five of them sedated to ensure good ultrasonographic access to the intrarenal arteries and seven of them not sedated so the pyelorenal arteries were assessed. Although there were sedated horses in the study, only the healthy non-sedated group was included in this review. The aim of this pioneer research was to establish a reference range for peak systolic and end diastolic velocities and for RI in the equine species.

Furthermore, Macrì et al.[11] included 13 healthy Thoroughbred horses in their research. The writers had the goal of determining a reference range for arterial renal RI in healthy non-sedated horses and to compare the results of right and left kidneys, mares and geldings as well as trained and untrained animals. Siwinska and peers[28] selected 45 Warmbloods horses and compared the renal RI among animals´ age groups (15 foals, 15 adults and 15 elderly), between the left and right kidneys, likewise the influence of age, body weight, heart rate and blood pressure on the intrarenal arteries RI. They also assessed repeatability by performing a second ultrasound two hours after the first measurements.

In addition, Freccero et al.[29] aimed to obtain the renal RI value of each kidney from 33 horses and nine donkeys, along with their breeds, age, body mass and body condition and compare all this information within itself. Since this systematic review is considering only horses, the data including donkeys were not acknowledged. Lastly, Siwinska and colleagues[30] obtained the renal RI of 30 healthy horses, 11 horses diagnosed with acute kidney injury (AKI) and 30 horses under risk of developing AKI. She and her group aimed to determine if animals with AKI would show a higher renal RI, as well as, to measure renal RI in horses at risk for AKI and investigate if potential nephrotoxic agents could impact renal RI. They compared the RI data from right and left kidneys between groups and checked for correlation between renal RI and blood and urine parameters. Also, the group evaluated the sensitivity and specificity of renal RI to AKI detection.

It is crucial to stablish that for this systematic review only the healthy non-sedated group of each study was considered, so the baseline characteristics and methodological features could be reliably compared between papers. The Table 2.1 summarizes the characteristics of the included information from the studies included in the review.

Table 2.1 - Characterization of studies (n = 5) selected to the systematic review

Reference	Country	Breed	Number of animals	Grouping	Body weight	Age
Hoffmann et al., 1997	Australia	Thoroughbreds Standardbreds	7	Non-sedated	NR	15 ± 4,6 years
Macrì et al., 2015	Italy	Thoroughbreds	13	Male (5) Female (8) Trained (5) Untrained (8)	420 – 500kg	3 – 24 years
Siwinska et al., 2019	Poland	Warmbloods	45	Foals (15) Adults (15) Elderly (15)	205 ± 38.8kg 558.4 ± 95.3kg 542.6 ± 74.6kg	Up to 6 months 4 – 10 years Above 18 years
Freccero et al., 2020	Italy	Trotters Thoroughbreds Warmbloods	33	Adults (18) Elderly (15) Trotters (23) Other breeds (10)	Horses: 370 – 645kg	Horses: adults up to 15 years and elderly above 15 years
Siwinska et al., 2021	Poland	Warmbloods	30	Healthy (15F/15M)	Healthy: 554 ± 80kg	Healthy: 14.7 ± 8,4 years

Abbreviations: NR not reported, F female, M male, AKI acute kidney injury.

2.3.3. Ultrasonographic examination

In view of renal ultrasonography, all studies reported the performance of the transabdominal approach with the horse standing and none of them have selected the transrectal access. All studies also have described the preparation of the animals prior to the examination (Table 2.2). This stage was very similar between studies and consisted of clipping or shaving the hair coat over the 14th or 15th to the 17th intercostal space (ICS) and the paralumbar fossa on the right side and over the 15th or 16th to the 17th ICS and the paralumbar fossa on the left side. Siwinska et al.[28,30] opted for washing the horses` skin with chlorhexidine and rinsing with alcohol, drying and applying water-soluble coupling gel while Freccero et al.[29] chose to use alcohol and coupling gel, similarly Hoffmann et al.[31] and Macrì et al.[11] applied only ultrasound gel. Concerning the technical information about the ultrasound machine and settings (Table 2.2), the five studies have reported the type of probe and its range of frequency, the sample volume (SV) and the wall filter (WF), except for Macrì et al.[11] that did not demonstrate data on the last two and for Hoffmann et al.[31] that reported only the WF.

Freccero et al.[29] have additionally presented information about the pulse repetition frequency (PRF). Siwinska et al.[30] used the same ultrasound protocol as Siwinska et al.[28].

In order to avoid stressing the animals, Siwinska et al.[28] had the foals included in their experiment kept together with mares during the examination, also adults and elderly horses were examined near or in front of their stalls. Freccero et al.[29] had the animals restrained in a handling box that horses had already been acclimated. It is important to minimize stress as it can affect the waveforms, and thus the RI value[35].

Table 2.2 - Characterization of patient preparation and ultrasound machine and settings of the studies included (n = 5) in the systematic review.

Reference	Patient preparation	US characteristics	Equipment settings
Hoffmann et al., 1997	Hair shaved over the 14th to 16th ITS and the PL fossa on the right side and over the 15th to 17th ICS and PL fossa on the left side. Use of coupling gel.	Opus 1 (Ausonics International). Split-crystal mechanical sector probe. 2.5MHz for B-mode and 1.9MHz for continuous wave Doppler	WF of 50Hz
Macrì et al., 2015	Hair was clipped. Water-soluble gel applied to the lumbar abdomen.	MyLab 50 Vet (Esaote, Italy). 1.9MHZ to 3,0MHz phased-array probe.	NR
Siwinska et al., 2019	Fur was clipped over the PL fossa and from the 16th to 17th ICS on the left side and from the 15th to 17th ICS on the right. Skin was washed with chlorhexidine soap, flushed with alcohol, dried and covered by coupling gel.	MyLab 30 Gold Vet (Esaote). 1MHz to 5MHz convex probe.	SV set at 5 to 10mm and decreased to small as possible WF of 50 or 100Hz
Freccero et al., 2020	Hair clipping over the 15th to 17th ICS and PL fossa on the left side and over the 14th to 17th ICS and PL fossa on the right side. Use of alcohol coupled with ultrasound gel.	MyLab 30 Gold (Esaote, Italy). Multifrequency curvilinear probe 3.5MHz to 5MHz.	SV set at 2mm WF of 50Hz PRF set lowest possible values
Siwinska et al., 2021	Fur was clipped over the PL fossa and from the 16th to 17th ICS on the left side and from the 15th to 17th ICS on the right. Skin was washed with chlorhexidine soap, flushed with alcohol, dried and covered by coupling gel.	MyLab 30 Gold Vet (Esaote). 1MHz to 5MHz convex probe.	SV set at 5 to 10mm and decreased to small as possible WF of 50 or 100Hz

Abbreviations: US ultrasonography, ICS intercostal space, PL paralumbar, NR not reported, WF wall filter, SV sample volume, PRF pulse repetition frequency

2.3.4. RI measurements

All five studies included in this review reported data on RI measurements in healthy horses and none of them reported PI values. A total of 128 healthy horses were assessed in the studies and characterization of papers is presented in Table 2.3. Among the five studies, one has reported a mean value for both kidneys whereas the other four have presented the values for left and right kidneys separately. Only Hoffmann et al.[31] has used pyelorenal arteries, all other authors have chosen intrarenal arteries, being those the arcuate and/or interlobar. All writers presented the RI values as mean and standard deviation. The higher value measured across studies was 0.58 ± 0.06 for the right kidney of untrained horses[11] and the lowest value was 0.46 ± 0.04 for the left kidney of foals[28]. Hoffmann and colleagues[31] accepted horses` RI normality value as being of the same order of humans, cats and dogs, in which 0.70 is considered to be the upper limit.

As a method to calculate a more reliable RI value, Hoffmann et al.[31] reported that at least seven continuous-spectral waves consisting of peak systolic and end diastolic velocities were attempted. Macrì and colleagues[11] repeated measurements three times on each kidney and Siwinska et al. [28,30] have acquired three adequate waveforms. Lastly, Freccero and peers[29] have selected at least one 8-second spectral tracing from each kidney.

Table 2.3 - Characterization of resistivity index of healthy horses' groups of the studies (n = 5) included in the systematic review.

Reference	Artery of interest	Grouping (n)	RI	
Hoffmann et al., 1997	Pyelorenal	Non-sedated (7)	0,512 ± 0,004	
Macrì et al., 2015	Interlobar and arcuate	Male (5) Female (8) Trained (5) Untrained (8) Total: 13	LK: Male: 0.50 ± 0.05 Female: 0.50 ± 0.06 Trained: 0.52 ± 0.04 Untrained: 0.48 ± 0.06	RK: Male: 0.56 ± 0.03 Female: 0.57 ± 0.06 Trained: 0.55 ± 0.01 Untrained: 0.58 ± 0.06
Siwinska et al., 2019	Interlobar or arcuate	Foals (15) Adults (15) Elderly (15) Total: 45	LK: Foals: 0.46 ± 0.04 Adults: 0.48 ± 0.05 Elderly: 0.52 ± 0.05	RK: Foals: 0.47 ± 0.05 Adults: 0.48 ± 0.05 Elderly: 0.52 ± 0.05

Continues…

Continued.

Reference	Artery of interest	Grouping (n)	RI	Reference
Freccero et al., 2020	Arcuate	Adults (18) Elderly (15) Trotters (23) Other breeds (10) Total: 33	LK: Adults: 0.51 ± 0.01 Elderly: 0.51 ± 0.004 Trotters: 0.51 ± 0.01 Other breeds: 0.51 ± 0.003	RK: Adults: 0.58 ± 0.004 Elderly: 0.59 ± 0.01 Trotters: 0.58 ± 0.01 Other breeds: 0.59 ± 0.01
Siwinska et al., 2021	Interlobar or arcuate	Healthy (15F/15M) Total: 30	LK: Healthy: 0.50 ± 0.05	RK: Healthy: 0.50 ± 0.04

Abbreviations: RI resistivity index, LK left kidney, RK right kidney, F female, M male.

2.3.5. Other findings

Hoffmann et al.[31] suggested that due to their findings on variation of RI values within the horse, repeated RI measurements over time could be used to monitor changes in the renal blood flow. Macrì et al.[11] reported differences in the RI values of right and left kidneys in the horses evaluated in their study which, according to the authors, is not seen in other species. Conjointly, this finding was not consistent in the reviewed studies, where only Freccero et al.[29] described the same result. Additionally, they found no difference in the RI values related to gender, corroborating the other studies included in this review. Siwinska et al.[28] assessed repeatability in their study and found a high correlation between measuring time-points. She and her group also noted a higher RI value among the elderly horses compared to adults and foals and, moreover, that RI is affected by the pulse pressure. All studies have considered the renal ultrasonographic examination for obtaining RI value as time consuming, dependent on the horse´s willing to cooperate and dependent on the operator´s experience to perform the exam.

2.3.6. Risk of Bias

The risk analysis applied in this systematic review was adapted from the SYRCLE tool[26] and the results are demonstrated in Table 2.4 below.

Concerning selection bias, allocation concealment was not included due to the types of studies. For allocation sequence, it was considered a high risk for all five studies[11,28,29,30,31] as the authors have selected animals after taking laboratorial tests to ensure horses' good health and so it cannot be considered as random allocation. In regard to baseline characteristics, Hoffmann et al.[31] did not describe in details the animals' conditions prior to ultrasound examinations as the other four studies[11,28,29,30] did, so to his study the risk was considered as unclear and to the others it was considered as low risk.

The performance bias of animal housing and management was addressed as high risk for one study[31] that did not report any data on this matter, as unclear risk for two studies[29,30] that informed incomplete data and as low risk to two studies[11,28] that described in details the housing, feeding, management, handling and exercising routine of the horses. Another source of bias was the outcome assessor and its randomness. In this matter, it was assessed the experience level of the evaluator and whether it was the same person to perform the examinations in all horses during the research. The study that did not report any information was considered as high risk, if there was at least one information available, it was considered as unclear risk and as low risk if there was enough data.

Regarding the risk of incomplete outcome data, it was assessed if the study has demonstrated the expected results, if all proposed RI measurements were concluded and if they were achieved in both kidneys and in all horses. Furthermore, to evaluate the risk of selective outcome reporting, it was considered if the study reported data about all proposed outcomes and methods of animal evaluation, if they mentioned the ethical committee protocol or equivalent and if it was clear whether all expected results were demonstrated. For both previously cited risks, the studies were considered as high risk if they did show any of the cited characteristics, as unclear if they reported a few information and as low risk if they presented all information needed.

Other high risks assigned to the retrieved articles were related to a small sample number[11,31], one-time observation on renal RI[11,29,30,31] and to not performing blood pressure measurement[11,30], as this parameter is known to potentially interfere with the renal RI value.

Table 2.4 - Assessment of methodological quality of reviewed reports. Symbols were addressed to each bias regarding the five evaluated studies, standing for high risk (- red), unclear risk (? yellow) and low risk (+ green).

		HOFFMANN, 1997	MACRÌ, 2015	SIWINSKA, 2019	FRECCERO, 2020	SIWINSKA, 2021
Selection bias	Allocation sequence	-	-	-	-	-
	Baseline characteristics	?	+	+	+	+
Performance bias	Animal housing and management	-	+	+	?	?
Detection bias	Outcome assessor	-	+	+	+	?
Attrition bias	Incomplete outcome data	-	?	+	-	-
Reporting bias	Selective outcome reporting	?	?	+	+	+
Other sources of bias	Small sample number	-	-	+	+	+
	Single moment of evaluation	-	-	+	-	-
	Blood pressure measurement	-	+	+	+	-

2.4. Discussion

Despite B mode ultrasound being largely used in the equine veterinary practice, its relevance to renal evaluation is limited as morphology does not always relate to function[32]. On the other hand, the noninvasive Doppler sonography is able to provide data in respect to renal blood flow and vascular resistance[16]. The RI derives out of the information given in the Doppler arterial waveforms and reflects the hemodynamics of the targeted tissue[12,33], which in turn, can reflect its function and a damaged function[17].

Although the RI is still not extensively used in veterinary medicine, it is being considered as an important tool to assess renal microcirculation in physiological and pathological situations[34], most widely considered today for dogs and cats[24]. However, a number of elements can influence renal RI, such as arterial characteristics (i.e. aortic stiffness), renal (i.e. interstitial and venous pressure) and systemic vascular wall properties (i.e. vascular compliance) and systemic hemodynamics factors (i.e. pulse pressure, heart rate)[17,33,34]. It is crucial to keep that information in mind when undertaking a RI study.

The lack of information on equine renal RI and its physiological range of value, even though its relevance in humans and other animal species have been demonstrated, motivated the accomplishment of this systematic review. A compilation of obtained renal RI values from healthy horses in five eligible studies was made, as well as a description of study methodology, equipment adjustments, technical information and patient preparation in order to develop a standardized method to perform renal Doppler ultrasonography and to possibly determine a reference range for renal RI values in horses. Unfortunately, due to the limited number of studies and the methodological variation between them, it was not possible to execute a meta-analysis.

Regarding patient preparation, the five selected studies[11,28,29,30,31] described this step minutely and were consentaneous about hair clipping, use of coupling gel and the location of examination. Also, Siwinska et al.[28] and Freccero et al.[29] described important strategies to minimize stress, as it can affect the waveforms, and thus the RI value[35].

The transabdominal approach was a successful method in all studies, using a 2 to 5MHz frequency curvilinear probe[11,28,29,30,31]. It has been already described in literature that this frequency range is more suitable for abdominal ultrasound imaging[36]. For ultrasound examination, all operators first performed the B mode evaluation of the kidneys, used the color Doppler to set the artery and then got the Doppler waveforms on spectral Doppler. Another important information concerns equipment settings as reason for better image resolution, lowering occurrence of artifacts and, thus, providing a more reliable result. In this light, pulse repetition frequency and wall filter should be set as lower as possible to avoid aliasing[37] and wall artifacts or clutter[38,39], respectively. Sample volume size should be adjusted to artery diameter and positioned in the vascular lumen[35], but it is still not standardized for horses.

Some authors[28,30,31] have found it difficult to retrieve a good quality image from left kidney in comparison to right kidney. Siwinska et al.[28] have attributed this difficulty to the anatomical location of the organ, that lays under the spleen and is surrounded by intestinal components. The target arteries (arcuate and interlobar) was the same in four studies[11,28,29,30], only Hoffmann and others[31] used the pyelorenal artery due to the equipment they had available. It is not well described in literature the ideal vessel for RI measurement in horses; however, it has been demonstrated that lower caliber arteries suffer more from flow changes, thus producing a greater variation in the RI value[14,40]. Also, it has been reported that it is necessary to acquire three to five RI measures to properly get a RI definitive value[41]. Two reviewed studies[11,31] did not mentioned if they managed to get all desired waveforms and the other three[28,29,30] have reached a lower number that they expected. Yet, all five studies managed to get a statistical reliable RI value.

Hoffmann et al.[31] noted that RI values did not diverge between left and right kidneys, so they pooled the results together, being found the mean value of 0.512 ± 0.004 from evaluated horses. Macrì and his peers[11] demonstrated their results as minimum, maximum and mean for each kidney according to sex as well as mean and standard deviation in order to evaluate the effect of training, sex and kidney side. They reported a higher RI value in the right kidney of geldings (left 0.50 and right 0.56), mares (left 0.50 and right 0.57), trained (left 0.52 and right 0.55) and untrained (left 0.48 and right 0.58) horses. As to Siwinska et al.[28], a higher RI value was found in the group of elderly horses (0.52 ± 0.05 vs 0.48 ± 0.05 in adults; 0.52 ± 0.05 vs 0.47 ± 0.05 for right kidney and 0.46 ± 0.04 for left kidney in foals) and no differences between kidneys nor regarding sex were observed. Freccero and colleagues[29] reported difference between left and right kidneys, corroborating with Macrì's group findings. Finally, Siwinska et al.[30] reported a mean RI value of 0.50 for both kidneys in healthy horses. All renal RI mean values from the five studies can be checked in Table 3 on topic 2.3.4 of this systematic review. When these findings are compared to human, dogs and cats' results, it can be noticed that they diverge; for equine, the RI values are mostly under 0.60 while for the other mentioned species the upper limit is considered to be 0.70[18,24,42].

For humans, a normal renal RI value of 0.60 ± 0.01(mean and SD) and a threshold of 0,70 is being accepted nowadays[43,44]. Also, there has been described changes in the RI value according to age, being higher in infants and in the elderly population[44-47]. Siwinska et al.[28] demonstrated a similar pattern for aged horses, yet the RI value did not reach the threshold set for humans. For cats, the normal renal RI values range between 0.64 and 0.72 and the influence of age is still unclear[20]. Further, the mean renal RI for dogs found in a study with 27 healthy animals was 0.62 ± 0.04[48] and the considered upper limit is the same as for humans[22]. For dogs, the age seems to influence the renal RI value during the firsts three weeks of life, being higher in this period and then slowly decreasing until adult values at 12 weeks[49].

Although at first it was not considered for appraisal by this systematic review, Siwinska et al.[30] also evaluated two other groups that can be interesting for our objectives: animals at risk of developing acute kidney injury (AKI) and animals with clinical AKI. Siwinska and her colleagues identified that even in the nonhealthy categories, the renal RI values did not reach or pass 0.70, the RI value used for humans and small animals, which brings the question whether this upper limit is suitable for the equine species. In this regard, more studies on the theme are still necessary to determine a reliable renal RI reference range for horses.

Siwinska et al.[28] have analyzed their results in correlation to a number of physiological parameters, such as age, heart rate, body weight and blood pressure. They found no interaction among the RI and cited parameters, however, there was an association between pulse pressure and the obtained renal RI values. Therefore, it can be advised for future studies the measurement of pulse pressure in order to perform a better evaluation of the renal RI results. Freccero et al.[29] compared renal RI values to physiological parameters as well, being age, breed, body weight and body condition and also did not find any correlation. All five studies[11,28,29,30,31] concluded that acquisition of renal RI trough transabdominal approach is time consuming and very dependent on the operator.

2.5. Conclusions and future directions

Although renal RI has been stablished over the years as an efficient tool in human medicine and has been a matter of interest for the last decade in regard to small animals, its usefulness in the equine medicine is still not demonstrated. It is crucial that a variety of studies can be conducted to ensure that renal RI has clinical utility in horses. Determining a normal range for equine renal RI is still necessary in a larger population, including comparisons with horses proven to present kidney disease, once all studies enrolled in this systematic review found a RI value between 0.50 and 0.64 (mean values) in the 128 examined animals, much lower than the upper limit considered for humans, cats and dogs of 0.70.

As future directions, we suggest a few patterns to be followed during transabdominal renal ultrasound examination. For animal preparation, hair clipping and use of coupling gel are very important, but if it is not possible to trim the horse, the haircoat should be abundantly rinsed with alcohol. A curvilinear probe is more suitable for the exam and the frequency range should be between 2 and 5MHz. The wall filter should be set at 50Hz, a sample volume of 2 to 4mm should be preferred and a lower as possible pulse repetition frequency should be set. In order to locate the artery to be assessed, the color or power Doppler must be employed. It is crucial that at least three waveforms are acquired from each kidney, so the RI value could be more reliable. The RI value can be presented as mean and standard deviation and it is important to notice that, for horses, these values are mostly under 0,70. Lastly, as the pulse pressure has been proven to affect the RI results, it should also be measured and taken into consideration when evaluating the kidneys. The writers of the present reviewing article believe and hope that in a short future other works on the topic will be presented and further evaluation will be made.

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3. CAPÍTULO 3 - EXPERIMENTAL STUDY IN HEALTHY ANIMALS

Renal Doppler Ultrasound in healthy horses: a comparison between transabdominal and transrectal approaches

Renal Doppler Ultrasound in healthy horses: a comparison between transabdominal and transrectal approaches

Abstract

Early detection of renal injuries and deficient renal function is imperative to determine the patient`s prognostic. Kidney diseases are being increasingly recognized in horses, so the relevance of these scenario is enormous. The ultrasound examination as a tool to evaluate the kidneys is already widely spread in humans and small animals medicine. In horses, the bidimensional grey scale ultrasound is already inserted in the clinical routine. In the equine, the renal ultrasonography can be performed through the abdominal wall or transrectally through palpation, being possible to analyze the morphology and the organs' hemodynamics through B mode and Doppler mode, respectively. By spectral Doppler mode, the resistivity index (RI) and the pulsatility index (PI) can be obtained. These indexes reflect arterial compliance and resistance, which are altered in cases of obstruction and vasoconstriction, for instance. To our knowledge, no reports on renal PI in horses are published to date. In this study, 29 healthy adult horses of various breeds were sonographicaly evaluated through abdominal wall and transrectal palpation for the measurement of renal RI and PI on intrarenal arteries. The mean values from the transabdominal technique for renal RI and PI in the right kidney were, respectively, 0.577 ± 0.072 and 0.950 ± 0.182, and for the left kidney they were 0.553 ± 0.077 and 0.884 ± 0.163. As for the transrectal technique, the mean values for RI and PI in the right kidney were 0.543 ± 0.096 and 0.848 ± 0.212, respectively, and for the left kidney they were 0.551 ± 0.089 and 0.888 ± 0.204. Results were analyzed using two-way ANOVA. No significant difference was noted between left and right kidneys nor between transabdominal and transrectal techniques. In conclusion, the Doppler indexes can be obtained from both sonographic routes, being chosen depending on the animal size and operator` skills.

Keywords: equine, kidney, Doppler indexes

3.1. Introduction

The kidneys are organs of major importance to the body due to their numerous functions. These organs are responsible for blood filtering and excretion of waste products; also, they play a major role in homeostasis, in water and electrolyte balance, maintenance of blood pH and still have endocrine functions[1]. The kidneys receive approximately 20% of the cardiac output, yet the renal medulla is irrigated with only 20% of this amount, what can render the equine kidney is susceptible to ischemia[1,2]. For this reason, the early detection of renal injury increases the probability of a successful treatment or, at least, a more effective management of the disease process.

The renal B mode ultrasonography is already a routine exam in human, small animal and equine practice. It is possible to evaluate the shape, size and different anatomic structures' echotexture in grey scale, as well as the blood flow and hemodynamics using the color and/or the spectral Doppler tools[3]. As Radermacher et al.[4] described, detecting changes in renal arterial resistance can help predict the degree of renal tissue damage. In that case, Doppler sonography and its' indexes are of great value when suspecting of renal injury[5]. The pulsatility and resistivity indexes (PI and RI, respectively) are calculated from the information given by the spectral waveform, being RI = (peak systolic velocity – final diastolic velocity)/peak systolic velocity and PI = (peak systolic velocity – final diastolic velocity/average velocity. These indexes express the vascular compliance and resistance, which can be increased due to obstruction or vasoconstriction[5,6], for instance, and have been related to numerous kidney injuries in other species[7-19].

Concerning large animals, there are two methods to perform the ultrasonographic exam of the kidneys, the transabdominal and the transrectal approaches[20]. There are a few studies published in the light of renal Doppler indexes in horses, being all of them about the renal RI and by the transabdominal approach only[21-25]. We aimed to evaluate healthy non-sedated horses of different breeds and ages in order to determine a normal range value for both indexes, RI and PI, in the studied population. Further, the kidneys from all horses were assessed through both methods, transabdominal and transrectal, to verify whether the renal RI and PI results would be influenced by the type of technique.

3.2. Materials and Methods

The present experimental study was accomplished in strict accordance with the recommendations of the Brazilian Legislation, the normative resolutions issued by the National Council for Animal Experimentation (CONCEA/MCTI) through the Brazilian Directive for the Care and Use of Animals in Teaching or Scientific Research and Euthanasia Practice Guidelines recommended by CONCEA/MCTI. This research project was approved under the registration number 72/2021 by the Ethics Committee on Animal Use of the Federal University of Viçosa (CEUA/UFV). The ultrasound evaluations were carried out in three facilities of the Federal University of Viçosa and a local training center, all located in Minas Gerais, Brazil.

3.2.1. Animal selection

Thirty-two adult (between 4 and 25 years) healthy horses of different breeds (13 cross-breed horses, 6 Mangalarga Marchador horses, 8 Draft horses, 3 Lusitano horses, one horse Mangalarga Paulista and one Campolina and Breton cross-breed horse) from both sexes (18 non-pregnant mares and 14 males, being 12 geldings and 2 stallions) were selected to the study. As so, management and training program were not consistent among the entire group.

Information on animal identification (name, age, sex and breed) were taken, as well as their previous clinical history. The horses’ body weight was assessed using a girth tape, which was the same during all experiment for all animals. Blood samples were collected for hematological and biochemical evaluations (complete blood count, serum creatinine, urea, total protein and fibrinogen) in order to certify horses` health condition. Also, a clinical exam was performed prior to the ultrasound examination. Urine was not collected for urinalysis due to operational concerns. Horses were included if they match the following eligible criteria: no history of kidney or cardiovascular diseases, no symptoms of disease by the time of evaluation, no alterations in the blood tests and no alterations in B-mode ultrasound of the kidneys.

3.2.2. Ultrasound examination

Two ultrasound equipment were used to perform the examinations, an Esaote MyLab 30 Vet Gold with a convex transducer (CA431) ranging from 1.3 to 6.6MHz, used for renal evaluation by the transabdominal technique and a Mindray Z50 Vet with a linear rectal transducer of 5 to 10 MHz for the transrectal technique. Each horse underwent renal ultrasound once, being attempted for both transabdominal and transrectal data collection from left and right kidneys. Animals were kept in quadrupedal position, restrained in a handling box and no sedative medication was administered. As the horses enrolled in the study were already acclimatized, we strongly believe that stress levels were minimum or zero during the procedure. Evaluations were taken by the same two operators, being one for the transabdominal and other for the transrectal examination, based on their technical skills with the equipment and rectal palpation and the need for operating the device as well.

Prior to the transabdominal ultrasound, horses were prepared by clipping the hair over the paralumbar fossa and from the 15^{th} to the 17^{th} intercostal space (ICS). Further, the skin was rinsed with alcohol and a layer of conductive gel were applied. In cases which the animal could not have the coat removed, alcohol was used in abundance. As to the examination, first, B-mode images were obtained, performed in the longitudinal and transversal planes of the organ, then their thickness on lateral and dorsoventral directions were measured. In addition, the renal parenchyma, its shape, size, architecture and echogenicity were evaluated to investigate the occurrence of lesions. After evaluating the architecture of the kidney, the intrarenal arteries (arcuate and/or interlobar) were located using the color Doppler. Finally, by means of spectral Doppler, the detection of the intrarenal arteries waveforms to determine Doppler indexes was accomplished.

As preparation to the transrectal ultrasound examination, horses were restrained in a handling box and then feces were removed from the rectum. Next, the transducer and the operator's arm were lubricated and the transducer positioned towards the direction of each kidney. Then, the procedure to obtain the arterial waveforms was similar to the transabdominal approach. For the two techniques, at least three adequate waveforms were attempted from each kidney.

The optimal acoustic window was selected depending on the kidney location in each horse. The focus was positioned at the level of the area of interest. The wall filter was set at 50MHz, the pulse repetition frequency (PRF) was set as lower as possible without causing artifacts. The gate was set at 1 or 2mm and the Doppler gain were adjusted according to need. The renal RI and PI were calculated automatically by the equipment after the waveforms were manually marked by the observer using the electronic caliper, done always by the same observer for all measurements. Tracings with at least two adequate waveforms were included. The mean value of renal RI and PI between the acquired waveforms were obtained.

3.2.3. Statistical analysis

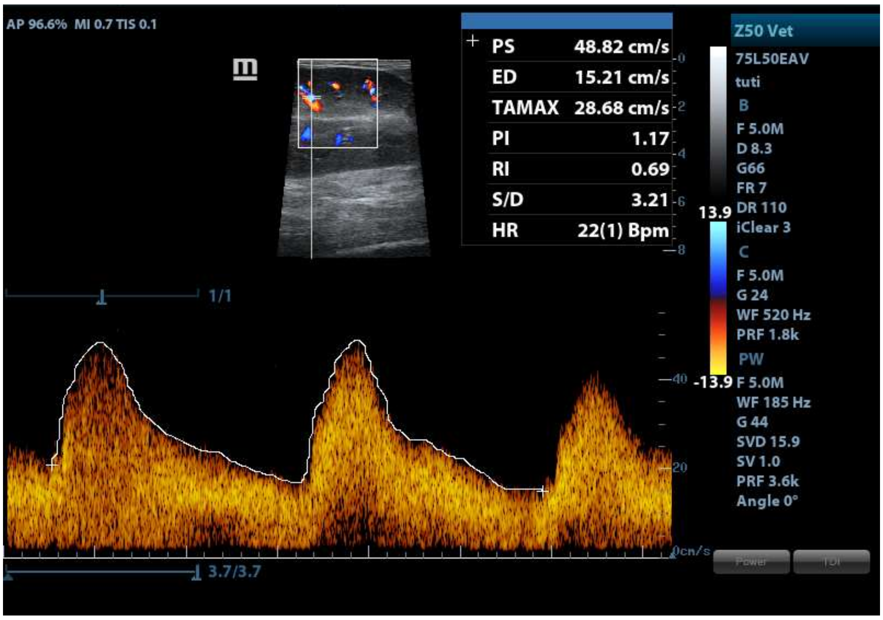
Statistical analysis was performed using the software R 4.2.1. The normal distribution of data was investigated using the Kolmogorov-Smirnov-Lilliefors test. Differences between kidneys (left and right) and between sonographic techniques (transabdominal and transrectal) were evaluated using two-way ANOVA. Statistical significance level was set at 5% (α=0.05). Variables are presented as mean and standard deviation (SD) as they were proved normally distributed.

3.3. Results

Thirty-two horses were enrolled in the study. The horses' mean age was 11.9 ± 6.2 (SD) and their mean body weight was 412 ± 70.7 (SD). In transabdominal approach, the left kidney was mostly identified at the 17^{th} ICS and the paralumbar fossa area, under the spleen. The right kidney was consistently identified at the 16^{th} and 17^{th} ICS. It was possible to distinctly define the cortex, medulla, corticomedullary junction, capsule and renal pelvis in all horses. None of the horses were diagnosed with renal disease through ultrasound examination.

At least 50 minutes per animal was necessary to perform the two approaches of renal ultrasound examination. Good quality images of the left kidney by transabdominal technique and of the right kidney by the transrectal approach were more difficult to obtain. In total, 71 waveforms were obtained from the left kidney and 63 from the right kidney through transabdominal technique, and 65 waveforms from left kidney and 33 from right kidney through transrectal technique. Figure 3.1 below shows the waveforms obtained through transrectal approach and the automatically calculated Doppler indexes.

Figure 3.1 - Waveforms acquired through transrectal approach in an adult horse. PS peak systolic velocity, ED end diastolic velocity, TAMAX average velocity, PI pulsatility index, RI resistivity index.



RI and PI were acquired in twenty-nine animals, being 24/29 of left kidney and 23/29 of right kidney from transabdominal technique and 25/29 of left kidney and 16/29 of right kidney from transrectal technique. Measurements from both kidneys in both evaluation approaches were acquired in 11/29 animals due to different problems with obtaining a satisfactory image of the kidney and arterial waveform. In the transabdominal technique, the mean values for RI and PI in the right kidney were, respectively, 0.568 ± 0.058 and 0.934 ± 0.137, and for the left kidney they were 0.545 ± 0.064 and 0.864 ± 0.132. As for the transrectal technique, the mean values for RI and PI in the right kidney were 0.557 ± 0.068 and 0.872 ± 0.147, respectively, and for the left kidney they were 0.554 ± 0.070 and 0.877 ± 0.158 (Table 3.1). In three horses the ultrasound examination could not be accomplished due to misbehavior, being two of them stallions. In general, difficulties in getting a good quality image were related to following: body movement, intestinal motility and gas content, overlapping organs, lack of animal cooperation and, lastly, due to animal size. As some horses were very large, the operator was not able to reach the kidney through palpation for the transrectal technique to be performed.

No significant differences between renal RI and PI between left and right kidneys were observed through transabdominal technique, as well as for left kidneys through transabdominal and transrectal techniques. No significant differences were observed for both RI and PI when comparing the right with the left kidneys and the technique, by the transrectal via or transabdominal approach.

Table 3.1 - Renal Doppler indices mean values and standard deviation obtained in evaluated horses through transabdominal and transrectal ultrasound approaches.

	Left kidney		Right kidney	
	TA (n=24)	TR (n=25)	TA (n=23)	TR (n=16)
RI	0.545 ± 0.064	0.554 ± 0.070	0.568 ± 0.058	0.557 ± 0.068
PI	0.864 ± 0.132	0.877 ± 0.158	0.934 ± 0.137	0.872 ± 0.147

Abbreviations: RI resistivity index, PI pulsatility index, TA transabdominal, TR transrectal.

3.4. Discussion

Doppler sonography is increasingly being used as a diagnostic tool in humans, dogs and cats[4-19]. It is a non-invasive, low cost and easily accomplished method to evaluate internal organs' perfusion and hemodynamics, such as the kidneys. In horses, it is more often performed in cardiology, orthopedics and reproduction areas[26-32]. In the past decades, five studies[21-25] have reported experimental data on equine renal RI. However, to our best knowledge, there is no published information on equine renal PI. The PI is believed to be more adequate to assess high resistance vessels[33]. Hence, this is probably why only RI values were contemplated when evaluating the kidneys, as these organs do not offer great vascular resistance[6]. Still, the renal PI is advantageous as it takes into consideration the average blood flow velocity, while RI takes only peak and final velocities. Therefore, renal PI reflects the entire cardiac cycle and it is more sensitive to changes in the arterial waveform[4].

We provide here mean values for equine renal RI and PI, as well as the comparison between the transabdominal and the transrectal approaches. The renal RI mean value of 0.56 obtained was similar to values obtained in previously published studies, ranging from 0.48 and 0.59[21-25]. As for equine renal PI, as far as we concern, there is no published data. A study with humans[17] showed that renal PI values over 1.55 can be linked to quicker loss of renal function. Concerning small animals, the renal PI upper limit is still heterogeneous among studies. For cats, renal PI cut-off is considered to be of 1.29[11], while for dogs it is of 1.52[4]. Our research group found a renal PI value ranging between 0.86 and 0.93, suggesting that renal PI for horses also might be of a lower threshold than humans, cats and dogs.

Considering a 95% confidence interval[34] of the probabilistic method that comprises the mean plus two times the standard deviation, described by Novelas et al.[4] as being ideal when setting the normal upper limit for RI and PI for cats and dogs, we suggest a mean RI value of 0.69 and a mean PI value of 1.18 as the normal upper limits for horses based on our results. However, a unique cut-off value may not be the best approach due to numerous reasons. Our results and others`[21-25] demonstrated that the RI and PI values are consistently lower than 0.70, which is stablished as the threshold for humans[6] and small animals[4,35,36]. Also, there is only one published study[24] regarding the evaluation of renal RI in horses with kidney injury. In this study, the RI was proved higher in the right kidney of horses with acute kidney injury, but still it did not exceed 0.70. Therefore, based on the present research and other publications[21-25], the upper limit of normality must be analyzed carefully and an intermediate value of risk of kidney damage may be evaluated.

Regarding the comparison between transabdominal and transrectal approaches, our hypothesis was that anatomical differences could influence the results. These differences are (1) the greater distance of left kidney from the transducer, once the spleen is located between this organ and the abdominal wall, influencing the transabdominal approach and (2) the longer distance to reach the right kidneys in larger horses. However, these anatomical limitations did not influence the values per se, but rather the capacity to obtain the values. Values were not obtained when the operator was not able to reach many of the horses' right kidneys and Doppler technique was impaired for left kidneys of horses with high body fat. Also, differences for the right and left kidney were not observed, differing from Macrì et al.[21] and Freccero et al.[23] that have found that the RI values for the right kidney were higher than for the left kidney, our data corroborates with Siwinska et al.[22,24] and Hoffmann et al.[25]. Further, other works on small animals and humans have not found differences between right and left kidneys regarding RI and PI values[4,7,11,12,33,35]. In addition, studies on horses` RI and PI in renal injury evaluation are lacking, so we suggest that further research is made in the field. To determine a normal range for RI and PI, it is crucial that their values in renal injury of different levels and etiologies are known. As for humans[5-7,17,19] and small animals[4,8,10,11], there are numerous studies demonstrating the usefulness and the alterations seen in both renal RI and PI in individuals with renal injury. We believe that it is important to carry out studies using horses with diagnosed renal injury by biopsy and histological examination of kidney tissue and then, evaluating the Doppler indexes and its clinical application.

Regarding our perception of ultrasound examination, it was more difficult to obtain good quality imagens from larger horses or the ones with a thicker layer of fat. Further, it was easier to obtain good quality images from the left kidney through the transrectal approach and from the right kidney through the transabdominal approach. The acquisition of adequate waveforms in the transabdominal approach was simpler, as the horses` movement would greatly interfere with the ability of the operator to stay still over the kidney area. Most of the horses enrolled in this study were used to transrectal palpation, still it was harder to retrieve the waveforms.

The limitation of this study was the inability to measure horses` pulse pressure. This parameter was demonstrated to influence on interpretation of renal RI by several authors[5,22,37,38]. Due to logistic limitations, we were unable to perform the blood pressure measurements non-invasively. Also, as RI and PI are influenced by sedation and direct measurement of blood pressure requires it, therefore, we chose not to perform it. Other limitations were related to methodologic issues, such as the operator not being able to reach the kidney transrectally, intestinal gas content, intestinal movement, respiratory movement, and animal´s lack of cooperation. In spite of that, our study has compared two renal ultrasound approaches and have measured renal PI, which has not been published before. Further, the sampling size was substantial, conferring reliability to our results.

3.5. Conclusions

This is the first study to obtain renal PI values, thus further information is needed in order to evaluate its clinical relevance. No relevant differences on renal RI and PI between ultrasound techniques or side of kidneys were found. We suggest that the upper limit for both Doppler indexes regarding the equine species should be set at 0.69 for RI and 1.18 for PI. The authors, similarly to others, recognize both renal ultrasound approaches as time-consuming and operator-dependent.

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4. CAPÍTULO 4 - EXPERIMENTAL STUDY IN HEALTHY ANIMALS AFTER 24 HOURS OF WATER AND FOOD FASTING

Short Communication: Renal Doppler ultrasound in healthy horses after 24 hours of fasting

Short Communication: Renal Doppler ultrasound in healthy horses after 24 hours of fasting

Abstract

The kidneys play a major role in water, electrolyte and acid-base balance. The renal function is closely related to renal blood flow, which can be reduced when the animal is dehydrated. To evaluate renal function and hemodynamics, the Doppler ultrasonographic exam is the chosen imaging method. It is possible to determine the renal resistive (RI) and pulsatility (PI) indexes from the arterial waveform provided by spectral Doppler mode. These indexes express arterial compliance and resistance, meaning that, when altered, the kidney might be under low perfusion and higher resistance to blood flow. The aim of this study was investigate whether low levels of dehydration could influence RI and PI values. For such, we evaluated renal RI and PI before and after a 24-hour period of hydric and food fasting (T1 and T2, respectively), as well as after a 12-hour period of rehydration (T3) with enteral electrolyte solution per intercaecal via. Four adult healthy horses were enrolled in the study. In total, horses were submitted to fasting nine times being one week apart between procedures when it was accomplished using the same animal. The ultrasound exam was performed transabdominally on the left kidney. The mean values found for renal RI in the T1, T2 and T3 periods were as follows: 0.599 ± 0.049, 0.593 ± 0.047 and 0.553 ± 0.085; as for PI they were 0.990 ± 0.138, 0.961 ± 0.112 and 0.854 ± 0.177, respectively. There was no significant difference between periods. The authors believe that this result is in virtue of the short period of fasting.

Keywords: Dehydration, Doppler ultrasound, horse, kidney, restive index, pulsatility index

4.1. Findings

The kidneys have a major role in the maintenance of body water and acid-base balance[1]. According to Toribio[1], the renal ability to control body water and acid-base balance are results of the renal blood flow, glomerular filtration, tubular reabsorption, diffusion and secretion. The renal blood flow is required to be high due to the large glomerular filtration process and the metabolic needs of the renal medulla[2]. Dehydration is associated with intrarenal vasoconstriction and, when the hypovolemia is severe, with lower glomerular filtration rate[3]. The most commonly used laboratorial tests for renal evaluation is the serum creatinine and serum urea. However, when these variables finally increase in laboratorial tests, the renal function is considered to be affected in 75%[1]. Apart from hematological and urinary tests, imaging resources can be used as a useful tool when assessing the kidneys.

Ultrasonography is an accessible non-invasive method to assess renal function through Doppler evaluation. The color Doppler function is able to provide information on renal perfusion[4]. As to spectral Doppler mode, it is possible to acquire arterial waveforms graphs and, through them, the resistive (RI) and pulsatility (PI) indexes[4]. These indexes express the intrarenal arterial compliance and resistance to blood flow[5]. The renal Doppler ultrasonography is already widely employed in human medicine for several purposes[6-8] as well as for dogs[9-11] and cats[9,11-13] The RI is known to be influenced by blood pressure, dehydration, pulse pressure, systemic vascular compliance and heart rate[12,14].

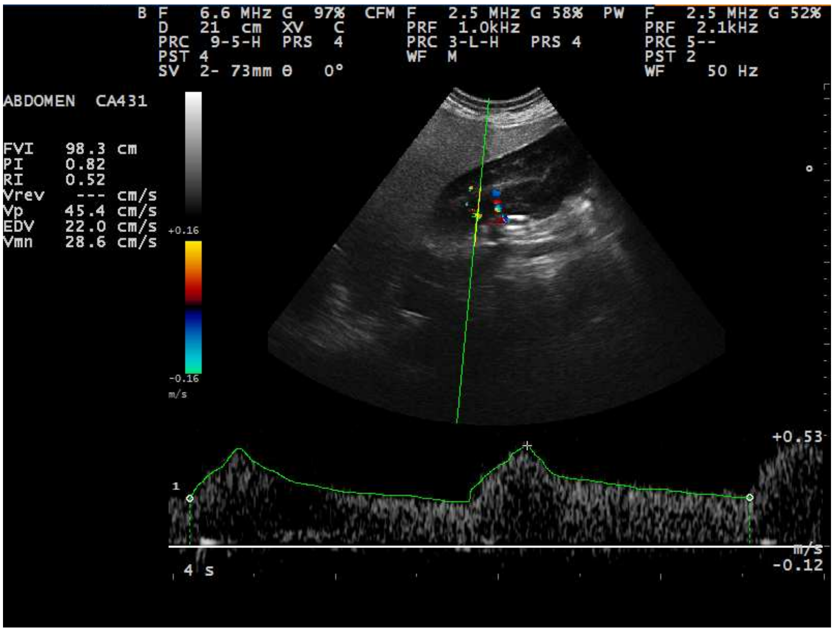
Dehydration results from an imbalance between water intake and loss of body fluids, being extremely dangerous when losses of total body water exceed 15%[15]. This water imbalance can be resultant of water consumption deprivation or fluid loss, for example through urinating, defecating, breathing and sweating processes. Further, dehydration is a clinical sign of numerous diseases and syndromes affecting the equine species, such as diarrhea, colic, intestinal obstruction, peritonitis and others[16]. Also, excessive exercise may lead to dehydration in horses due to exaggerated sudoresis[17]. Determining the patient's dehydration level is a key point when deciding the treatment protocol. However, it is difficult to precisely make that decision based exclusively on clinical signs[15].

In equine practice, there are a few published articles regarding renal RI[18-22]. Still, as far as we concern, there is no published data on effects of water and food deprivation in renal Doppler indexes. In that light, the aim of this study was to evaluate the effects of a 24 hour-period of fasting in the renal RI and PI of healthy non-sedated horses. We also assessed the renal Doppler indexes after rehydration with enteral electrolyte solution.

Our work on Doppler indexes is a sub-project of the main project on hydration through a cecal cannulation (approved by Ethic Committee in Animal Use, protocol number 17/2015) and the methods are detailed here only on what concerns the present report. Four healthy adult horses were included in the study, being 3 mares and one gelding. The animals ranged between 5 and 10 years old and weighted between 305 and 375kg. Horses' management was standardized throughout the study period. Animals were housed in stalls, they were fed hay and roughage, had free access to water and mineral salt. Horses were submitted to a surgical procedure of cecal fistulation and canulation according to Ferreira et al.'s[23] description. Prior to ultrasound Doppler examinations, animals underwent a 24-hour period of hydric and food fasting, procedure considered to induce a low level of dehydration. They were posteriorly submitted to a 12-hour period of rehydration using enteral electrolyte solutions through the caecum canula. The ultrasound Doppler evaluations were accomplished immediately before beginning of fasting (T1), at the end of the 24-hour period of fasting (T2) and at the end of the 12-hour rehydration procedure (T3).

Renal Doppler evaluation was carried out on the left kidney through transabdominal approach using an Esaote MyLab 30 Vet Gold with a convex transducer (CA431) ranging from 1.3 to 6.6MHz of frequency. The right kidney was not assessed due to the positioning site of the cannula. As animals' preparation, the hair was clipped from the left paralumbar fossa to the 15th intercostal space, the skin was rinsed with alcohol and coupling gel was applied deliberately. First, the kidney was located using B mode ultrasound and kidney's size, shape and echotexture were analyzed. Then, the intrarenal arcuate arteries were assessed using color Doppler and finally, the waveforms were obtained through spectral Doppler mode. The ultrasound equipment settings, such as gain, wall filter, sample volume size and pulse repetition frequency, were adjusted as needed to get a better image resolution. At least three complete waveforms were attempted from each horse. The RI and PI indexes were automatically obtained after manually tracing the waveform using the caliper on the ultrasound equipment (Figure 4.1). The three-time Doppler measurements (T1, T2 and T3) were taken nine times from the four equine enrolled in the study. Each repetition was considered as a new measurement even though it was from the same animal. Ultrasound evaluations were performed by the same operator throughout the entire study.

Figure 4.1 - Intrarenal arterial waveform obtained from a mare. The green line was traced using the caliper. PI: pulsatility index, RI: resistive index, Vp: peak velocity, EDV: end diastolic velocity, PRF: pulse repetition frequency, WF: wall filter.



Statistical analysis was performed using the R software (version 4.2.1). Variables (renal RI and renal PI) were checked for normal distribution using the Kolmogorov-Smirnov-Lilliefors test. As they were proved normally distributed, repeated-measures ANOVA test was used to compare the three measurement periods (T1, T2 and T3), at 95% (α=0.05) significance level.

The left kidney was consistently visualized over the paralumbar fossa area, the 16th and 17th intercostal spaces deep to the spleen. Using B mode ultrasound, the renal capsule, cortex, medulla, pelvis and corticomedullary junction were clearly identified. The renal width, length and thickness were measured. By means of spectral Doppler ultrasound, the renal RI and PI acquired in all nine examinations and in all three periods. Three waveforms from each animal at each time-point were retrieved and evaluated, so the mean renal RI and PI values were more reliable. The obtained renal RI values were similar to the ones found in other studies[18-22]. The renal RI and PI values are presented as mean and standard deviation in Table 4.1 below. The mean values were calculated from the nine times that renal Doppler examinations were undertaken. No significant difference ($\alpha<0.05$) between the three time-points for both RI and PI (T1, T2 and T3). However, we have noted that it was more difficult to locate the intrarenal arteries on T2 period than on T1 and T3 periods.

Table 4.1 - Mean and standard deviation values of renal resistive (RI) and pulsatility (PI) indexes of the left kidney though transabdominal approach from animals submitted to 24-hour fasting. T1: immediately before fasting, T2: after 24-hour fasting, T3: 12 hours after rehydration.

	T1	T2	T3
RI	0.599 ± 0.049	0.593 ± 0.047	0.553 ± 0.085
PI	0.990 ± 0.138	0.961 ± 0.112	0.854 ± 0.177

Abbreviations: RI resistivity index, PI pulsatility index.

Regarding the renal ultrasound exam through transabdominal approach, a few limitations can be cited. In respect to the animal, body movement, intestinal gas content, intestinal and splenic overlapping, breathing movement and lack of cooperation can interfere with renal evaluation and could possibly prevent the examination from being executed[18,19]. Concerning technical features, the operator's experience is a key point, likewise the equipment employed, as the frequency and probe type will affect directly on image resolution[9,19]. Nevertheless, the renal transabdominal sonographic exam was proved as feasible, reliable and reproducible for renal function evaluation[18,20].

Hoffmann et al.[22] stated that Doppler ultrasonography is a valuable tool to detect renal blood flow changes within a timeframe. The blood flow in intrarenal arteries is related to renal function, whilst renal perfusion alterations are associated with early stages of kidney malfunction in humans, cats and dogs[11]. The Doppler indexes are calculated to quantify the arterial resistance to blood flow, so these indexes can evaluate renal hemodynamics[9]. For humans and small animals, the upper limit for renal RI is considered to be 0.70[11]. For the equine species, this limit might be lower[18-22], but more studies are still needed to determine a reliable reference value. To our knowledge, we are the first group to study renal PI for horses.

Fluid depletion, such as in fasting and dehydration, may lead to hypovolemia and thus renal hypoperfusion[24]. In this sense, the measurement of renal RI and PI could help to evaluate patient’s dehydration state but also on the contrary, in other uses such as evaluation kidney diseases, dehydration may affect RI and PI values regardless of the kidney disease itself. However, in our study, the renal RI and PI did not significantly change between time-points. We believe that low levels of dehydration and its short period of time (24 hours) do not yet affect kidney perfusion. Indeed, to induce medium to severe dehydration in horses a fasting period 36 to 72 hours is reported to be needed[15,25]. These protocols have also used furosemide when dehydrating the animals. Future studies with severe dehydration in horses are still needed to determine the degree of influence on RI and PI.

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