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Validation of ultrasonography for measurement of cartilage thickness in the equine carpus

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Abstract

Articular cartilage thinning is an important hallmark of osteoarthritis (OA), and ultrasonography (US) is a clinically accessible tool potentially suitable for repeated evaluation. The aim of the present prospective methods comparison study was to validate US as a tool for measuring cartilage thickness in the carpus of the horse. Eight Standardbred trotters underwent US examination with 9 and 15 MHz linear transducers. Six anatomical locations in the radiocarpal joint (RCJ) and middle carpal joint (MCJ) were examined. The same joints were assessed by ultrahigh field (9.4 Tesla) magnetic resonance imaging (MRI) and histology. Associations between measurements obtained by the different modalities were assessed by ANOVA, Deming regression, Pearson correlation and Bland–Altman plots. Histologically assessed total cartilage thickness (the noncalcified cartilage (NCC) plus the calcified cartilage zone (CCZ)) overestimated thickness compared to MRI ($P < 0.01$) and US ($P < 0.01$). US 15 MHz had substantial agreement with MRI and NCC histology, and repeatability was acceptable (coefficient of variation = 8.6–17.9%) when used for assessment of cartilage thickness in the RCJ. In contrast, 9 MHz US showed poorer agreement with MRI and NCC histology, as it overestimated the thickness of thin cartilage and underestimated the thickness of thicker cartilage in the RCJ and MCJ. Moreover, repeatability was suboptimal (coefficient of variation = 10.4–26.3%). A 15 MHz transducer US is recommended for detecting changes in RCJ cartilage thickness or monitoring development over time, and it has the potential for noninvasive assessment of cartilage health in horses.

KEYWORDS
front knee, histopathology, horse, orthopedic, ultrasound

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1 | INTRODUCTION

Osteoarthritis (OA) in the carpal joint is commonly seen in Thoroughbred and Standardbred racehorses.[1,2] In racehorses, OA is thought to be a stress-related syndrome caused by repetitive overloading of the articular surface of the carpal bones.[3,4] OA is a degenerative joint disease characterized by fibrillation and gradual thinning of the articular cartilage as a consequence of joint inflammation[5–7], and measurement of cartilage thickness is therefore potentially a valuable tool for diagnostic and prognostication purposes. Ultrasonography (US) has great potential as a tool for measuring cartilage thickness,[8,9] however, the assessment of cartilage thickness of the equine carpus by US has never been validated.

Many imaging modalities have been used for the assessment of articular changes; nevertheless, there are several limitations that make them less useful in the detection and monitoring of OA in horses. Radiography is cheap and available to the general clinic but does not allow direct visualization of cartilage.[10] Changes that can be seen on radiographs include joint space narrowing and collapse, enthesophytes, osteophytes, subchondral cyst-like lesions, erosion, and sclerosis.[3,10] These are changes that only develop in late-stage OA.[5–7] Therefore, radiography is not ideal to diagnose and monitor the early stages of OA.[10] Magnetic resonance imaging (MRI) offers noninvasive three-dimensional imaging, which allows assessment of the entire joint.[3,11,12] Although MRI-based quantitative and qualitative cartilage measurement has been continuously improved for diagnosis and monitoring of the progression of OA in both human patients and horses,[12–14] MRI is not always available to the equine practitioner. MRI is expensive, and general anesthesia is often needed. Therefore, MRI is less attractive for clinical routine examinations, surveillance of OA progression, or serial evaluations for research purposes.[8] Compared to the abovementioned techniques, US has several advantages: high spatial resolution,[15] noninvasiveness, and no ionizing radiation exposure.[8,16] US is cost-effective and easily implementable in equine clinics and hence theoretically ideal for day-to-day clinical and research-related surveillance of joint health, including measurements of cartilage thickness.[8,16] US has been shown to correlate well with MRI measurements of cartilage thickness in several human joints, including the shoulder, knee, ankle and wrist.[16–19] US has also been shown to correlate well with cartilage thickness measured by histology in the stifle of foals[20] and the human knee.[8] However, the ultrasound beam angle and accessibility of the cartilage region of interest are key determinants of the success of cartilage measurements.[21] Therefore, the complex nature of the equine carpus with several differently oriented articular surfaces and very thin cartilage[22] makes the accessibility for US difficult compared to the fetlock and stifle. While MRI is considered the gold standard for cartilage thickness measurement in the human knee,[17,23] this has not been established in the equine carpus. Histologic preparation of bone/cartilage specimens involves the risk of shrinkage or stretching of the tissue,[24] which is why histology is also not considered the gold standard. The needle indentation technique is considered the gold standard for in vitro cartilage thickness measurement in animal models,[25,26] but this technique was not available for this study. Therefore, US cartilage thickness measurements were compared to both MRI and histology in the current study.

The aim of this study was to evaluate and validate the use of US for measuring cartilage thickness in the dorsal aspect of the radiocarpal joint (RCJ) and middle carpal joint (MCJ) in horses in vivo. US, postmortem MRI and histology were used to assess the correlation and association of cartilage thickness measurements between modalities. Our hypothesis was that cartilage thickness measurements would correlate well between US and MRI and between US and histologic measurements.

2 | MATERIALS AND METHODS

2.1 | Animals

Eight young Standardbred trotters, which were enrolled in another study and euthanized for reasons unrelated to the present study, were included. The sample size for our study was based on the sample size of the aforementioned study and hence the number of horses available to us. Lameness examinations were performed for all horses by a veterinarian with a minimum of 10 years of clinical experience with equine lameness as well as a completed residency in large animal surgery (ECVS). Only horses with a lameness of or less than grade 2 on the AAEP (American Association of Equine Practitioners) lameness scale on any leg were included in the study. None of the included horses had pathological changes as seen on US, gross pathology, or histopathology. The study was approved by the Local Ethical and Administrative Committee of the Department of Veterinary Clinical Sciences, University of Copenhagen (permit # 2018-005) and the Danish Animal Experiments Inspectorate (permit # 2016-15-0201-01128). All horses were Standardbred trotters, aged 4 to 10 years (mean 6.0 years; median 5.5 years), five mares and three geldings, with a weight range of 431–555 kg (mean 495 kg; median 499 kg).

2.2 | Study design

This was a prospective, nonrandomized methods comparison study. Six anatomical locations of the RCJ and MCJ of the right carpus were chosen for US, MRI, and histological evaluation. These were the most distal point of the medial parasagittal radial ridge (RR), the most proximal points of the intermediate and medial radial facets (IRF and MRF, respectively), the proximal dorsal articular surface of the radial carpal bone immediately opposite the MRF (prRCB), the distal dorsal articular surface of the radial carpal bone 5 mm medial to the lateral edge of the bone (diRCB), and the distal dorsal articular surface of the intermediate carpal bone 5 mm lateral to the medial edge of the bone (ICB) (Figures 1 and 2). For locations IRF and MRF, both the most proximal point and the points 2 mm lateral and medial to the most proximal point were assessed, and in later analysis, an average of those three measurements was used.
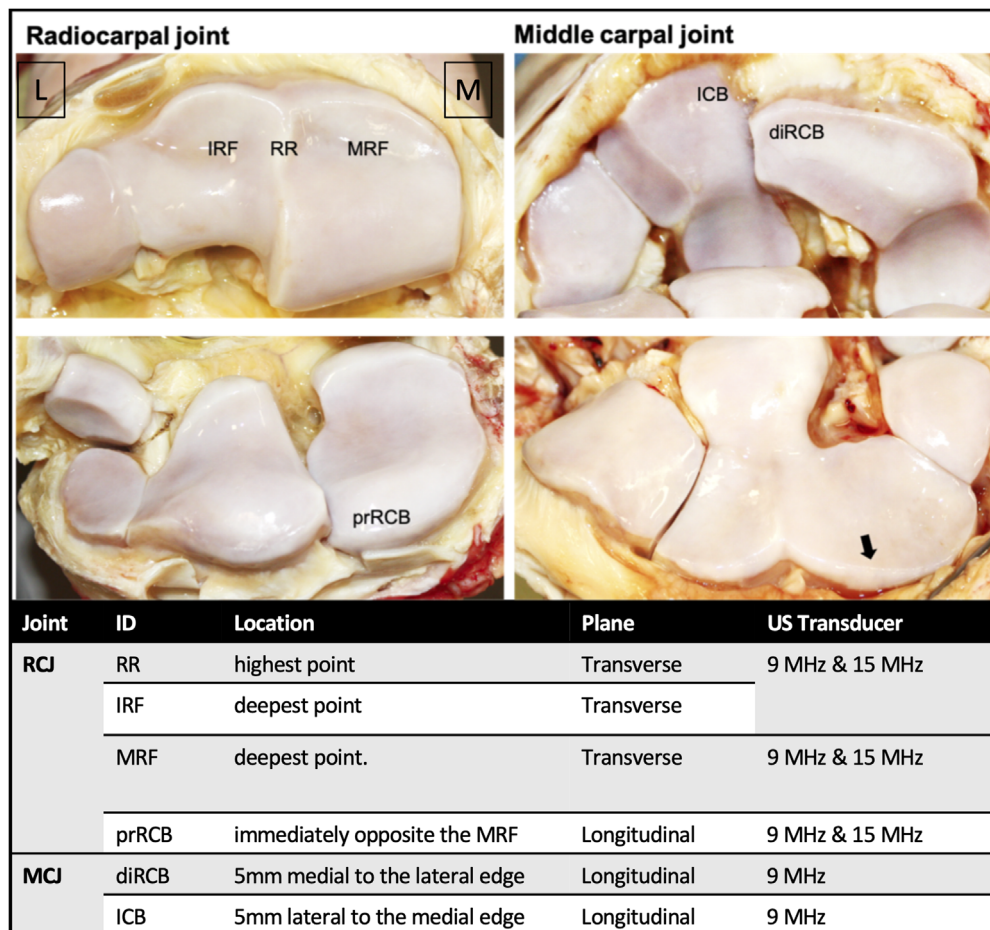
FIGURE 1 Anatomical locations for cartilage thickness measurement. Abbreviations: RCJ, radiocarpal joint; MCJ, middle carpal joint; RR, radial ridge; IRF, intermediate radial facet; MRF, medial radial facet; prRCB, proximal radiocarpal bone; diRCB, distal radiocarpal bone; ICB, intermediate carpal bone. The cartilage of the third carpal bone could not be adequately visualized (arrow). Lateral is to the left

2.3 | Ultrasonographic measurements

All horses underwent US examination performed 1–2 days prior to euthanasia by an equine clinician with 10 years of experience with orthopedics and US who had completed all modules of the ISELP (International Society of Equine Locomotor Pathology- http://www.iselp.org) Certification offered by Prof. Dr. Jean Marie Denoix (SØ). The dorsal aspect of the carpus was clipped, and the skin was washed with soap and warm water and moistened with alcohol and ultrasound coupling gel (Bluescan, Zealand Coating APS, Bjaeverskov, Denmark). To ensure the same degree of flexion in all horses in the study, the right carpus was flexed and held in place with a fixed-angle device at an angle of 24 degrees placed between the caudal aspect of the antebrachium and the palmar aspect of the metacarpus. All ultrasonographic examinations were performed using a LOGIQ E9 (GE Healthcare) with a linear array 9 MHz transducer with a spatial pulse length (SPL) of 0.8556 mm and an axial resolution of 0.4278 mm (personal communication with GE Healthcare) (GE Healthcare, 9L), and measurements of the anatomical locations in the RCJ were repeated with a linear array 15 MHz transducer with an SPL of 0.3593 mm and an axial resolution of 0.1797 mm (GE Healthcare, ML6-15). Initially, examination using the 15 MHz transducer was also attempted in the two locations in the MCJ, but the image quality was inadequate to allow proper assessments; therefore, it was abandoned. All measurements of the articular surface of the radius were obtained in the transverse plane (RR, MRF and IRF), whereas the rest of the anatomical locations were scanned in the longitudinal plane (prRCB, diRCB, and ICB; Figure 1.) Settings were standardized (Table S1). At each anatomical location, cartilage thickness was measured from the thin hyperechoic line at the synovial fluid-cartilage interface to the hyperechoic line at the cartilage-bone interface (Figures 3 and 4). The on-board calipers of the US scanner could measure with an accuracy of 0.01 cm. The cartilage thickness was measured three times at each anatomical location. These three repetitions were used to assess repeatability (coefficient of variance). The mean ± standard error of the mean (SEM) of the three repetitions was used for comparison of cartilage thickness with the other modalities.
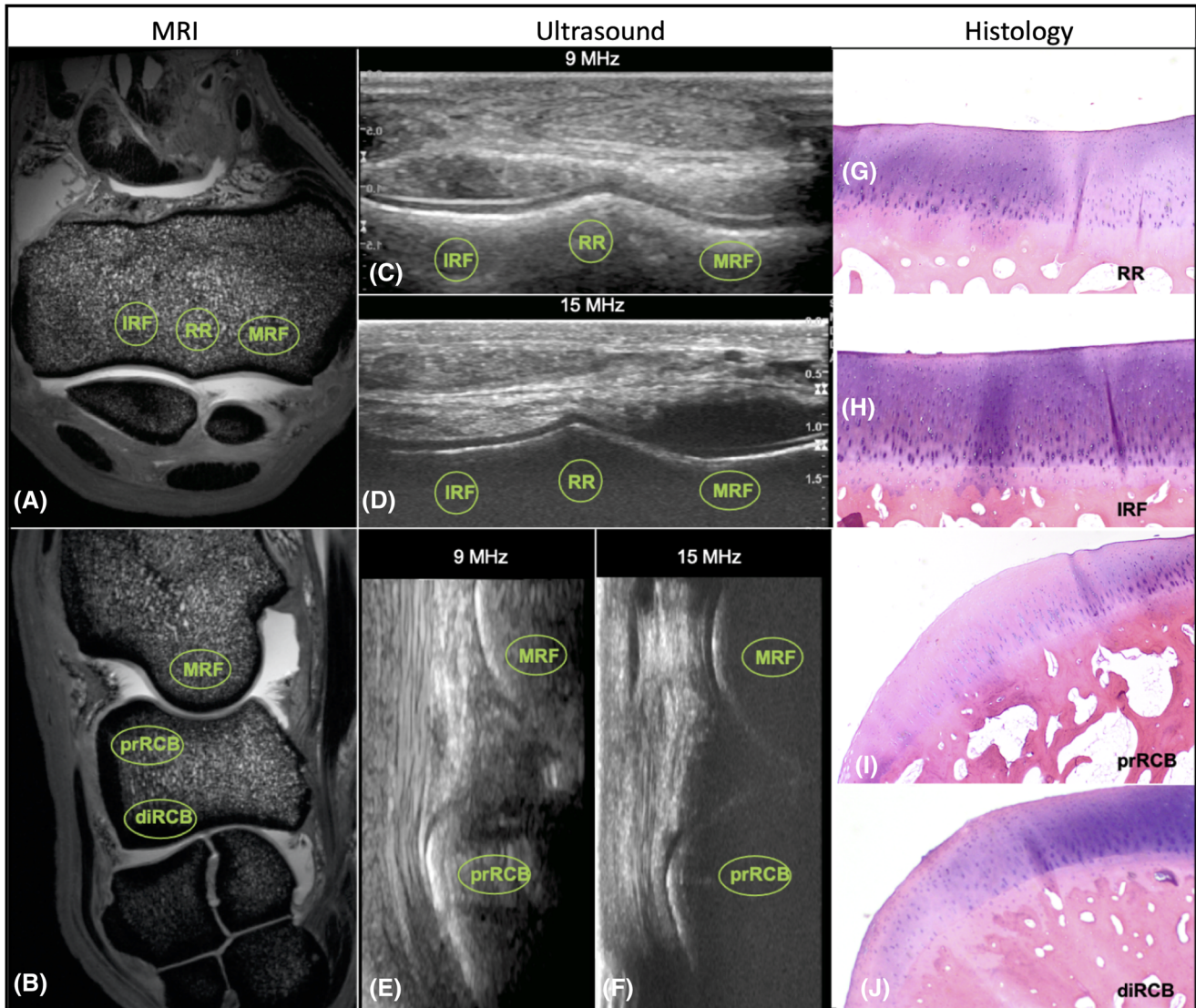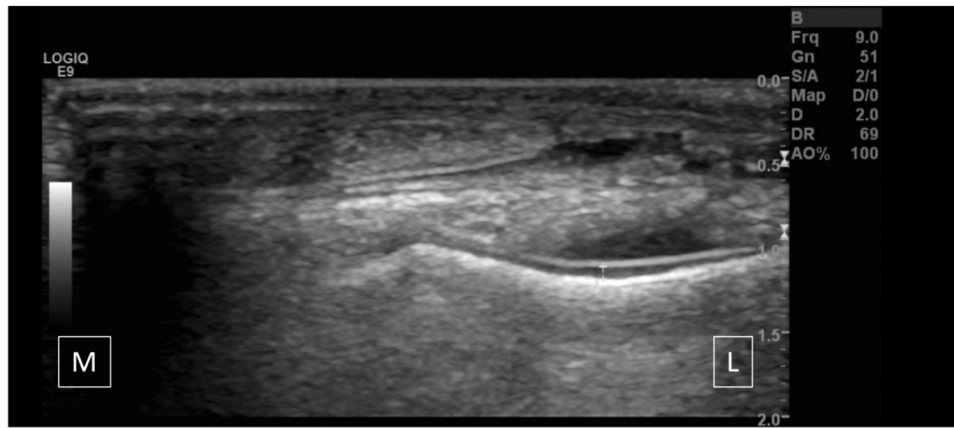
FIGURE 2 Representative images of the measured anatomical locations by dorsal (A) and sagittal (B) MRI, 9 MHz transverse (C) and 15 MHz transverse (D) ultrasound, 9 MHz longitudinal (E) and 15 MHz longitudinal ultrasound and histology (G–J). Thickness was measured at the most distal point for the location RR (G) and at the most proximal point for MRF and IRF (H), whereas the thickness was measured on the dorsal rim of the bone for all remaining locations (I,J). All locations are not shown with all modalities. RR, radial ridge; IRF, intermediate radial facet; MRF, medial radial facet; prRCB, proximal radiocarpal bone; diRCB, distal radiocarpal bone; MRI, magnetic resonance imaging

2.4 | MRI measurements

Postmortem MRI scans were performed immediately after euthanasia using a 9.4 Tesla preclinical horizontal bore scanner (BioSpec 94/30 USR, Bruker BioSpin, Ettlingen, Germany) equipped with a 100 mT/m gradient coil (BGA-20S, Bruker). Imaging was performed with a 154 mm-inner-diameter volume resonator and a 4-channel surface quadrature array receiver coil. In a preliminary session, we used three sequences: T2*-weighted 3D-fast low angle shot (3D-FLASH), T2-weighted 2D-rapid acquisition with relaxation enhancement (2D-RARE), and 3D gradient-spoiled fast imaging with steady state precession (3D-FISP) (Suppl. Table 2). The 3D-FISP revealed superior contrast between cartilage and synovial fluid (Figure 2); therefore,

only the 3D-FISP (TR = 6 ms, TE = 3 ms, number of averages = 12, flip angle = 20°, FOV 150 mm × 128 mm × 110 mm, matrix = 514 × 340 × 328, image resolution = 0.29 mm × 0.38 mm × 0.34 mm, acquisition time = 150 min) was used for the subsequent measurements. Cartilage thickness was assessed through manual measurements using Osirix (ver. 10.0.4, Pixmeo, Geneva, Switzerland) by a Diplomate of the American College of Veterinary Radiology–Equine Diagnostic Imaging (J.G.). The MRI observer was blinded to the US measurements. Cartilage thickness was defined as the perpendicular distance between the outer interface between cartilage and synovial fluid and the inner interface of the cartilage layer and bone (Figure 5). The distance was measured with the integrated "Length Measurement Tool" with an accuracy of 0.01 mm. All measurements were obtained in the plane that

FIGURE 3 Representative ultrasound image transverse view showing measurement of the cartilage thickness at the medial radial facet with a 9 MHz linear transducer. Cartilage thickness is measured between the two hyperechoic lines representing the cartilage-fluid interface and the cartilage-bone interface. All measurements were repeated three times, and an average was used for analysis. Medial is to the left
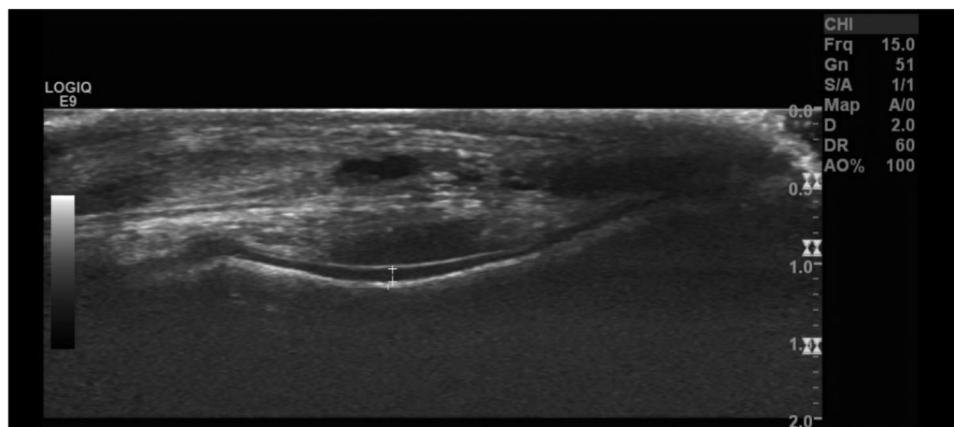
FIGURE 4 Representative ultrasound image transverse view showing measurement of the cartilage thickness at the medial radial facet with a 15 MHz linear transducer. Medial is to the left
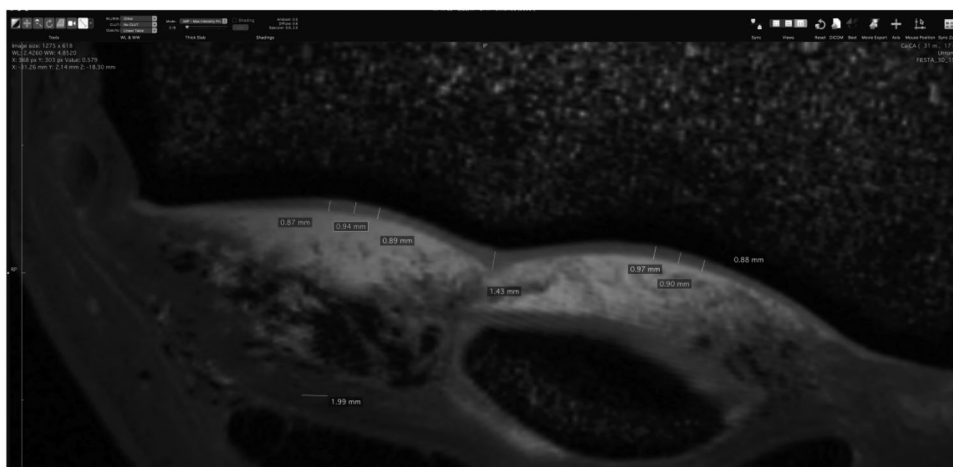
FIGURE 5 Representative magnetic resonance image dorsal view showing measurements of the cartilage thickness of the three anatomical locations of the radius. Cartilage thickness is measured between the low-intensity subchondral bone and the high-intensity synovial fluid
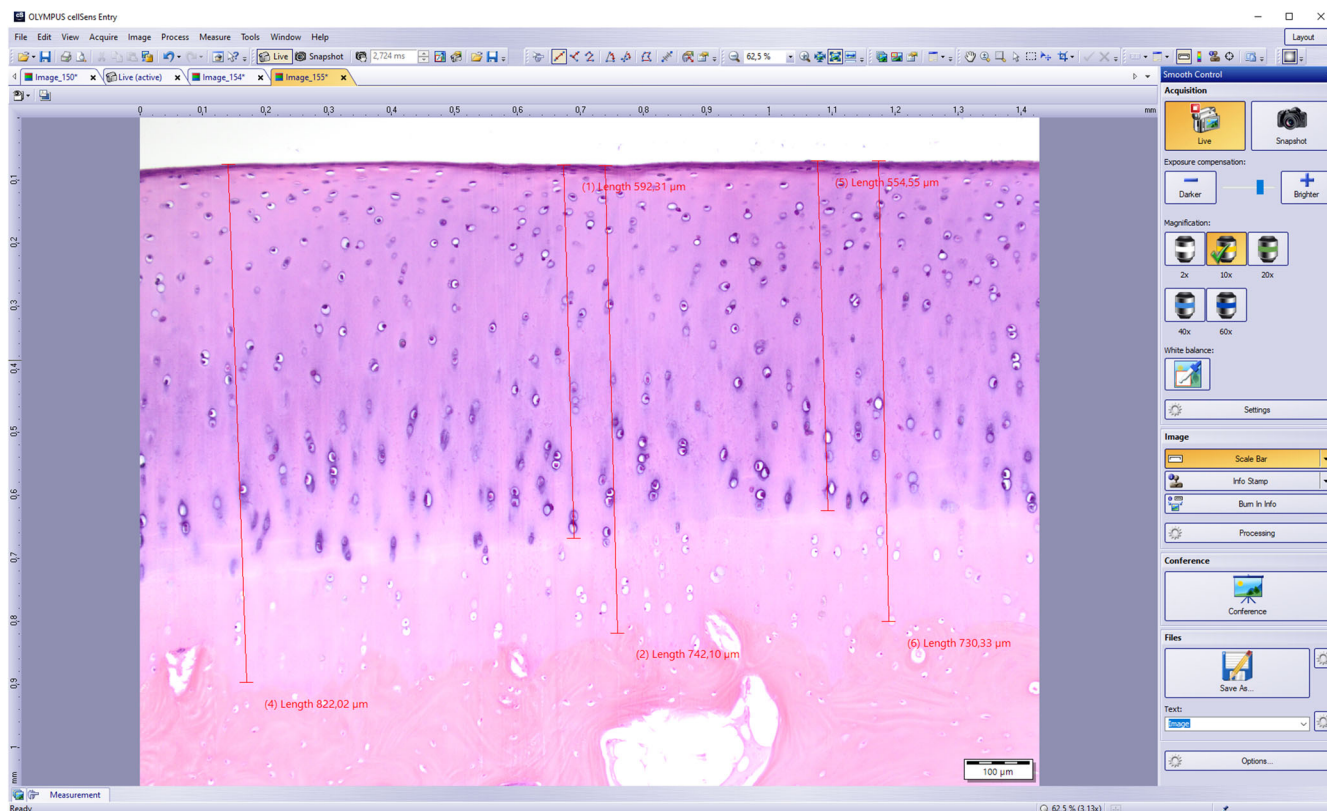
FIGURE 6 Representative histologic image showing hematoxylin and eosin-stained cartilage at the medial radial facet sagittal view. The noncalcified cartilage is measured between the surface and the sharply delineated tidemark line. Total cartilage thickness was measured between the surface and the interface between the calcified cartilage zone and the subchondral bone. All measurements were repeated three times, and an average was used for analysis

corresponded to the plane of the ultrasound measurements (Figure 1) so that the orientation of the 3D MRI images would mimic the ultrasound beam: the anatomical locations RR, MRF, and IRF were measured on images in the dorsal view, and the prRCB, diRCB, and ICB were measured on sagittal images.

ness of the noncalcified cartilage (NCC) and the total cartilage thickness (the NCC plus the calcified cartilage zone (CCZ)) were each measured three times for every location (Figure 6). The mean ± SEM of the three repetitions was used for comparison of cartilage thickness with the other modalities.

2.5 | Histology measurements

Immediately after MRI, the carpal joints were opened by sharp dissection carefully to avoid any damage to the cartilage. Osteochondral wedge sections (5 × 5 × 10 mm) were cut out using an oscillating saw. The samples were fixed in formaldehyde, decalcified in formic acid, embedded in paraffin, cut into 2 μm sections and stained with hematoxylin-eosin. All samples were sliced in the sagittal plane. Images of the sections were obtained using an Olympus BX45 microscope and an Olympus UC30 camera and processed with Olympus cellSens Entry 2.1 software (Figure 2). The cartilage thickness was measured with the "Arbitrary Distance" tool with an accuracy of 0.01 μm (Figure 6). The histopathology observer was blinded to the US and MRI measurements. Histopathology measurements were performed by one of the authors (CA) after thorough training and alignment by and with a Diplomate of the European College of Veterinary Pathologists. The thick-

2.6 | Statistical analysis

All statistical analyses were performed with a statistical software package (R, version 3.6.1, The R Foundation for Statistical Computing). Selection and completion of statistical tests was performed by a PhD Fellow (C.A.) who had completed advanced training in statistics and a professor, Dipl. ECVS (S.T.J.), with extensive experience in statistics, with supervision by a PhD-fellow and a full professor of statistics. Normality of data and residuals was confirmed on histograms and Q-Q plots. The overall difference between modalities was assessed by ANOVA (only for locations in the RCJ, which were measured by all modalities). Pearson correlation analysis was used to quantify the pairwise correlation between measurements obtained by US and MRI and between US and NCC histology. Deming regression was used to analyze the pairwise association between US and MRI and between US and NCC histology. Visual inspection of Bland–Altman plots was

TABLE 1 ANOVA and corresponding p values for pairwise comparisons of cartilage thickness in the equine radiocarpal joint

	US 9 MHz	US 15 MHz	MRI	Histology NCC	Histology Total
US 9 MHz	–				
US 15 MHz	0.026*	–			
MRI	0.040*	0.858	–		
Histology NCC	0.010*	0.725	0.595	–	
Histology Total	2.94e-07***	0.002**	0.001**	0.006**	–

Abbreviations: US, ultrasound; MRI, magnetic resonance imaging; NCC, noncalcified cartilage. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

used to evaluate bias between modalities. A mixed model analysis with fixed effects of modality and location and random effects of the individual horse was used to calculate the estimated cartilage thickness for each location by each modality. Histologic total cartilage thickness (NCC plus the CCZ) was not included in the mixed model analysis. The coefficient of variation (CV% = SD/Mean × 100%) was calculated between repeated US measurements to assess repeatability. A CV of less than 15% was considered to represent satisfactory agreement between repetitions.[18,27] A P-value < 0.05 was considered statistically significant.

3 | RESULTS

3.1 | Histological total cartilage thickness was significantly different from US and MRI measurements

An ANOVA showed that histologically measured total cartilage thickness (NCC + CCZ) was significantly thicker (1.03 ± 0.05 mm) than values obtained with US or MRI and with histologic measurements of the NCC only (US 9 MHz, 0.69 ± 0.03 mm, $P < 0.001$; US 15 MHz, 0.83 ± 0.04 mm, $P < 0.01$; MRI, 0.82 ± 0.05 mm, $P < 0.01$; NCC histology, 0.86 ± 0.05 mm, $P < 0.01$; Table 1). Therefore, histologically measured total cartilage thickness was discarded from subsequent analysis, and US was only compared to NCC histology and MRI. All cartilage thickness measurements presented as boxplots with medians, interquartile ranges, and outliers are shown in Figure 7.

3.2 | Ultrasonographic cartilage thickness measurement was dependent on frequency

The same ANOVA showed that 9 MHz US measurements were significantly thinner than values obtained from all other imaging modalities (US 15 MHz, $P < 0.05$; MRI, $P < 0.05$; NCC histology, $P < 0.05$). In contrast, cartilage thickness assessed by 15 MHz US showed no significant difference compared to NCC histology ($P = 0.725$) and MRI ($P = 0.858$). Additionally, there was no significant difference between NCC histology and MRI ($P = 0.595$; Table 1).

3.3 | Association and correlation between imaging modalities

The slope of the Deming regression line of the association between US and NCC histology and between US and MRI did not differ significantly from 1 for the 15 MHz transducer (NCC histology slope = 1.35 (95% CI: 0.84–1.86), intercept = −0.27 (95% CI: −0.68–0.14); MRI slope = 1.40 (95% CI: 0.65–2.14), intercept = 0.39, (95% CI: −1.01–0.23), while for the 9 MHz transducer, the slope of the regression line differed significantly from 1 (NCC histology, slope = 2.24 (95% CI: 1.52–2.96), intercept = −0.69 (95% CI: −1.19 to −0.21; MRI, slope = 2.38 (95% CI: 1.62–3.13), intercept = −0.83 (95% CI: −1.34 to −0.32; Figure 4). Pearson correlation showed substantial agreement between modalities for all comparisons (US 15 MHz versus NCC histology $r = 0.70$, (95% CI: 0.47–0.85); US 15 MHz versus MRI $r = 0.61$ (95% CI: 0.34–0.79); US 9 MHz versus NCC histology $r = 0.62$, (95% CI: 0.40–0.77); MRI versus US 9 MHz $r = 0.69$, (95% CI: 0.49–0.82)) (Figure 4). Bland–Altman plots showed good agreement between US 15 MHz and NCC histology and between US 15 MHz and MRI, while there seemed to be proportional and systematic bias when US 9 MHz was compared to NCC histology and MRI. The US 9 MHz seemed to overestimate smaller cartilage thicknesses and underestimate larger cartilage thicknesses relative to the other two modalities (Figure 4). Complete results of the mixed model analysis of cartilage thickness estimates can be seen in Table 2. The residual deviance of the mixed model analysis was 0.05 mm.

3.4 | Repeatability of US measurements

The repeatability of US 15 MHz was acceptable (CV% = 8.6–17.9%) when used for the assessment of cartilage thickness in the RCJ. The repeatability of measurements made by US 9 MHz of the cartilage of the radius was acceptable (CV% = 8.6–10.6). At all other locations, repeatability was poor (CV% = 19.1–26.3%) (Table 3).

A post hoc power calculation showed a power of 7.2% based on the difference in the mean and pooled standard deviation of the comparison between US 9 MHz and NCC histology, which were our main outcomes. We would have required a total of 222 horses enrolled in this study to achieve a power of 80%.

Summary of all locations
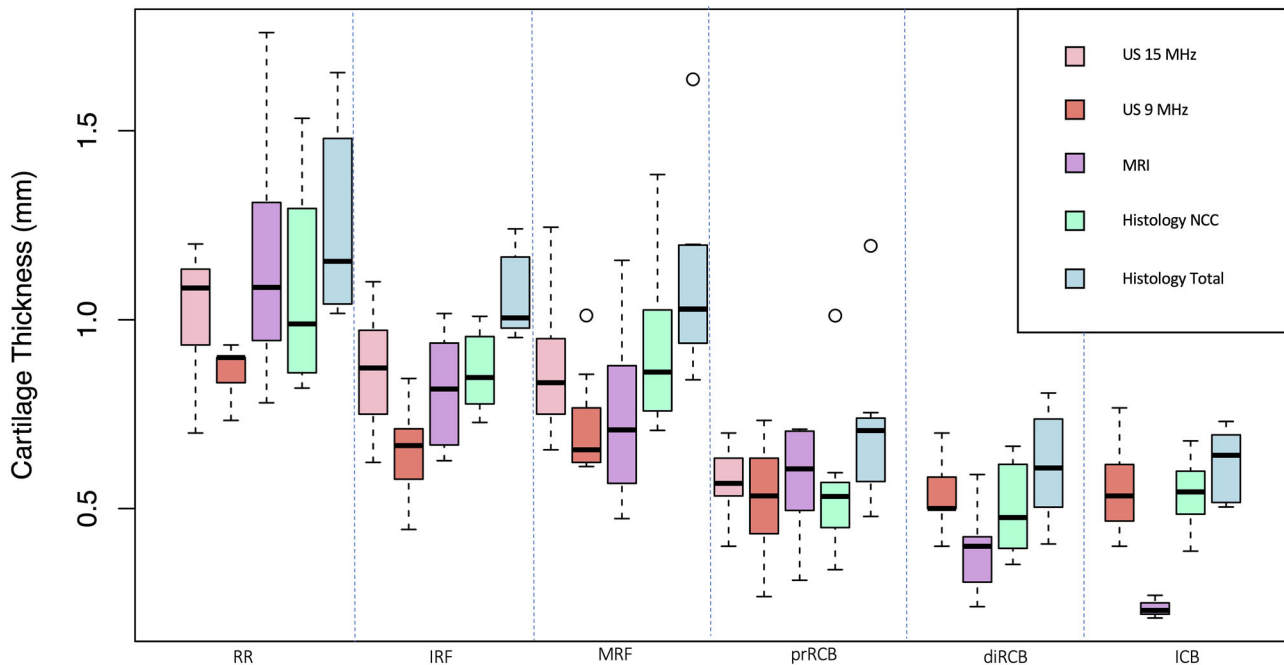
FIGURE 7 All cartilage thickness measurements presented as boxplots with medians, interquartile range, and outliers. Measurements are divided by anatomical location and by measurement method. diRCB and ICB were not measured by 15 MHz ultrasound. Abbreviations: US, ultrasound; MRI, magnetic resonance imaging; NCC, noncalcified cartilage, RR, radial ridge; IRF, intermediate radial facet; MRF, medial radial facet; prRCB, proximal radiocarpal bone; diRCB, distal radiocarpal bone; ICB, intermediate carpal bone

TABLE 2 Estimated cartilage thickness for each anatomical location based on a mixed model analysis

Joint	RCJ				MCJ	
	RR (mm)	IRF (mm)	MRF (mm)	prRCB (mm)	diRCB (mm)	ICB (mm)
MRI	1.01	0.78	0.79	0.54	0.46	0.48
Histology NCC	1.08	0.84	0.86	0.60	0.52	0.54
US 9 MHz	0.97	0.74	0.75	0.50	0.42	0.43
US 15 MHz	1.06	0.83	0.85	0.59	Not performed	Not performed

Abbreviations: RCJ, radiocarpal joint; MCJ, middle carpal joint; RR, radial ridge; IRF, intermediate radial facet; MRF, medial radial facet; prRCB, proximal radiocarpal bone; diRCB, distal radiocarpal bone; ICB, intermediate carpal bone (distal articular surface). US, ultrasound; MRI, magnetic resonance imaging; NCC, noncalcified cartilage.

4 | DISCUSSION

There was substantial agreement between cartilage thickness determined by US and cartilage thickness measured by MRI or NCC histology for the anatomical locations chosen in this study, which were the radial ridge; the intermediate and medial radial facets; the proximal dorsal articular surface of the radial carpal bone; the distal dorsal articular surface of the radial carpal bone; and the distal dorsal articular surface of the intermediate carpal bone. This was particularly true for measurements obtained at 15 MHz. In contrast, cartilage thickness measured by US 9 MHz was less reliable, with the US 9 MHz measurements overestimating the thickness of thinner cartilage and underes-

timating the thickness of thicker articular cartilage (Figure 7), as suggested by the ANOVA (Table 1) and Bland–Altman plots (Figure 8). Particularly in the thinner cartilage of the MCJ, US 9 MHz had low precision (a high CV%) (Table 3).

To the best of the authors' knowledge, this is the first time that US has been investigated for the assessment of carpal articular cartilage thickness in the adult horse. US has been used for the assessment of articular cartilage thickness in human patients and has been shown to correlate well with other modalities such as MRI[16,17,19] and histology.[8] Until now, US measurement of equine cartilage thickness has only been investigated in the femorotibial joint of foals.[20] In that study, cartilage thickness of the lateral and medial trochlear ridges

TABLE 3 Coefficient of variance (CV%) between three repetitions of ultrasound (US) measurements of the same anatomical location in the same horse. A CV of less than 15% was considered to represent satisfactory agreement between repetitions[18,27].

Joint	RCJ				MCJ	
Location	RR	IRF	MRF	prRCB	diRCB	ICB
CV% US 9 MHz	15.3	10.4	11.6	26.3	24.8	19.1
CV% US 15 MHz	10.6	9.8	8.6	17.9	Not performed	Not performed

Abbreviations: RCJ, radiocarpal joint; MCJ, middle carpal joint; RR, radial ridge; IRF, intermediate radial facet; MRF, medial radial facet; prRCB, proximal radiocarpal bone; diRCB, distal radiocarpal bone; ICB, intermediate carpal bone (distal articular surface).
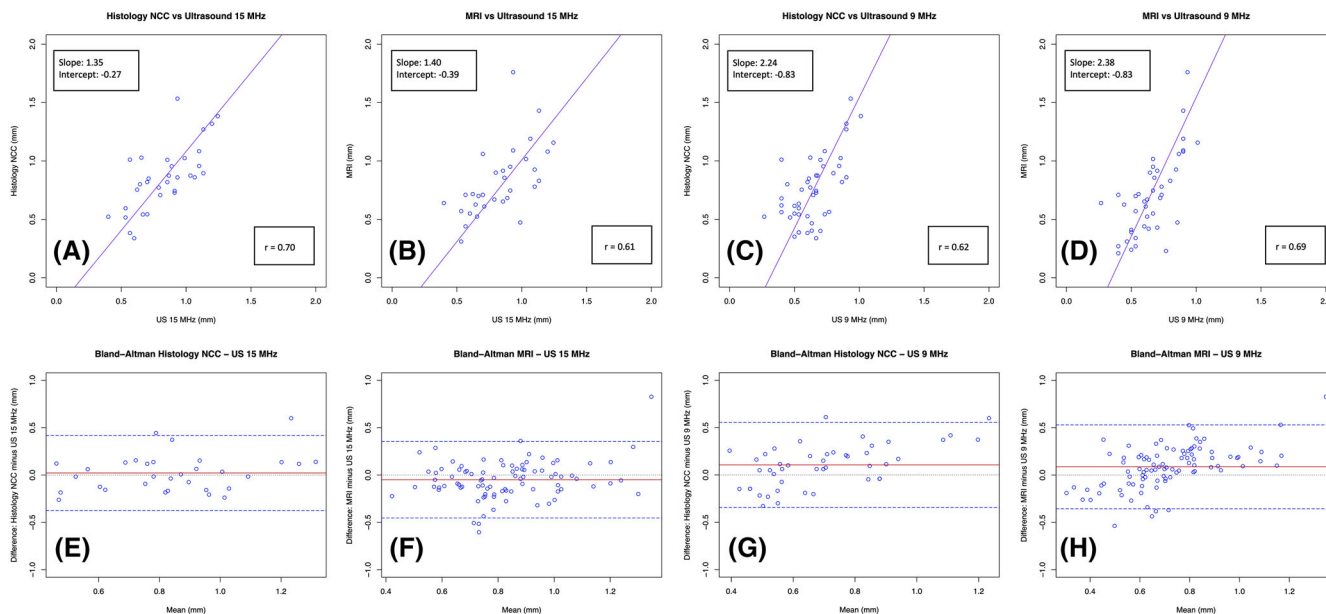
FIGURE 8 Deming regression (A–D) with regression line, slope and intercept. Pearson's correlation coefficient (r) for each comparison is shown. Bland–Altman plots (E–H) visualizing the association between cartilage thicknesses measured by histology NCC, MRI, US 9 MHz, and US 15 MHz. There is a better association between modalities between US 15 MHz and both histology NCC (A) and MRI (B) than with the 9 MHz transducer (C) and (D). For the Bland–Altman plots, the mean difference is shown in red, and 2× SD is shown in blue. There is a tendency to a proportional bias when US 9 MHz is compared to both histological NCC and MRI (G) and (H). Abbreviations: US, ultrasound; MRI, magnetic resonance imaging; NCC, noncalcified cartilage [Color figure can be viewed at wileyonlinelibrary.com]

of the femur of cadavers of neonatal foals was measured by US with a 10 MHz linear transducer and showed excellent correlation to histologic measurements.[20] However, there is a substantial difference between the thick immature epiphyseal growth cartilage in the stifle of foals[28] and the thin layer of articular cartilage in the carpus of adult horses,[29] and these results cannot be directly translated to mature horses and other anatomical locations. Therefore, further studies similar to the present one are needed.

The anatomical locations examined in this study were chosen based on the predilection for OA development in racehorses.[3,30] In the RCJ, osteochondral changes are most often seen on the dorsoproximal aspect of the RCB and ICB.[30] In the MCJ, changes are most often seen in the dorsodistal aspect of the RCB and on the dorsoproximal aspect of the third carpal bone.[3] Osteoarthritis in the carpometacarpal joint is much less common and occurs mainly in older horses with no specific occupational- or breed-related predisposition.[3] In general, we were able to visualize the cartilage relatively deep within the RCJ with the

reported US techniques and approach. Unfortunately, we were not able to include the third carpal bone in the scanning protocol because we could not reliably achieve the perpendicular direction of the US beam onto the articular cartilage. In the MCJ, we could only assess the cartilage on the dorsal rim of the articular surfaces and only when using the 9 MHz transducer. As lesions most often develop in the dorsal part of the joint[3,30] examinations of this region are important for OA surveillance.

Articular cartilage is composed of the NCC and CCZ layers. The latter forms the interface between articular cartilage and subchondral bone.[31,32] Histologic measurements of total cartilage thickness, including the CCZ, were consistently larger than both measurements made by US and MRI. These findings were expected, as cartilage thickness was measured with US as the hypoechoic band between two hyperechoic lines (Figures 3 and 4). It is generally assumed that the second hyperechoic reflection line in an US image of the osteochondral unit comes from the tidemark and hence delineates the boundary between

the NCC and the CCZ.[33] The same is true for MRI sequences; because of the high mineral content, the CCZ has intrinsically short T2/T2* values, which makes it difficult to make contrast between the CCZ and underlying bone.[34] Thinning of the NCC is a hallmark of OA and is caused by both erosion and fibrillation of the superficial layer and by expansion of the CCZ into the NCC.[5] Consequently, surveillance of NCC thickness could be used as an indicator of cartilage health. Our results show that US measurements of certain regions could be used to monitor NCC thickness but with careful observation of the limitations of the scanner type and transducer.

Cartilage thickness measured by histology may not fully correlate with true thickness because of possible shrinkage and manipulation of histological specimens.[24] Therefore, we compared US measurements with both histology and MRI scanning with an ultrahigh field 9.4 Tesla preclinical scanner. For MRI measurements, the spatial resolution was limited by a voxel size of $0.29 \times 0.38 \times 0.34$ mm. Even with this excellent resolution, which was much better than standard clinical MRI in equine practice, it was still challenging to measure the very thin cartilage of the carpus, especially that of the MCJ, where we found an estimated cartilage thickness of approximately or less than 0.5 mm (Figure 7 and Table 2). A study by Cheng et al. indicated that cartilage thickness measurement should be possible if the cartilage thickness is at least twice the voxel size.[35] For our study, this is only true for measurements made in the RCJ and not in the MCJ. Ultrasound typically has better spatial resolution than clinical MRI and should therefore be more reliable, but differences in scan plane and off-incidence beam geometry may affect reliability.[21] US measurement of cartilage thickness has been shown to be smaller than the thickness measured by MRI in both the human ankle[27] and knee.[17,36] In those studies, low-frequency transducers were used, and our results showed that the 9 MHz transducer underestimated thickness compared to MRI and histology in the thicker cartilage found in the RCJ (RR, IRF, and MRF). In contrast, our results suggest that excellent agreement may be achieved when a 15 MHz transducer is used.

In our study, we used a 9 MHz transducer with an axial resolution of 0.4278 mm and a 15 MHz transducer with an axial resolution of 0.1796 mm. The poorer axial resolution of the 9 MHz transducer can explain why it did not perform as well as the 15 MHz transducer. This also explains why both transducers performed better in the relatively thicker cartilage of the radius than in the thinner cartilage of the MCJ. In some horses, we measured a cartilage thickness of approximately 0.4 mm in the MCJ, and at these locations, the cartilage layer was very difficult to differentiate from other tissues, especially with the 9 MHz transducer. The very thin cartilage of the equine carpal joints offers great challenges in regard to the accuracy of thickness measurements and in regard to the on-board calipers of the US scanner (Figures 3 and 4), which could measure intervals of 0.01 cm. This means that a difference of one unit will account for up to 25% of the total thickness in some locations. Therefore, even very small differences in caliper placement will affect the result and the repeatability very much, which we also experienced in this study. We may have achieved better results with a finer caliper, for example, one that was able to measure differ-

ences under 0.1 mm, as we could with histology (0.01 μm) and MRI (0.01 mm).

The repeatability of measurements made with the 15 MHz transducer in the RCJ was satisfactory, thereby indicating that it may be possible to follow the same patient over time, potentially allowing repeated cartilage health monitoring in an inexpensive and noninvasive manner. For the 9 MHz transducer, repeatability was acceptable only for measurements of the cartilage thickness of the articular surface of the radius (Table 3). This means that intraindividual variation is too great to accurately follow cartilage thinning in the rest of the carpal joint in the same animal over time with a 9 MHz transducer. Ultrasound measurements in the RCJ were generally easier to perform than in the MCJ, both because the cartilage in the RCJ is thicker and because the RCJ has a greater degree of flexion, which makes it easier to access the articular surface perpendicularly with the ultrasound probe.

The US measurements were only obtained by one observer in this study. Therefore, interobserver reliability was not assessed, and the results may not be generalizable for other observers, as the observer is an important source of variance.[37] Another limitation of this study is that locations in the MCJ were only examined by US with the 9 MHz transducer and not the 15 MHz transducer. We could not measure cartilage thickness in the MCJ with the 15 MHz transducer because we could not establish adequate contact between the wide footprint of the linear probe and the small, curved carpal bones. We also tried to improve the probe contact with a silicon standoff; however, it did not work well (data not shown). We might have been able to obtain more accurate measurements with a high-frequency transducer, and we may have been able to include the cartilage of the third carpal bone with a differently designed transducer or with more optimization of the scanning protocol. For future studies, it would be interesting to measure the cartilage thickness of the entire joint with a narrower high-frequency transducer. This may require improvements in image quality and correction for the nonperpendicular US beam angle.[21] Additionally, we may have been able to obtain better ultrasound images with individual optimization for each horse with regard to the angle of carpal flexion or ultrasound settings such as gain. In this study, however, we chose to standardize these conditions for enhanced repeatability. An additional limitation is related to the study population. It is possible that cartilage measurement in horses with naturally occurring carpal osteoarthritis will be more difficult because of variable lesion location, decreased range-of-motion, or loss of anatomical landmarks (e.g., erosion of the subchondral bone plate).

5 | CONCLUSION

Cartilage thickness in the equine RCJ assessed ultrasonographically using a 15 MHz transducer corresponded well with thickness determined by MRI and histology (the NCC portion of the articular cartilage), and precision of repeated measurements was within acceptable limits. The more commonly available 9 MHz transducer

overestimated the thickness of thinner cartilage and underestimated the thickness of thicker cartilage and had poor repeatability (CV% 19.1–26.3) in the smaller cartilage thickness found in the prRCB, diRCB and ICB. To detect changes in RCJ cartilage thickness or monitor development over time, a 15 MHz transducer is recommended. Further studies are suggested to improve cartilage assessment with US in the MCJ.

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(b) Acquisition of Data: Andersen, Østergaard, Mori, J Griffin IV.

(c) Analysis and Interpretation of Data: Andersen, Jacobsen.

Category 2

(a) Drafting the Article: Andersen, Jacobsen.

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Category 3

(a) Final Approval of the Completed Article: Lindegaard, Jacobsen, Walters, Østergaard, Mori, Griffin IV, Andersen.

Category 4

(a) Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved: Lindegaard, Jacobsen, Walters, Østergaard, Mori, Griffin IV, Andersen.

CONFLICT OF INTEREST

The authors have declared no conflict of interest.

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