Received: 16 May 2019 | Revised: 21 October 2019 | Accepted: 8 November 2019

DOI: 10.1111/vop.12730

ORIGINAL ARTICLE

WILEY

Clinical utility, dose determination, and safety of ocular contrast-enhanced ultrasonography in horses: A pilot study

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Abstract

Objective: To determine efficacy of contrast-enhanced ultrasonography (CEUS) using different sulfur hexafluoride (SF6) doses to assess blood flow and perfusion in equine eyes and to evaluate safety of SF6 in horses.

Procedures: Ocular B-mode and contrast-enhanced ultrasonography were performed bilaterally in nine sedated university-owned horses. Intravenous SonoVue® bolus injections of 5, 10, 15, 20, 25, and 30 mL were administered for 2/18, 5/18, 6/18, 3/18, 1/18, and 1/18 eyes, respectively. Doses were increased based on ascending bodyweight. Each eye within one horse was examined utilizing a different dose. Qualitative blood flow and quantitative perfusion were analyzed. Heart and respiratory rates were monitored nonsedated, sedated, and during first and second minutes of CEUS.

Results: Qualitative contrast enhancement (CE) was visible in 7/9 animals. Quantitative CE was measurable bilaterally in four horses, unilaterally in three individuals, and not detected in two animals. In all horses with unilateral CE, the positive eye received the higher dose. Fifteen mL dose resulted in significantly shorter time to peak than 10 mL ($P < .05$). Peak intensity, maximum signal increase, and corresponding area under the curve were significantly higher for 15 and 20 mL doses compared with 10 mL ($P < .05$). Uveal and retinal tissues were enhanced frequently. Twenty-five and 30 mL doses revealed no CE. Only sedation reduced heart rates significantly ($P < .05$). Clinically relevant changes in respiratory rates or adverse reactions following SF6 application were not observed.

Conclusions: Contrast enhancement was in most instances dose-dependent. Fifteen mL appeared appropriate to assess equine ocular perfusion. The reliability in horses remains questionable; however, CEUS was well-tolerated.

KEYWORDS

contrast-enhanced ultrasonography, dose comparison, eye, horse, safety, sulfur hexafluoride

1 | INTRODUCTION

Ultrasonography is the most common imaging technique when ocular structures are clinically not visible.[1] Intraocular and orbital tissues can be hidden by anatomy or with opacification of transparent media. Horses' prey species behavior and the exposed globe position elevate their risk for vision-threatening injuries.[2] Globe rupture,[2] lens luxation,[1,2]

lens rupture,[1,3] retinal detachment,[1-4] and retrobulbar lesions[1,3] have been depicted by conventional ultrasonography. Despite being noninvasive, cost-effective, and widely available,[3] the B-mode echogenicity is often equivocal[5] which indicates extended modalities (Doppler and contrast imaging),[6] providing additional information on blood flow and perfusion.[7-9]

Contrast-enhanced ultrasonography (CEUS) proved its clinical value in eyes of humans,[10-12] dogs, and cats.[13] Fundamental research regarding CEUS blood flow assessment in sedated horses,[7] birds,[14] and anesthetized[8] and conscious[9] beagles was recently published. The tolerability of CEUS was unknown until Seiler et al[7] examined equine soft tissue structures of the distal limb by intravenous and regional intra-arterial perfluorobutane. In experimental ophthalmology, CEUS visualized normal vs impaired choroidal perfusion,[15] and correlated with histologic size and vascularity of uveal melanomas in rabbits[16] and mice.[17] The characterization of perfusional features of intraocular masses such as melanoma, hemangiosarcoma,[18] medulloepithelioma/retinoblastoma,[19] or lymphoma might be a future CEUS indication in horses. Especially in uveal hemangiosarcoma and lymphoma diagnosis from clinical appearance can be challenging. Anterior segment angiography in horses using indocyanine green (ICG) and sodium fluorescein (SF) failed to visualize the major iridal arterial circle, and SF enhancement was compromised by ocular pigment and dye extravasation.[20]

Second generation CEUS agents pass the pulmonary capillaries and remain exclusively intravascular (blood pooling) which are beneficial properties.[21] The diagnosis of equine recurrent uveitis (ERU) is made clinically but encompasses different manifestations.[22] Blood flow and microvasculature alterations occur inherently with different types and stages of canine uveitis.[23] It is unknown whether contrast-enhanced qualitative or quantitative assessment of uveal perfusion might facilitate ERU diagnosis and monitoring.[20]

Ultrasonography is frequently required after ocular trauma with subsequent periorbital cellulitis, corneal edema, hyphema, aqueous flare, miosis, and lens and vitreal opacifications.[1-3] In humans, dogs, and cats, CEUS has been superior to B-mode and Doppler ultrasonography to distinguish retinal detachment from vitreous membrane.[11,13]

To the authors' knowledge, the clinical utility of ocular CEUS and sulfur hexafluoride (SF6) application in horses have not been investigated. Thus, the primary objective of this pilot study was to determine whether different SF6 doses resulted in consistent qualitative and quantitative contrast enhancement of equine ocular structures. An additional goal was to evaluate the safety of SF6 in horses. We hypothesized that recommended dosages in dogs[24-26] and cats[27-29] apply to the horse and that SF6 would be safe based on previous studies in various species.[14,30-33]

2 | MATERIALS AND METHODS

2.1 | Animals studied

This descriptive, explorative, and prospective study enrolled ten university-owned horses. Animals' signalment and SF6 microbubble contrast agent, SonoVue® (Bracco), dosages can be found in Table 1. Before and during this trial, none of the horses were used for procedures that might have altered the evaluated variables.

Prior to inclusion, subjects underwent a complete physical examination and a nonsedated ophthalmic examination including menace response, pupillary light reflexes, dazzle reflex, slit-lamp biomicroscopy (Kowa SL-15®; Kowa), fluorescein staining (Fluorotouch Ophthalmic Strips®; Eickemeyer), tonometry (Tonopen Vet®; Eickemeyer), and direct ophthalmoscopy (Heine Beta200 LED Ophthalmoscope®; Heine) after pupillary dilation with tropicamide (Mydriatikum®; Agepha Pharma). Horses without ocular or respiratory abnormalities based on initial examinations were included, and a right jugular vein catheter (Intraflon 2 12G®; Vygon) was placed. Hematocrit and total protein values were determined. All procedures were conducted by a veterinary ophthalmology resident (KOB) under supervision of an ECVO diplomate (BN). This study was approved by the Ethics and Animal Welfare Committee and the National Authority (GZ: BMBWF-68.205/0086-V/3b/2018).

2.2 | B-mode and contrast-enhanced ultrasonography

Standing sedation was provided by a single detomidine hydrochloride dose (10 µg/kg IV, Equidor® 10 mg/mL; Richter Pharma). For eyelid akinesia, 2% mepivacaine hydrochloride (1 mL SC, Mepinaest purum 2%®; Gebro Pharma) was injected bilaterally over the auriculopalpebral branch of the facial nerve. The horse's head was supported in a steady position at withers height. Topical 0.4% oxybuprocaine hydrochloride (Novain®; Agepha Pharma) was applied as needed. Transcorneal ocular B-mode ultrasonography utilizing a 12-5 MHz linear array transducer (iU22 Philips®, Philips) was performed in axial plane with vertical and horizontal probe position to confirm the absence of ocular and orbital pathologies.

Perfusion of both eyes was evaluated by contrast harmonic imaging with the same linear probe (12-5 MHz) using SonoVue® (Lot-number: 17A062E). Horses were assigned to different doses, such that the right eye received different dose to the left eye. A crossover allocation of the lower and higher dose applied to the right and left eye, respectively, was chosen to avoid confounding effects on dose comparison (Table 1). These doses were arbitrarily chosen based on

TABLE 1 Signalment of the n = 9 horses undergoing ocular CEUS (n = 18 eyes), SonoVue® dosages (mL/kg) and allocation, and contrast enhancement of each eye

Horse	Breed	Age (y)	Gender	BWT (kg)	SonoVue OD (mL)	SonoVue OD (mL/kg)	CE OD	SonoVue OS (mL)	SonoVue OS (mL/kg)	CE OS
1	Haflinger	6	Male c.	524	5	0.010	Yes	10	0.019	Yes
2	Trotter	6	Male c.	530	10	0.019	Yes	5	0.009	No
3	Haflinger	19	Female	534	10	0.019	Yes	15	0,028	Yes
4	Trotter	18	Female	575	15	0.026	No	10	0.017	No
5	Haflinger	15	female	580	10	0.017	Yes	15	0.026	Yes
6	Trotter	18	male c.	584	15	0.026	Yes	20	0.034	yes
7	Warmblood	17	male c.	617	20	0.032	Yes	15	0.024	No
8	Warmblood	12	Male c.	623	15	0.024	No	20	0.032	Yes
9	Trotter	14	Female	693	25	0.036	No	30	0.043	No

Note: All dosages (mL/kg) were rounded to three decimal figures.

Abbreviations: BWT, bodyweight; c., castrated; CEUS, contrast-enhanced ultrasonography; CE, contrast enhancement; n, number of animals or eyes; OD, right eye; OS, left eye.

preliminary observations (K.-O. Blohm, unpublished data) and were increased with ascending bodyweight. The heaviest horse received 25 and 30 mL volumes. The right eye was always examined initially and the opposite eye at least five minutes afterward. Consequently, SonoVue® was applied twice in each horse.

Bolus injections of 5, 10, 15, 20, 25, and 30 mL were administered intravenously to evaluate 2/18, 5/18, 6/18, 3/18, 1/18, and 1/18 eyes, respectively, followed by a rapid 10 mL saline flush (NaCl 0.9%®; B. Braun). The required amount of SonoVue® was prepared, and a respective luer-lock syringe was filled immediately before each injection. This syringe was attached to the three-way stopcock port in straight direction of the catheter extension (B. Braun 30 cm luer-lock®; B. Braun). All globes were scanned transcorneally in axial plane with horizontal probe orientation and nasal marker position (Figures 1 and 2). A steady probe orientation was attempted for each entire sequence.

The recording timer was set to 120 seconds (853 frames) and started simultaneously with contrast injection. Video acquisition settings including mechanical index (0.11), depth (6 cm), focus (2.75 cm), and a 86% contrast gain value were fixed throughout the study. The same researcher (KOB) with comprehensive expertise in ocular CEUS performed all examinations supervised by a board-certified ophthalmologist (BN).

2.3 | Qualitative and quantitative analysis

All sequences were assessed using a specific CEUS software (QLAB Release 10.7®; Philips). Qualitative real-time enhancement pattern of ocular and adnexal structures was evaluated. Criteria were homogeneity and distribution of contrast medium.

Region sizes (mm^2) for ocular and adnexal structures with observed contrast enhancement were individually delineated avoiding inclusion of adjacent tissues (Figures 1 and 2). The following quantitative time-intensity curve (TIC) parameters were measured: contrast arrival time (slope time) and time to peak (TTP) in seconds (sec), peak intensity (PI) of the fitted TIC and maximum signal increase (MI) of the original TIC in decibel (db), and the area under the curve (AUC) as db x sec.

A 3-point semiquantitative TIC grading was applied for all obtained regions of interest (ROIs) and TIC quality was classified as poor (1 point): marked oscillations of signal intensities, good (2 points): mild to moderate oscillations of signal intensities, and excellent (3 points): minimal oscillations of signal intensities. Dose group indices were calculated by means of dividing the total score of corresponding ROIs by the number of horses which received the respective doses. Sequences were evaluated full length at least two times by the first author (KOB) individually and once in consensus with a second observer (BN).

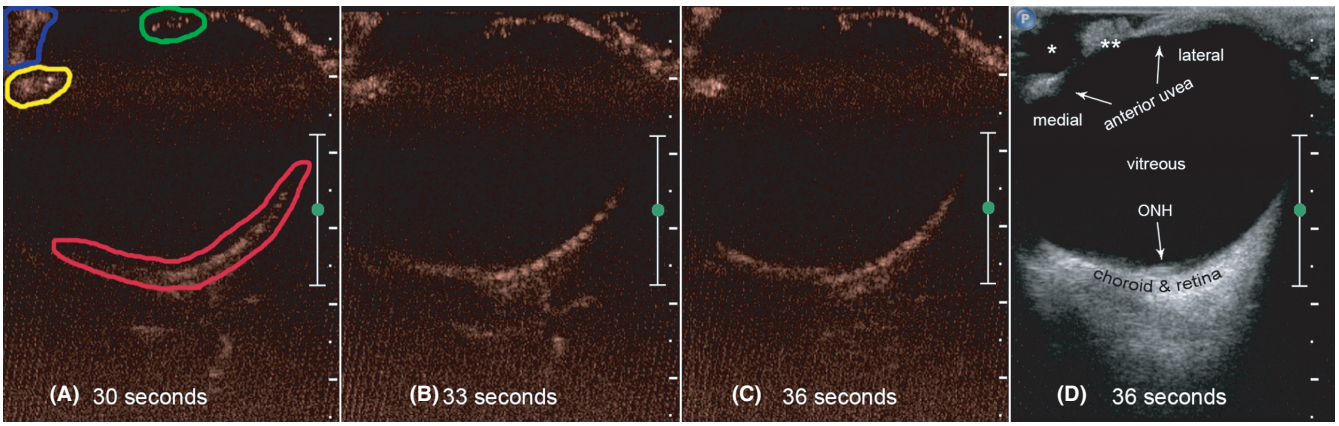

FIGURE 1 Ocular CEUS sequence (Horse 5) of the left eye (15 mL SonoVue®) at (A) 30, (B) 33, and (C) 36 s after bolus injection. (A) Regions of interest are delineated: medial (yellow) and lateral (green) anterior uvea, choroid and retina (red), and third eyelid (blue). (D) Reference gray scale image at 36 s. Asterisk indicates the anterior chamber, double asterisk indicates the anterior lens capsule and corpora nigra, and ONH shows the optic nerve head. Marker position ‚P' was medial. (A-D) Due to mild medial globe rotation, at illustrated time points, the lateral anterior uvea is slightly off-focus

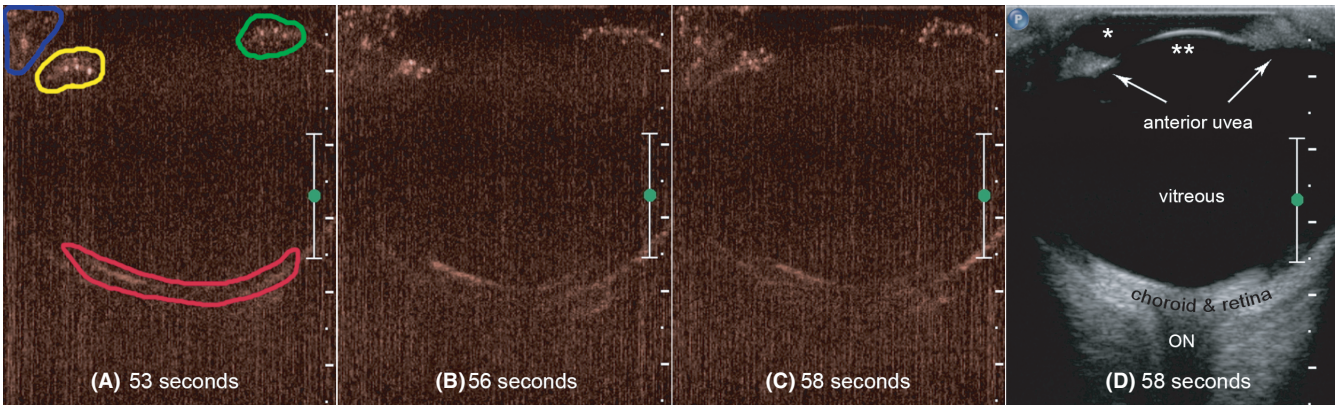

FIGURE 2 Ocular CEUS sequence (Horse 6) of the right eye (15 mL SonoVue®) at (A) 53, (B) 56, and (C) 58 s after bolus injection. (A) Regions of interest are delineated: medial (yellow) and lateral (green) anterior uvea, choroid and retina (red), and third eyelid (blue). (D) Reference gray scale image at 58 s. Asterisk indicates the anterior chamber, double asterisk indicates the anterior lens capsule, and ON shows the optic nerve. Marker position ‚P' was medial. (A-D) The globe orientation is straight which provides representative comparison of the medial and lateral anterior uvea

2.4 | Heart rate, respiratory rate, and post-procedure monitoring

Initial heart and respiratory rates were obtained before sedation (baseline). Time points for subsequent monitoring were ten minutes after standardized detomidine (10 µg/kg IV) application, and throughout the first and second minute after each SF6 bolus injection. All animals were hospitalized overnight until physical and ophthalmic examinations were repeated the following morning (KOB).

2.5 | Statistical analysis

Normality for metric parameters was evaluated with a Kolmogorov-Smirnov test. The eyes examined by 5, 10, 15, 20, 25, and 30 mL doses were grouped together. Data of descriptive statistics were expressed as mean ± standard deviation. In order to determine the effect of 10, 15, and 20 mL doses on region size, slope time, TTP, PI, MI, and AUC parameters, a mixed-effects model analysis was performed using Šidák's alpha-correction as post hoc procedure. To investigate systemic adverse effects related to SF6, heart and respiratory rates at the predefined time points were compared by mixed-effects models. The level of significance was set to 0.05 for all analyses. Statistical testing was done using IBM SPSS v24® (IBM Corp.).

3 | RESULTS

3.1 | Animals studied

Nine out of 10 horses were eligible for study inclusion. One horse was excluded because thorough ophthalmic

examination was not possible without sedation. The study population comprised five geldings and four mares belonging to three different breeds (four Trotters, three Haflingers, and two Warmbloods). The mean age was 13.9 ± 5.0 years (range 6-19 years) and bodyweights ranged from 524 to 693 kg (mean 584.4 ± 54.3 kg). Physical examinations revealed no abnormalities, except in Horse 1 which had a holosystolic heart murmur grade 3/6 with punctum maximum located in the right and left fourth intercostal space. Echocardiography verified a 3 x 3.5 cm proximal, perimembranous ventricular septum defect beneath the aortic valve, and a mild aortic valve regurgitation. Ophthalmic examinations, hematocrit (mean 37.3% ± 4.0%), total protein (mean 6.5 g/dL ± 0.5 g/dL), and ocular B-mode sonograms were within normal limits for all examined horses.

3.2 | Qualitative and quantitative contrast enhancement

Qualitative contrast enhancement after intravenous SF6 bolus injections was visible in 7/9 (78%) horses and 11/18 eyes (61%), respectively. Overall, the ROIs showing subjective contrast enhancement included: medial (ROI 1) and lateral anterior uvea (ROI 2), choroid and retina (ROI 3), third eyelid (ROI 4), and bulbar conjunctiva (ROI 5). The wash-in phase displayed distinct microbubble movements rapidly extending within the posterior ROI 3 and almost simultaneously traveling from the periphery of ROI 1 and 2 toward the pupillary margin (Figures 1 and 2). The chorioretinal region appeared homogenously hyperenhanced (Figure 1) though signal intensity faded quickly (Figure 2). Blood flow to the choroidal and retinal layer could not be differentiated. Both anterior uveal regions developed marked, slightly heterogenous enhancement and were nearly isoenhanced (Figure 2). The third eyelid and bulbar conjunctiva showed homogenous enhancement with rather hypoenhanced ROI 4 and brighter signals of ROI 5. In contrast, the anterior chamber and vitreous body remained nonenhanced for each entire sequence in sharp distinction to the adjacent ocular structures. The wash-out pattern was characterized by centrifugal microbubble movements, and contrast intensity during this phase was low but relatively long-lasting.

Bilateral and unilateral quantitative contrast enhancement was measured in 4/9 (45%) and 3/9 (33%) horses, respectively (Table 1). In Horses 4 and 9 (22%), no postcontrast intensity levels were detected. Time-intensity curve parameters for the different ROIs are summarized in Table 2. Quantitative enhancement of ROI 3 (four eyes), ROI 4 (four eyes), and ROIs 1 and 2 (two eyes each) has been perceived bilaterally in Horses 3, 5, and 6. Unilateral contrast measurements in Horses 2, 7, and 8 were consistently attributed to the higher dose.

The 5 mL dose only yielded contrast enhancement of ROIs 1 and 4 within the right eye in Horse 1 whereas the 25 and 30 mL doses revealed no contrast enhancement in Horse 9. Consequently the 10, 15, and 20 mL dose groups contributed to statistical comparison (Table 3). Mean region size with respect to all doses was 85.92 ± 61.09 mm^2 (range 18.00-218.10 mm^2). Region sizes of eyes examined by 10, 15, and 20 mL doses were not significantly different ($P > .05$). Mean slope time and TTP concerning all doses were 42.54 ± 12.97 seconds (range 19.70-79.69 sec) and 48.16 ± 12.75 seconds (range 26.33-86.59 seconds), respectively. Slope time and TTP following 15 mL administration were significantly shorter compared with 10 mL ($P < .05$) but not significantly different to the 20 mL dose ($P > .05$). Figure 3, showing TICs of the left eye in Horse 5 administered 15 mL of SonoVue®, reflects on the one hand relative consistency of TTP and on the other hand decibel differences between the ROIs.

Mean PI of fitted TICs and MI of original TICs as for all doses were 3.33 ± 1.38 db (range 0.93-6.99 db) and 4.56 ± 1.98 db (range 1.25-9.63 db), respectively. Peak intensity and MI utilizing 10 mL were significantly lower compared with 15 mL (PI: $P = .002$; MI: $P < .05$) and 20 mL (PI: $P = .001$; MI: $P < .05$) applications. Mean AUC regarding all doses was 63.49 ± 40.10 db x sec (range 12.42-161.93 db x sec). Differences in AUC for 10 mL compared with 15 mL ($P = .003$) and 20 mL ($P = .002$) were significant.

TABLE 2 Descriptive statistics of CEUS perfusion parameters (mean ± SD) for enhanced ROIs in n = 7 sedated horses (n = 11 eyes)

	Med. anterior uvea	Lat. anterior uvea	Choroid and retina	Third eyelid	Bulbar conjunctiva
Region size (mm^2)	45.92 ± 27.75	65.52 ± 26.87	139.23 ± 65.97	73.73 ± 52.41	33.65 ± 22.13
Slope time (sec)	41.68 ± 11.23	40.52 ± 12.34	47.51 ± 17.08	38.09 ± 10.50	40.76 ± 0.69
TTP (sec)	47.09 ± 9.67	47.37 ± 8.98	52.91 ± 17.50	43.91 ± 12.08	44.28 ± 0.30
PI (db)	3.08 ± 1.28	3.76 ± 1.43	3.51 ± 1.66	2.64 ± 1.18	4.20 ± 0.23
MI (db)	5.34 ± 2.89	5.66 ± 1.23	4.08 ± 1.92	3.54 ± 1.56	5.61 ± 0.23
AUC (db x sec)	56.22 ± 50.45	64.12 ± 40.85	65.61 ± 45.45	53.72 ± 34.65	96.52 ± 20.17
Total ROIs (n = 27)	5	5	9	6	2

Note: Abbreviations: CEUS, contrast-enhanced ultrasonography; SD, standard deviation; ROIs, regions of interest; n, number of animals or eyes or ROIs.

TABLE 3 Dose group comparison of CEUS perfusion parameters (mean ± SD) for 10, 15, and 20 mL SonoVue doses in n = 7 sedated horses (n = 10 eyes)

Dose (mL)	Region size (mm^2)	Slope time (s)	TTP (sec)	PI (db)	MI (db)	AUC (db x sec)
10	88.02 ± 45.25	52.54 ± 13.58[b]	57.13 ± 15.02[b]	2.22 ± 0.56[b, c]	3.10 ± 0.97[b, c]	30.69 ± 16.90[b, c]
15	87.03 ± 76.23	34.20 ± 11.14[a]	41.40 ± 10.84[a]	4.06 ± 0.71[a]	5.60 ± 1.73[a]	84.60 ± 27.83[a]
20	92.76 ± 65.54	43.43 ± 8.14	49.02 ± 7.91	4.35 ± 1.30[a]	5.41 ± 1.65[a]	91.23 ± 37.74[a]

Note: Superscript letters a, b, and c indicate significant difference (a) to 10 mL, (b) to 15 mL, and (c) to 20 mL at $P < .05$.

Abbreviations: CEUS, contrast-enhanced ultrasonography; SD, standard deviation; n, number of animals or eyes.

Time-intensity curve grading pertaining to all doses is shown in Table 4. Dose group indices yielded 5 mL: 2.5, 10 mL: 2.8, 15 mL: 4.2, and 20 mL: 5.3 demonstrating an increased TIC quality with the higher doses. Contrary to this, 25 and 30 mL doses in Horse 9 revealed no visible qualitative or measurable quantitative contrast enhancement.

3.3 | Safety assessment

Mean baseline heart rate was 38.2 ± 3.5 bpm. Ten minutes following sedation, and in the first and second minute of CEUS, heart rates were consistently lower evaluating the right (10 minutes sedated: 31.3 ± 5.2 bpm; +1. minute CEUS: 30.4 ± 4.2 bpm; +2. minutes CEUS: 31.1 ± 3.3 bpm) and left eye (10 minutes sedated: 30.4 ± 3.0 bpm; +1. minute CEUS: 32.2 ± 3.8 bpm; + 2 minutes CEUS: 32.2 ± 3.1 bpm) compared with baseline values ($P < .05$). Mean respiratory rate 10 minutes following sedation 11.3 ± 1.0 brpm was statistically higher than during the second CEUS minute of the left eye 10.4 ± 0.9 brpm ($P < .05$). Mean respiratory rates at all other time points did not vary significantly ($P > .05$). None of the horses had systemic or ocular adverse reactions to SF6 application within the monitoring period.

4 | DISCUSSION

This pilot study was designed to determine the applicability of CEUS using different doses of SonoVue® to assess ocular blood flow and perfusion in horses. Our current results indicate that the 15 mL dose may provide the highest diagnostic value whereas all doses were well-tolerated. To the authors' knowledge, this is the first trial evaluating the clinical utility and safety of different SF6 doses to examine equine eyes.

Despite being a straightforward technique in small animals the applicability of ocular CEUS in horses was uncertain. In anesthetized[8] and conscious[9] beagles using commercial decafluorobutane (Sonazoid®) and SF6 (SonoVue®), respectively, ocular CEUS resulted in 100% visible and measurable enhancement. Technically, it was feasible to perform ocular CEUS in sedated horses of our study population. However, qualitative and quantitative enhancement was only observed in 78% animals and 61% eyes, respectively. Although dosages comparable to our canine research (0.03 mL/kg)[9] were used in several horses, remarkable differences in bodyweight, blood volume, and the distance from injection site to the heart and eventually the eye must be taken into consideration. In the present study, contrast enhancement was in most instances significantly associated with the dose of intravenous SF6 with the exception of the 25 and 30 mL doses in Horse 9. The direct comparison of both eyes within the same horse showed that the higher dose yielded successful enhancement in all animals, which exhibited unilateral contrast detection only. A confounding accumulative effect in the second eye, after the elapse of at least five minutes between bolus injections, is unlikely according to our previous results of bilateral ocular CEUS in dogs[9] and the crossover allocation of the lower and higher dose in this study.

The implications of different SonoVue® doses regarding contrast enhancement were investigated. The association between quantitative ocular intensity parameter (PI, AUC) increase by 5 mL dose increments from 5 up to 20 mL holds true with the conjecture of a dose-related blood concentration of microbubbles. The TIC quality gradings also revealed a considerable improvement comparing 5 and 10 mL with 15 and 20 mL doses. The expense of the SF6 diagnostic agent SonoVue® is a limitation particularly in horses. Therefore, our aim was to find the most efficient volume among different SF6 doses. Seiler et. al[7] investigated CEUS using custom-made decafluorobutane to assess blood flow and perfusion in the equine distal limb. Their subjective degree of contrast enhancement was similar for lower (5 mL) and higher (10 mL) intravenous doses, even though the 10 mL resulted in slightly better scores. It is worth noting that 33% of horses showed no fetlock joint region enhancement after intravenous 5 and 10 mL contrast injections.[7] Compared to our study this percentage was higher than the proportion of animals without qualitative ocular contrast enhancement (22%) but slightly lower than the percentage of eyes without quantitative (39%) contrast enhancement. The linear probes utilized in the two studies had a comparable frequency range, and the tissues of interest were approximately in the same depth. Although a different contrast agent has been utilized

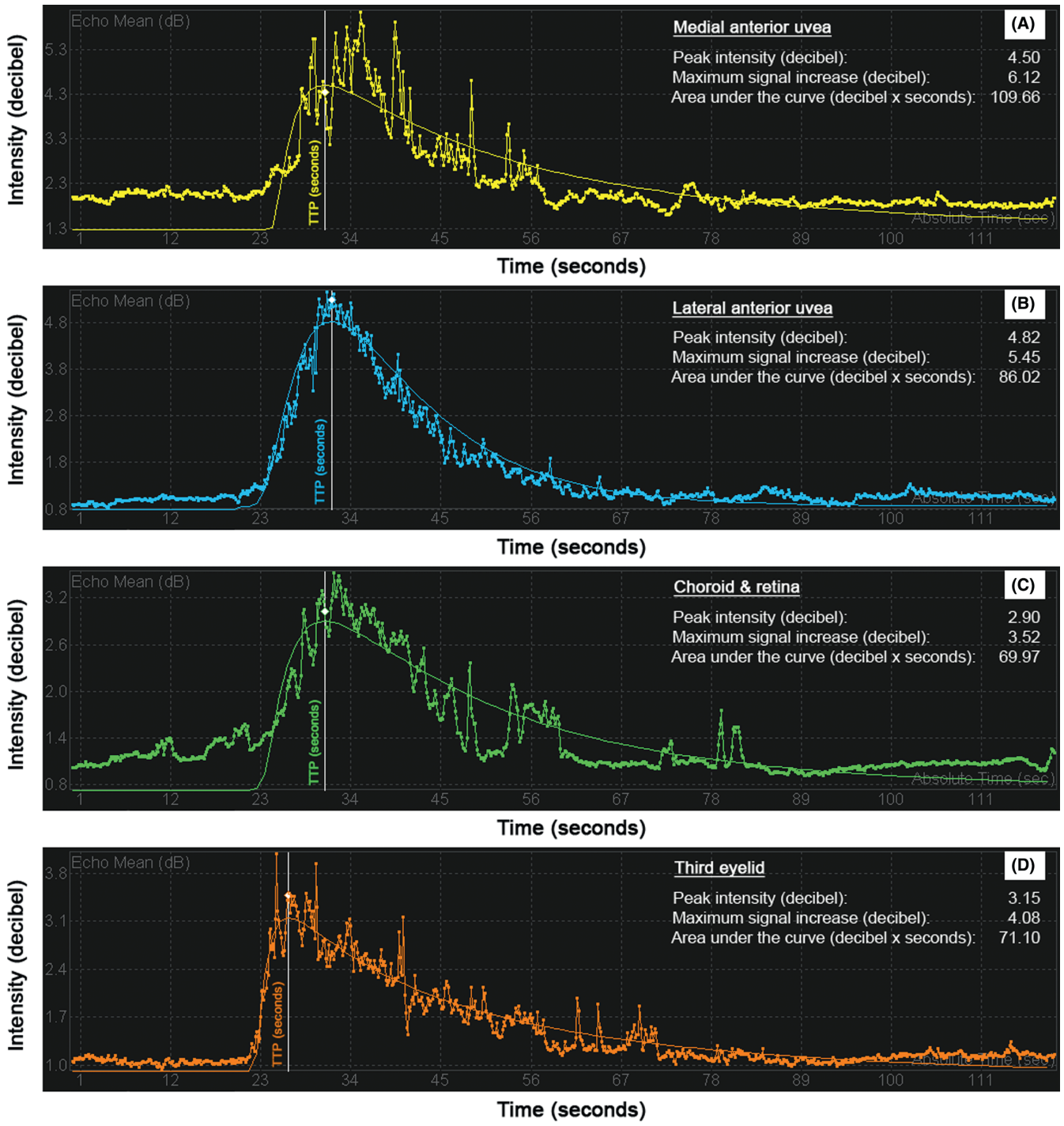

FIGURE 3 Time-intensity curves (Horse 5) of the left eye (15 mL SonoVue®) measured at (A) the medial and (B) lateral anterior uvea, (C) the choroid and retina, and (D) the third eyelid. Time to peak (white line) was almost constant whereas intensity parameters differed between ROIs with highest values at the anterior uveal regions

by Seiler et al,[7] preliminary results of a recent diagnostic accuracy comparison in human focal liver lesions indicated that commercial decafluorobutane is noninferior to SF6.[34] Since ocular structures are more delicate than parenchyma in the fetlock or palmar pastern region, this factor might have contributed to lack of contrast enhancement in equine eyes. Lack of contrast enhancement does not necessarily imply insufficient microbubble concentration in the ROI as failure due to the sonographer or equipment is possible. However, in case of successful contrast enhancement detection performing consecutive examinations with the same settings and technique this error becomes less likely.

Horse 9 in the current study received 25 and 30 mL doses and no qualitative or quantitative enhancement was

TABLE 4 Time-intensity curve quality scores for 5, 10, 15, and 20 mL SonoVue doses in n = 7 sedated horses (n = 11 eyes)

	5 mL	10 mL	15 mL	20 mL
Poor	-	3	-	1
Good	1	4	5	3
Excellent	1	1	5	3
Total ROIs (n = 27)	2	8	10	7

Note: Abbreviations: n, number of animals or eyes or ROIs; ROIs, regions of interest.

identified. This animal was examined last and was the only subject examined solely that day. On all other days, at least two horses were examined. Consequently, animals showing contrast enhancement served as a certain positive control for the setup. Even though the authors strictly followed the standardized examination protocol for Horse 9, a false negative enhancement due to unknown technical factors might explain this contradictory result. Taking cost-effectiveness into account, further studies will be necessary to evaluate the effect of 25 and 30 mL SonoVue® volumes in a larger equine population in order to define the diagnostic value. The CEUS examination of Horse 9 indicates that these higher doses are well-tolerated.

The perfusion parameter comparison of target structures is an important merit of CEUS. Quantitative TIC parameter analysis of this study yielded an overall mean slope time (43 seconds) and TTP (48 seconds) similar to intravenous ICG (38 seconds) and SF arrival times (47 seconds) for equine anterior segment angiography.[20] The 15 mL SF6 dose resulted in significantly faster enhancement (slope time, TTP) and significantly higher contrast intensities (PI, MI, AUC) than the 10 mL dose whereas the 20 mL dose revealed later wash-in and no significant increase of PI, MI, and AUC parameters compared with the 15 mL dose. Time-intensity curve parameters are related to the region sizes, but those were not significantly different which allowed accurate comparison between 10, 15, and 20 mL doses. Finally, the 15 mL SF6 dose was most appropriate for intravenous ocular CEUS in horses based on our data at this point. According to the bodyweights of individual horses that received 15 mL, this dose was equivalent to dosages from 0.024 to 0.028 mL/kg. Although the decibel values cannot be compared directly to previous canine studies,[8,9] contrast enhancement, as a measure of microbubble concentration, seemed to be less intense in equine eyes. Strikingly, diagnostic unilateral and bilateral ocular CEUS sequences were obtained in horses administered 0.019, 0.026, 0.028, and 0.034 mL/kg, whereas the eyes of other individuals showed no contrast enhancement following dosages of 0.024, 0.026, and even 0.036 and 0.043 mL/kg. It can be speculated that this was associated with an inter-individual cardiovascular response to the standardized alpha-2 agonist dosage. A markedly deep level of sedation featured by constant leaning against the stocks and buckling of the limbs[35] before the SF6 bolus injections and a comparatively lower head height above the ground[36] after head support removal was observed in Horse 4. Contrast enhancement was bilaterally negative for this animal.

This pilot study investigated if SF6 dosages utilized in other species do apply to the horse. The current findings caused us to scrutinize our hypothesis that SF6 dosages used for CEUS in feline (0.03 mL/kg)[29] and canine (0.03 mL/kg; 0.05 mL/kg)[9,37] eyes can be extrapolated to examine ocular perfusion in horses. In a clinical study,[13] 0.1 mL/kg was given to distinguish retinal detachment vs vitreous membrane in cats and dogs, but applying corresponding 50 mL of SonoVue® in a horse is barely affordable. Due to this dilemma our dosages were about 0.01 to 0.04 mL/kg which higher range still exceeded clinical doses of SF6 successfully utilized to diagnose choroidal melanomas in humans.[38]

The implementation of conventional delivery contrast imaging in large animals, whose blood volume is many times more than in other species, is challenging. Pirie et al[20] compared intravenous and intra-arterial ICG and SF for anterior segment angiography and recognized a significant effect of dilution after intravenous injection before ocular vascular is reached. Their study population comprised only three horses but no drawbacks other than mild hemorrhage and minimal additional time were reported with ultrasonographically guided temporary common carotid artery catheterization.[20] The results of previous research proved superior contrast enhancement after intra-arterial microbubble[7] or dye[20] administration which warrants further investigation of this route for ocular CEUS in horses. A rapid wash-in and a stronger contrast enhancement are expected leading to a higher diagnostic utility. For contrast-enhanced computed tomography of the equine head, low-dose intra-arterial and high volume intravenous administration resulted in comparable soft tissue enhancement.[39] If smaller intra-arterial SF6 doses would be efficient, CEUS may be more conceivable in equine ophthalmology.

Ocular CEUS applications focusing on different ROIs demonstrated diagnostic potential. Qualitative CEUS was superior to Doppler imaging detecting slow blood flow at the perfusion level.[8,11,13] Vitreal opacities are highly variable in appearance on conventional equine ocular ultrasonography and subjective assessment associated with clinical findings determined their significance.[5] The detection of perfusion by selective microbubble visualization could facilitate differentiation of atypical retinal detachment vs blood, fibrin strands, and vitreous membranes which is vital guiding prognosis and therapy after ocular trauma. In this pilot study, the uvea and the retina were the main tissues of interest. Nineteen out of 27 (70%) qualitatively and quantitatively enhanced ROIs belonged to the anterior uvea, choroid, and retina. This is a promising fact, considering CEUS as a potential tool to aid

diagnosis, monitoring, and potentially treatment (eg, targeted microbubbles) of ERU.

At present, the reliability of intravenous CEUS as a diagnostic technique to assess ocular perfusion in horses appears questionable. This can be attributed to several intricacies besides the mere difficulty of a sufficient SF6 dose. The equine pulmonary intravascular macrophages (PIM) are adherent to the capillary endothelium and can produce vasoactive substances.[40] A major advantage of poorly soluble microbubbles is that they remain within the blood pool, even so an interference with histiocytes in the vascular bed of the lungs or other organs, prior to reaching remote ocular perfusion, cannot be ruled out. Phagocytosis of particles and red blood cells, which are about the same size as SF6 microbubbles, by PIMs were described in other animals.[41,42] Moreover, a high affinity of hepatic Kupffer cells for decafluorobutane microbubbles has been reported in humans.[34]

SonoVue® demonstrated a favorable safety profile in various species.[9,14,30-33] The phospholipid shell lacks denatured albumin or other compounds of anaphylactoid potential. Nevertheless, horses are known for sensitive PIMs and severe cardiovascular shock responses. The monitored mild heart and respiratory rate decreases in our study population were interpreted as a direct effect of detomidine sedation[43]; however, owing to the absence of a control group it is difficult to determine whether these findings could be due to SonoVue® application. No systemic or ocular adverse reactions were clinically observed after intravenous SF6 administration and CEUS at a low mechanical index. The sensitivity to SF6 application into the common carotid artery remains uncertain although decafluorobutane injections in the lateral palmar digital artery were safe in horses.[7]

Despite providing necessary baseline data and several relevant findings, some inherent limitations to this pilot study must be addressed. The relatively small sample size, especially for the lowest and the two highest doses, caused that statistical tests included only the 10, 15, and 20 mL doses. This prevented conclusions for the other doses; hence, future studies based on our preliminary results providing increased statistical power are needed. All scans were performed by the same clinician (KOB) to minimize inter-examiner variation whereas also the sequence analysis was primarily carried out by this author who was not masked to the doses. Even though no second observer conducted independent measurements, all CEUS sequences were reviewed by two authors in order to increase accuracy. The safety evaluation monitoring was reduced to essential vital parameters without blood pressure measurements because in the previous study investigating CEUS in horses mild, transient increases of systolic blood pressures occurred but remained within normal physiologic ranges.[7]

In conclusion, our preliminary results indicated that qualitative enhancement and postcontrast intensity parameters were in almost all instances dose-dependent. The 15 mL SF6 dose was most appropriate to assess equine ocular perfusion. The reliability of intravenous ocular CEUS in horses and therefore its clinical utility is questionable owing to pronounced inter-individual variations and lack of reproducibility. Contrast-enhanced ultrasonography by SF6 was well-tolerated, and factors influencing CEUS' consistency and evaluation of equine eyes with vascular alterations merit further investigation.

ACKNOWLEDGMENTS

The authors thank Ass. Prof. Dr. K. M. Hittmair and Prof. Dr. E. Ludewig (Dipl. ECVDI) for subject-specific discussions, and organizational and technical support. We also appreciate the provided assistance of A. Herk (technician) and M. Stipić (cand. med. vet.) in handling the horses and equipment.

CONFLICTS OF INTERESTS

The authors did not receive any direct or indirect funding for this study and have no conflicts of interests to declare.

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How to cite this article: Blohm K-O, Tichy A, Nell B. Clinical utility, dose determination, and safety of ocular contrast-enhanced ultrasonography in horses: A pilot study. *Vet Ophthalmol*. 2020;23:331–340. https://doi.org/10.1111/vop.12730