Journal of Animal Science and Veterinary Medicine
Volume 11(5), pages 435-445, October 2026
Article Number: 3750FC758
ISSN: 2536-7099
https://doi.org/10.31248/JASVM2025.689
https://integrityresjournals.org/journal/JASVM
Full Length Research

Haematological effects of repeated femoral exposure to different durations of ultrasound in New Zealand White rabbits

Sulaiman A.[1]*, Bappah M. N.[2], Abdurrahman M.[2] and Lawal M.[2]

[1]Department of Animal Health, Federal College of Horticulture, Dadin Kowa, Gombe State, Nigeria.
[2]Department of Veterinary Surgery and Radiology, Ahmadu Bello University, Zaria, Kaduna State, Nigeria.

*Corresponding author. Email: ayubasulaiman83@gmail.com; Tel: +2347035021006.

Copyright © 2026 Sulaiman et al. This article remains permanently open access under the terms of the Creative Commons Attribution License 4.0, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Received Date: 13 September 2026 | Accepted Date: 26th October 2026 | Published Date: 30 October 2026

ABSTRACT: Diagnostic ultrasound examinations are becoming longer and more frequent, yet the effect of scanning duration on circulating blood is poorly defined. This exploratory study examined how the duration of uninterrupted diagnostic brightness-mode scanning over the femoral region affects blood cell parameters in New Zealand White rabbits. Twelve male rabbits were allocated to four groups of three: a control group handled for 30 minutes with the scanner switched off, and groups scanned for 10, 20 or 30 minutes daily for five consecutive days. Blood was collected at baseline and immediately after each session, and red cell, platelet and white cell indices were analysed by repeated-measures analysis of variance with comparisons against the control. Packed cell volume, haemoglobin concentration and red blood cell count were lower after exposure, most clearly in the 30-minute group (packed cell volume 30.61 ± 3.45% versus 35.89 ± 0.70% in controls). Group differences were not significant, but the day and group-by-day effects were, and the 30-minute group fell significantly below its baseline from day 3 to day 5, with a less consistent decline at 20 minutes and no sustained change at 10 minutes. Monocyte counts were higher after 30 minutes than in controls (0.42 versus 0.10 × 10^3/µl; p = 0.003). Platelet counts were lower in the 30-minute group than in controls (p = 0.030), but counts rose above baseline in every group and the difference reflected high control values. Lymphocyte and eosinophil differences occurred only at 20 minutes and were not graded. Other indices did not change meaningfully. Scanning for 30 minutes, beyond a routine examination, was therefore associated with progressive declines in red cell indices and higher monocyte counts. Restraint stress cannot be excluded as a contributor, and acoustic output was not measured, so larger controlled studies are needed.

Keywords: Exposure duration, diagnostic ultrasound, haematological parameters, bioeffects, New Zealand White rabbits.

INTRODUCTION

Ultrasound imaging uses high-frequency sound waves, above the range of human hearing, that are sent into the body and reflected by tissues to form an image. In brightness mode (B-mode), the most common form, the echoes are displayed as a real-time, two-dimensional grey-scale picture.

Diagnostic ultrasound is one of the most widely used imaging modalities in modern medicine (Sidhu *et al*., 2023; van Neer *et al*., 2024). It is valued for being non-invasive, relatively inexpensive, free of ionising radiation, and capable of producing real-time soft-tissue images, making it indispensable in obstetrics, cardiology, abdominal imaging, musculoskeletal assessment, and, increasingly, point-of-care bedside diagnosis (Han *et al*., 2024). Advances in transducer technology and the growing availability of portable and handheld devices have driven a marked expansion in how often, and for how long, patients are scanned (Martins *et al*., 2024). Although ultrasound is generally considered safe when used within accepted diagnostic exposure limits, it is not biologically

inert (Srivastava *et al*., 2026). Ultrasound waves interact with living tissue through two broadly recognised mechanisms: thermal effects, arising from the absorption of acoustic energy and consequent local tissue heating, and non-thermal (mechanical) effects, which include acoustic cavitation, radiation force, and streaming (Quarato *et al*., 2023; American Institute of Ultrasound in Medicine, 2023).

The bioeffects literature covers exposures that differ widely in intensity, mode, and purpose, which limits comparison between studies. High-intensity focused ultrasound (HIFU) and focused ultrasound for blood–brain barrier opening use focused, high-pressure exposures, with prolonged or repeated sonication in HIFU ablation, to disrupt tissue or vasculature deliberately (Bachu *et al*., 2021; McMahon *et al*., 2024). Haemolysis, platelet activation and microvascular haemorrhage have been reported mainly with gas bodies or contrast agents (Bader *et al*., 2025; Izadifar *et al*., 2017). Low-intensity pulsed ultrasound (LIPUS) is applied therapeutically, for example in bone and tissue repair (Mehrpour *et al*., 2016; Uddin *et al*., 2021). The present study differs from these: it used a diagnostic B-mode scan at 3.5 MHz over the femoral region, without focusing or a contrast agent, as used in clinical imaging, but applied here for longer than a routine examination.

Haematological data for low-intensity exposures are limited and differ in direction. Continuous ultrasound used to treat acute muscle injury was associated with higher segmented neutrophils and eosinophils and lower erythrocytes (Plentz *et al*., 2008), whereas low-intensity pulsed ultrasound after an incisional muscle lesion in rats lowered total leucocytes without affecting erythrocytes (Signori *et al*., 2011). Reductions in red blood cell count, haemoglobin, and platelets were reported by Mehrpour *et al*. (2016) and, after prolonged prenatal insonation, by Idris *et al*. (2020). In contrast, LIPUS delivered to the rat femoral metaphysis increased bone marrow nucleated cells and circulating white blood cells (Wang *et al*., 2021), and similar protocols improved blood count recovery in chemotherapy-induced myelosuppression models (Luo *et al*., 2021; Tan *et al*., 2021). These studies used therapeutic settings, injured or compromised tissue, or prenatal exposure, so they are not directly comparable with direct diagnostic exposure of an adult animal.

Haematological indices are sensitive, fast-responding indicators of physiological status and can reflect stress, inflammation and physical insult before overt clinical signs appear (Witeska *et al*., 2023; Didkowska *et al*., 2024). In the rabbit, they shift measurably with physiological state (Chmurska-Gąsowska *et al*., 2021). Despite the use of rabbits in ultrasound research, few studies, to the best of our knowledge, have compared graded durations of diagnostic-range scanning with haematological parameters in this model. As examinations become longer and more frequent, duration-specific data are needed to inform safety guidance.

This study aimed to evaluate how the duration of continuous (uninterrupted) diagnostic B-mode scanning (pulsed-echo imaging, Sonostar S10, 3.5 MHz) over the femoral region, applied for 10, 20, or 30 min, affects haematological parameters in New Zealand White rabbits compared with sham controls. This study hypothesised that, under the specified exposure settings, longer insonation would be associated with measurable differences in selected haematological indices compared with handling controls. Because acoustic output and tissue dose can differ substantially across ultrasound modes, comparisons with pulsed or high-intensity studies are interpreted cautiously. It was found that scanning for 30 minutes, beyond a routine examination, was therefore associated with progressive declines in red cell indices and higher monocyte counts.

MATERIALS AND METHODS

Ethics/animal housing

This experimental study was conducted in the Department of Veterinary Surgery and Radiology, Ahmadu Bello University, Zaria, Nigeria, in accordance with the rules established for experimental research in laboratory animals by the University. Ethical approval was provided by the Ahmadu Bello University Committee on Animal Use and Care (ABUCAUC) with approval number ABUCAUC/2025/079.

The rabbits were housed in the Animal House of the Department of Veterinary Public Health and Preventive Medicine, Ahmadu Bello University, Zaria, Nigeria. Before commencement of the experiment, the rabbits were allowed to acclimatise to the housing environment for two weeks under strict supervision. The rabbits were kept in deep litter enclosures with adequate space according to their groups and provided with a specially formulated feed and clean water ad libitum. Housing conditions were maintained in accordance with animal-care protocol. They were monitored throughout the experiment period for general behaviour, feed and water intake, and signs of discomfort or distress. Only those that showed no clinical signs of disease and had normal baseline haematology values were considered for this study.

Animal grouping

Twelve clinically healthy male New Zealand White rabbits with an average age of 18 months and body weight ranging from 3–4 kg were used in the study and randomly allocated into four experimental groups, with three rabbits per group (n = 3). Random allocation was performed using Microsoft Excel.

Group 1 was exposed to ultrasound for 10 minutes, Group 2 for 20 minutes, and Group 3 for 30 minutes. Group

4, serving as the control or control group, underwent identical handling for 30 minutes without any ultrasound exposure (device off). Throughout the experiment, the ultrasound settings remained constant across all experimental groups, ensuring methodological consistency. The sample size was selected partly to facilitate the scheduling of experimental procedures at a consistent time of the day for each rabbit across the experimental period to minimise potential time-related variations. Given the limited sample size, and handling time before blood sampling was not identical across groups, the study was considered exploratory, and the findings were interpreted cautiously because of the potential limitations in statistical precision and the ability to detect differences between groups.

Ultrasonographic procedure

To prevent any movement of animals during US exposure, both experimental and control groups were properly restrained using a customised V-shaped apparatus. The medial aspect of the right femoral zone of both experimental and control animals was shaved (from the anterior medial upper thigh/groin to the knee) 24 hours before commencement of the experiment. The animals were exposed to ultrasound using a portable ultrasound machine (Sonostar S10®) equipped with an R60 transcutaneous curvilinear probe operating at a frequency of 3.5 MHz; the machine was operated in B-mode across the skin overlying the medial aspect of the entire length of the right femur continuously for the entire duration assigned to each rabbit group. Ultrasound coupling gel was applied liberally at the time of each ultrasound session. The transducer probe was held with light continuous contact and mild constant movement to prevent overheating of tissue. The femur appears as a hyperechoic line with acoustic shadowing. Machine settings were normal (preset), which included a gain (G80), depth setting (D100), Frame correlation (FC4), Line density (LN2), and frame rate (FR12). The depth scale was 170mm. As is standard for diagnostic B-mode ultrasound, the system operates in pulsed mode with a low duty cycle (typically <1%). Exact acoustic output parameters were not available from the manufacturer at the time of submission. The procedures were done for five consecutive days at about the same time of day for each rabbit.

Blood sample collection

A primary 2 mL blood sample was collected from the ear vein for all subjects two (2) days before commencement of the experiment and served as a baseline haematology for the study. Blood samples (2 mL) were collected daily for five days (a total of 12 mL over the entire experiment) immediately after completion of the assigned ultrasound exposure duration for haematological analysis. All procedures were performed at about the same time of day. The sampling schedule comprised one baseline collection and five post-insonation collections, with the latter corresponding to the five daily exposure sessions. The collected blood was immediately transferred into an EDTA container to prevent clotting. The sample bottle was gently inverted after the lid was closed 8–10 times to mix the blood with the anticoagulant. The samples were well labelled and referred to the laboratory in a cool box. Red Blood Cells (RBC), White Blood Cells (WBC), platelets (PLT), haemoglobin (HGB), haematocrit (HCT), Mean Corpuscular Volume (MCV), and Mean Corpuscular Haemoglobin (MCH) were measured using an Auto-Haematology Analyser (Mindray BC-3600). The analyser was operated according to the manufacturer's instructions and was not independently calibrated for this study. For Reticulocyte counts, 1000-2000 RBCs were counted across consecutive fields on at least two new methylene blue-stained smears per rabbit and reported as ($\times 10^9$/L) according to Bain and Leach (2025), and differential leucocyte counts were measured manually on Giemsa-stained blood smears, counting 200 leucocytes per sample, as described by Oikonomidis *et al*. (2021). Counts were performed by one observer blinded to group allocation.

Data analysis

Data are presented as mean ± standard deviation (SD), representing the arithmetic mean and standard deviation of all observations within each exposure group. The experimental unit was the rabbit. We analysed haematological parameters (erythrocytic parameters: PCV, Hgb, RBC, MCV, Reticulocytes, PLT, MCH; and leucocytic parameters: heterophils, Lymphocytes, Basophils, Monocytes, Eosinophils) using two-way repeated measures ANOVA, with group as the between-subject factor and day as the within-subjects factor. The Group × Day interaction was examined. We performed post-hoc comparisons with the control group using Dunnett's test; day comparisons used day 0 (baseline) as the reference. A p-value ≤ 0.05 was considered statistically significant.

RESULTS

Erythrocytic and platelet parameters

Packed cell volume (PCV), haemoglobin and red blood cell (RBC) count were lower after exposure than before it, most clearly in the 30-min group (Table 1). The group effects were not significant (PCV $p = 0.124$; haemoglobin $p = 0.371$; RBC $p = 0.416$), but the day effects ($p = 0.001$, $p < 0.001$ and $p = 0.039$) and the group × day interactions (p

Table 1. Erythrocytic and platelet parameters of New Zealand white rabbits following exposure to different insonation durations.

Parameter	Group	Baseline values	Mean ± SD	Group p-value	Day p-value	Group × Day p-value
PCV (%)	Control	36.07 ± 0.82	35.89 ± 0.70[a]	0.124	0.001	0.007
	G1 (10 min)	35.97 ± 0.85	32.94 ± 6.25[a]			
	G2 (20 min)	34.83 ± 2.26	30.87 ± 3.87[a]			
	G3 (30 min)	36.23 ± 4.50	30.61 ± 3.45[b]			
Hgb (g/dL)	Control	10.87 ± 0.75	10.87 ± 0.60[a]	0.371	<0.001	0.010
	G1 (10 min)	11.63 ± 0.70	10.73 ± 1.13[a]			
	G2 (20 min)	10.73 ± 1.27	10.18 ± 1.77[a]			
	G3 (30 min)	11.87 ± 1.42	9.67 ± 1.12[a]			
RBC (10^{12}/L)	Control	5.37 ± 0.42	5.27 ± 0.29[a]	0.416	0.039	0.019
	G1 (10 min)	5.20 ± 0.68	4.83 ± 0.61[a]			
	G2 (20 min)	5.24 ± 0.50	4.80 ± 0.99[a]			
	G3 (30 min)	5.72 ± 0.70	4.63 ± 0.63[a]			
Reticulocytes (× 10^9/L)	Control	297.71 ± 2.69	298.12 ± 0.71[a]	0.04	0.952	0.490
	G1 (10 min)	297.04 ± 1.61	298.00 ± 0.60[a]			
	G2 (20 min)	298.88 ± 2.69	297.60 ± 0.90[b]			
	G3 (30 min)	298.10 ± 1.23	299.42 ± 0.82[b]			
MCV (*f*L)	Control	65.93 ± 1.18	68.50 ± 2.39[a]	0.051	0.119	> 0.30
	G1 (10 min)	65.17 ± 0.82	65.94 ± 1.13[a]			
	G2 (20 min)	66.57 ± 2.01	65.65 ± 2.86[a]			
	G3 (30 min)	65.37 ± 2.31	65.36 ± 2.08[a]			
MCH (pg)	Control	22.20 ± 0.85	22.16 ± 0.85[a]	0.441	0.155	> 0.30
	G1 (10 min)	21.60 ± 0.80	21.62 ± 3.53[a]			
	G2 (20 min)	22.23 ± 0.75	22.24 ± 0.74[a]			
	G3 (30 min)	20.77 ± 0.70	21.08 ± 0.76[a]			
Platelet (10^9/L)	Control	296.33 ± 41.79	516.39 ± 350.01[a]	0.024	0.043	0.370
	G1 (10 min)	322.33 ± 15.01	450.22 ± 207.90[a]			
	G2 (20 min)	192.67 ± 71.50	323.50 ± 186.68[a]			
	G3 (30 min)	244.33 ± 115.90	297.67 ± 33.58[b]			

Values with different superscript letters along the same column for the same parameter differ significantly at $p < 0.05$.

= 0.007, p = 0.010 and p = 0.019) were, indicating that the decline developed over the exposure days and differed between groups. In Dunnett comparisons, PCV in G3 was borderline relative to the control (30.61 ± 3.45% against 35.89 ± 0.70%; p = 0.050), whereas G1 (p = 0.273) and G2 (p = 0.062) were not. No exposed group differed significantly from the control for haemoglobin (G3 9.67 ± 1.12 against 10.87 ± 0.60 g/dL) or RBC (G3 4.63 ± 0.63 against 5.27 ± 0.29 × 10^{12}/L). Relative to their own baseline, G3 rabbits had significantly lower PCV on days 3 to 5 (−7.3, −7.4 and −11.2 percentage points; p = 0.012, 0.011 and 0.001), haemoglobin on days 3 to 5 (−2.6, −2.4 and −2.6 g/dL; p = 0.013, 0.017 and 0.013) and RBC count on days 3 to 5 (−1.3, −1.2 and −1.9 × 10^{12}/L; p = 0.009, 0.010 and 0.001) (Appendix 6). G2 showed a similar but less consistent decline: PCV was lower on days 2 to 5 (p = 0.032, 0.010, 0.001 and 0.009), haemoglobin on days 3 to 5 (p = 0.019, 0.005 and 0.043) and RBC count on day 4 only (−1.2 × 10^{12}/L; p = 0.042). G1 showed no sustained change; an isolated haemoglobin difference on day 2 (p = 0.049) did not persist. Mean corpuscular volume (MCV; group p = 0.051; G3 against control p = 0.055) and mean corpuscular haemoglobin (MCH; group p = 0.441) did not differ significantly. The control mean for MCV (68.50 ± 2.39 fL) was above its baseline (65.93 ± 1.18 fL), whereas MCV in G3 (65.36 ± 2.08 fL) was unchanged from its baseline (65.37 ± 2.31 fL). Reticulocyte counts were close to 298 × 10^9/L in all groups. The group effect was significant (p =

Table 2. Leucocytic parameters of New Zealand white rabbits following exposure to different insonation durations.

Parameters	Group	Baseline values	Mean ± SD	Group p-value	Day p-value	Group × Day p-value
Total white blood cell (10^3/µl)	Control (0 min)	5.60 ± 1.24	5.73 ± 1.05[a]	0.344	0.740	>0.30
	G1 (10 min)	6.77 ± 3.70	6.55 ± 2.44[a]			
	G2 (20 min)	8.53 ± 2.83	7.06 ± 1.71[a]			
	G3 (30 min)	5.60 ± 3.64	7.24 ± 2.60[a]			
Heterophils (10^3/µl)	Control (0 min)	2.86 ± 0.06	2.73 ± 0.50[a]	0.056	0.025*	0.26
	G1 (10 min)	4.83 ± 0.89	2.91 ± 0.84[a]			
	G2 (20 min)	2.37 ± 1.16	3.29 ± 1.05[a]			
	G3 (30 min)	3.75 ± 0.61	4.27 ± 1.12[a]			
Lymphocyte (10^3/µl)	Control (0 min)	2.54 ± 0.09	2.72 ± 0.51[a]	0.001	0.883	0.032
	G1 (10 min)	2.71 ± 0.70	3.21 ± 1.31[a]			
	G2 (20 min)	3.46 ± 0.85	3.36 ± 1.21[b]			
	G3 (30 min)	2.89 ± 0.51	2.35 ± 0.99[a]			
Basophils (10^3/µl)	Control (0 min)	0.09 ± 0.06	0.10 ± 0.04[a]	0.134	0.472	0.69
	G1 (10 min)	0.33 ± 0.36	0.07 ± 0.03[a]			
	G2 (20 min)	0.21 ± 0.05	0.04 ± 0.02[a]			
	G3 (30 min)	0.09 ± 0.04	0.06 ± 0.02[a]			
Eosinophils (10^3/µl)	Control (0 min)	0.06 ± 0.00	0.12 ± 0.03[a]	0.008	0.581	0.20
	G1 (10 min)	0.05 ± 0.05	0.12 ± 0.04[a]			
	G2 (20 min)	0.01 ± 0.00	0.01 ± 0.05[b]			
	G3 (30 min)	0.13 ±0.17	0.07 ± 0.02[a]			
Monocytes (10^3/µl)	Control (0 min)	0.09 ± 0.06	0.10 ± 0.06[a]	0.027	0.217	0.95
	G1 (10 min)	0.21 ± 0.05	0.24 ± 0.22[a]			
	G2 (20 min)	0.33 ± 0.36	0.30 ± 0.14[a]			
	G3 (30 min)	0.09 ± 0.04	0.42 ± 0.03[b]			

Values with different superscript alphabets along the same column for the same parameter differ significantly at $p < 0.05$.

0.04), with G2 (297.60 ± 0.90; p = 0.003) and G3 (299.42 ± 0.82; p = 0.033) differing from the control (298.12 ± 0.71 × 10^9/L), but these differences were about 0.5 and 1.3 × 10^9/L, less than 0.5% of the mean, and the day (p = 0.952) and group × day (p = 0.490) effects were not significant.

Platelet counts differed between groups (group p = 0.024; day p = 0.043; group × day p = 0.370). Only G3 differed from the control in Dunnett comparisons (297.67 ± 33.58 against 516.39 ± 350.01 × 10^9/L; p = 0.030; G1 and G2 p = 0.800 each). However, mean post-exposure counts were above baseline in every group, including the control (296.33 to 516.39 × 10^9/L), and the day comparisons showed higher counts than at baseline on day 1 (+195.2 × 10^9/L; p = 0.019) and day 2 (+258.9 × 10^9/L; p = 0.002), but not on days 3 to 5 (p = 0.189, 0.363 and 0.055; Appendix 5). The lower G3 mean relative to the control therefore reflects a high and variable control mean (SD 350.01 × 10^9/L), not a fall in G3 below its own baseline (244.33 ± 115.90 × 10^9/L).

Leucocytic parameters

Total white blood cell count did not differ between groups (group p = 0.344; day p = 0.740; group × day p > 0.30; Table 2). Heterophil counts were highest in G3 (4.27 ± 1.12 × 10^3/µl) and lowest in the control (2.73 ± 0.50 × 10^3/µl), with intermediate values in G1 (2.91 ± 0.84) and G2 (3.29 ± 1.05). The group effect was borderline (p = 0.056), and no Dunnett comparison was significant (G2 p = 0.077; G1 and G3 p > 0.05). The day effect was significant (p = 0.025) and came from day 5 alone (p = 0.015 against baseline; days 1 to 4 p = 0.196, 0.750, 0.663 and 0.291; Appendix 8). Monocyte counts differed between groups (p = 0.027), and G3 had higher counts than the control (0.42 ± 0.03 against 0.10 ± 0.06 × 10^3/µl; p = 0.003). This was a rise from the G3 baseline of 0.09 ± 0.04 × 10^3/µl; G2 (p = 0.086) and G1 (p > 0.05) did not differ from the control, and neither the day nor the interaction effect was significant.

Lymphocyte counts differed between groups (p = 0.001)

with a significant group × day interaction (p = 0.032; day p = 0.883). Only G2 differed from the control (3.36 ± 1.21 against 2.72 ± 0.51 × 10^3/µl; p < 0.001); G3 (2.35 ± 0.99) did not. Eosinophil counts also differed between groups (p = 0.008), with only G2 below the control (0.01 ± 0.05 against 0.12 ± 0.03 × 10^3/µl; p = 0.032); G1 (p = 0.246) and G3 (p = 0.189) did not. In both cases, G2 had numerically different baseline values from the control (lymphocytes 3.46 against 2.54; eosinophils 0.01 against 0.06 × 10^3/µl), although baseline differences were not statistically significant (Appendix 11).

DISCUSSION

This study asked whether uninterrupted diagnostic B-mode scanning over the femoral region for 10, 20 or 30 min on five consecutive days alters the haematological profile of New Zealand White rabbits. With three animals per group, it was designed as an exploratory comparison. The clearest changes were lower PCV, haemoglobin and RBC count and a higher monocyte count after 30 min of scanning, with smaller and less consistent changes after 20 min and little change after 10 min. Platelets and the 20-min lymphocyte and eosinophil differences are better explained by group differences at baseline and in the control group, as discussed below, than by exposure duration.

The decline in PCV, haemoglobin and RBC count was graded by exposure duration: sustained in G3 from day 3, partial and less consistent in G2, and absent in G1. Because the group effects were not significant and only the day and group × day effects were, the pattern is better described as a time-dependent divergence from baseline than as a settled group difference, and the borderline PCV result (p = 0.050) should be read in that light. The unchanged MCV and MCH indicate that the cells remaining were of normal size and haemoglobin content, which fits a loss of cells or a change in plasma volume more than a change in cell properties. Reticulocytes did not rise: the statistically significant group effect rested on differences below 0.5% of the mean, which are unlikely to be biologically meaningful, so no regenerative response was detected over five days. That window may be too short to show one.

Several explanations are possible, and this study cannot separate them. Mechanical effects of ultrasound on red cells, including cavitation, microstreaming and shear, can damage membranes and cause haemolysis, mostly in the presence of gas bodies or contrast agents (Izadifar *et al*., 2017; Bader *et al*., 2025). Cavitation is unlikely at diagnostic B-mode output without contrast, and acoustic output was not available for the scanner used. Tissue heating and bone absorption near the probe could contribute (Quarato *et al*., 2023), but temperature was not measured. Haemodilution, sequestration, and handling or restraint effects are further possibilities. Haemolysis markers, plasma volume, marrow, and temperature were not measured, so these remain hypotheses.

The lower G3 platelet mean relative to the control should not be read as thrombocytopenia or platelet consumption. Platelet counts were higher than baseline in every group, including the control, and the group difference arose from an elevated, highly variable control mean. Ultrasound can activate platelets and promote aggregation (Izadifar *et al*., 2017; Liu and Yang, 2021), but these data do not show a reduction attributable to exposure. They also illustrate how numerical baseline differences and small groups can produce a nominally significant comparison, and why platelet counts should be confirmed in a larger study. The rise across all groups may reflect repeated handling and venipuncture; sampling-related effects were not assessed.

Total leucocyte count was unchanged. The late rise in heterophils (day 5 only, with the group effect borderline) is compatible with a mild inflammatory or stress-related response, but it was not significant against the control and is not a robust finding. Monocytes were higher in G3, rising from a baseline equal to the controls, and this was the one leucocyte change that was significant against the control and not explained by baseline differences. A monocyte increase could reflect clearance of damaged cells or mildly injured tissue, or a stress-related redistribution of leucocytes (Witeska *et al*., 2023); no tissue or inflammatory markers were measured to distinguish these.

The lymphocyte and eosinophil differences in G2 were not graded, since G3 did not follow the same pattern. They should not be taken as evidence of an exposure effect. Basophils were low in all groups and did not change.

Restraint is a plausible alternative explanation. Handling and restraint can alter leucocyte distribution through neuroendocrine responses, including activation of the hypothalamic–pituitary–adrenal axis, and haematological indices respond to stress (Witeska *et al*., 2023). Restraint time differed among groups (10, 20, or 30 min for scanned groups; 30 min for the control), so scanning duration and restraint duration were partly confounded, and the changes cannot be attributed exclusively to ultrasound. The 30-min scanned group and the control were restrained for the same time, so the comparison between them is the least confounded. Cortisol and catecholamines were not measured.

Previous haematological findings after ultrasound are mixed. Reductions in RBC count, haemoglobin and platelets were reported by Mehrpour *et al*. (2016) and, after prolonged prenatal insonation, by Idris *et al*. (2020), while leucocyte responses have varied with the exposure and the tissue condition (Plentz *et al*., 2008; Signori *et al*., 2011). Low-intensity pulsed ultrasound over the femur has instead raised marrow and circulating white cell counts (Wang *et al*., 2021). Our red cell findings point the same way as the first group of studies, whereas our platelet and lymphocyte data do not support them. Direct comparison is limited, because those studies differed in species, exposure mode and intensity, injury status and exposure

site, and none used diagnostic B-mode scanning with graded durations in healthy adult rabbits. To our knowledge, comparable data remain scarce.

Groups of three rabbits give little statistical precision and no formal power calculation, and only males were used. Acoustic output, mechanical and thermal indices and tissue temperature were not available. Restraint time was not equal across groups. Baseline values differed numerically between groups for several variables, and baseline was not used as a covariate, so isolated significant results should be treated as hypothesis-generating. Haemolysis markers, marrow, histology, cavitation and stress hormones were not measured, and follow-up was short.

The findings do not show that clinical diagnostic scanning is hazardous. They suggest that prolonged, uninterrupted scanning beyond usual examination times may be associated with lower red cell indices in rabbits, which is worth testing in larger studies with equal restraint time across groups, measured acoustic output, baseline-adjusted analysis, plasma volume and haemolysis markers, and a stress measure.

Conclusion

In this small exploratory study, 30 min of uninterrupted diagnostic B-mode scanning over the femur on five consecutive days was associated with progressive declines in PCV, haemoglobin and RBC count and a higher monocyte count, whereas 10 min had little effect. Platelet, lymphocyte and eosinophil differences were mainly attributable to baseline and control-group differences, and restraint stress cannot be excluded as a contributor. Larger controlled studies with measured acoustic output are needed.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

ACKNOWLEDGEMENT

We acknowledge the assistance of Mannir Sani of the Department of Veterinary Pathology, Ahmadu Bello University, Zaria, Nigeria.

REFERENCES

American Institute of Ultrasound in Medicine. (2023). AIUM official statement for the statement on biological effects of therapeutic ultrasound. *Journal of Ultrasound in Medicine, 42*(12), E68–E73. https://doi.org/10.1002/jum.16315

Bachu, V. S., Kedda, J., Suk, I., Green, J. J., & Tyler, B. (2021). High-intensity focused ultrasound: A review of mechanisms and clinical applications. *Annals of Biomedical Engineering, 49*(9), 1975–1991. https://doi.org/10.1007/s10439-021-02833-9

Bader, K. B., Padilla, F., Haworth, K. J., Ellens, N., Dalecki, D., Miller, D. L., Wear, K. A., & Bioeffects Committee of the American Institute of Ultrasound in Medicine. (2025). Overview of therapeutic ultrasound applications and safety considerations: 2024 update. *Journal of Ultrasound in Medicine, 44*(3), 381-433. https://doi.org/10.1002/jum.16611

Bain, B. J., & Leach, M. (2025). Blood cells: A practical guide (7th ed.). Wiley. https://doi.org/10.1002/9781394332083

Chmurska-Gąsowska, M., Bojarski, B., & Szała, L. (2021). Haematological changes in rabbits (*Oryctolagus cuniculus* f. *domesticus*) in the course of pregnancy. *Animal Reproduction, 18*(2), e20210013. https://doi.org/10.1590/1984-3143-AR2021-0013

Didkowska, A., Klich, D., Anusz, K., Wojciechowska, M., Kloch, M., Perlińska-Teresiak, M., Bielecki, W., & Olech, W. (2024). Determination of hematological and biochemical values blood parameters for European bison (Bison bonasus). *PLOS ONE, 19*(5), e0303457. https://doi.org/10.1371/journal.pone.0303457

Han, B., Liu, Y., Zhou, Q., Yu, Y., Liu, X., Guo, Y., Zheng, X., Zhou, M., Yu, H., & Wang, W. (2024). The advance of ultrasound-enabled diagnostics and therapeutics. Journal of Controlled Release, 375, 1-19. https://doi.org/10.1016/j.jconrel.2024.08.039

Idris, S. Y., Lawal, M., Enam, J. S., Muhammed, M. S., Muhammad, Y., & Saleh, A. (2020). Alterations in haematological parameters of multiple in-utero insonated rabbits (*Oryctolagus cuniculus*). Sokoto Journal of Veterinary Sciences, 18(3), 144–149. https://doi.org/10.4314/sokjvs.v18i3.4

Izadifar, Z., Babyn, P., & Chapman, D. (2017). Mechanical and biological effects of ultrasound: A review of present knowledge. *Ultrasound in Medicine & Biology, 43*(6), 1085–1104. https://doi.org/10.1016/j.ultrasmedbio.2017.01.023

Liu, T., & Yang, F. (2021). Study on the intervention of platelet biological activity by ultrasound. Sheng Wu Yi Xue Gong Cheng Xue Za Zhi, 38(4), 709–715. https://doi.org/10.7507/1001-5515.202005036

Luo, D., Chen, W., Wang, W., Chen, J., Xu, H., Chen, J., & Wang, Y. (2021). Low-intensity pulsed ultrasound alleviating myelosuppression of Sprague-Dawley rats after combined treating by paclitaxel and carboplatin. Translational Cancer Research, 10(3), 1181–1190. https://doi.org/10.21037/tcr-20-3035

Martins, J. G., Waller, J., Horgan, R., Kawakita, T., Kanaan, C., Abuhamad, A., & Saade, G. (2024). Point-of-care ultrasound in critical care obstetrics: A scoping review of the current evidence. Journal of Ultrasound in Medicine, 43(5), 951-965. https://doi.org/10.1002/jum.16425

McMahon, D., Jones, R. M., Ramdoyal, R., Zhuang, J. Y. X., Leavitt, D., & Hynynen, K. (2024). Investigation of sonication parameters for large-volume focused ultrasound-mediated blood–brain barrier permeability enhancement using a clinical-prototype hemispherical phased array. Pharmaceutics, 16(10), 1289. https://doi.org/10.3390/pharmaceutics16101289

Mehrpour, M., Shakeri-Zadeh, A., Basir, P., Jamei, B., Ghaheri, H., & Shiran, M. B. (2016). Effects of low-intensity continuous ultrasound on hematological parameters of rats. *Journal of Biomedical Physics and Engineering, 6*(3), 195-200. https://pubmed.ncbi.nlm.nih.gov/27853727/

Oikonomidis, I. L., Milne, E., & Piccinelli, C. (2021). Differential

white blood cell counts in rabbits: A comparison of the Advia 2120 and a manual method. *Journal of Veterinary Diagnostic Investigation, 33*(4), 670–676. https://doi.org/10.1177/10406387211007877

Plentz, R., Stoffel, P., Kolling, G., Costa, S., Beck, C., & Signori, L. (2008). Hematological changes produced by 1 MHz continuous ultrasound, applied during the acute phase of iatrogenic muscle injury in rats. *Brazilian Journal of Physical Therapy, 12*(6), 495–501.

Quarato, C. M. I., Lacedonia, D., Salvemini, M., Tuccari, G., Mastrodonato, G., Villani, R., Fiore, L. A., Scioscia, G., Mirijello, A., Saponara, A., & Sperandeo, M. (2023). A review on biological effects of ultrasounds: Key messages for clinicians. *Diagnostics, 13*(5), 855. https://doi.org/10.3390/diagnostics13050855

Sidhu, P. S., Ewertsen, C., Piskunowicz, M., Secil, M., Ricci, P., Fischer, T., Gaitini, D., Mitkov, V., Lim, A. K. P., Lu, Q., Chong, W. K., & Clevert, D. A. (2023). Diversity of current ultrasound practice within and outside radiology departments with a vision for 20 years into the future: A position paper of the ESR ultrasound subcommittee. *Insights into Imaging, 14,* Article 202. https://doi.org/10.1186/s13244-023-01548-w

Signori, L. U., da Costa, S. T., Saurin Neto, A. F., Pizzolotto, R. M., Beck, C., Sbruzzi, G., da Silva, A. M. V., & Plentz, R. D. M. (2011). Haematological effect of pulsed ultrasound in acute muscular inflammation in rats. *Physiotherapy, 97*(2), 163-169. https://doi.org/10.1016/j.physio.2010.06.004

Srivastava, S., Yu, B., Giraud, D., Dighe, M., Dhyani, M., & Barr, R. G. (2026). Overview of diagnostic ultrasound safety: Review for research and institutional review boards. Journal of Ultrasound in Medicine, 45(3), 487–496. https://doi.org/10.1002/jum.70085

Tan, Y., Guo, Y., Reed-Maldonado, A. B., Li, Z., Lin, G., Xia, S.-J., & Lue, T. F. (2021). Low-intensity pulsed ultrasound stimulates proliferation of stem/progenitor cells: What we need to know to translate basic science research into clinical applications. *Asian Journal of Andrology, 23*(6), 602–610. https://doi.org/10.4103/aja.aja_25_21

Uddin, S. M. Z., Komatsu, D. E., Motyka, T., & Petterson, S. (2021). Low-intensity continuous ultrasound therapies: A systematic review of current state-of-the-art and future perspectives. *Journal of Clinical Medicine, 10*(12), 2698. https://doi.org/10.3390/jcm10122698

van Neer, P. L. M. J., Peters, L. C. J. M., Verbeek, R. G. F. A., Peeters, B., de Haas, G., Hörchens, L., Fillinger, L., Schrama, T., Merks-Swolfs, E. J. W., Gijsbertse, K., Saris, A. E. C. M., Mozaffarzadeh, M., Menssen, J. M., de Korte, C. L., van der Steen, J.-L. P. J., Volker, A. W. F., & Gelinck, G. H. (2024). Flexible large-area ultrasound arrays for medical applications made using embossed polymer structures. Nature Communications, 15, Article 2802. https://doi.org/10.1038/s41467-024-47074-1

Wang, W., Chen, J., Luo, D., Chen, J., Xu, H., Chen, W., & Wang, Y. (2021). Effects of low-intensity pulsed ultrasound on myelosuppression of rats induced by chemotherapy drugs with cell cycle specificity. Journal of Ultrasound in Medicine, 40(9), 1811–1822. https://doi.org/10.1002/jum.15562

Witeska, M., Kondera, E., & Bojarski, B. (2023). Hematological and hematopoietic analysis in fish toxicology—A review. Animals, 13(16), 2625. https://doi.org/10.3390/ani13162625

Appendix 1. Table showing the Dunnett post hoc test for erythrocytic parameters.

Dependent Variable	(I) Exposure Duration	(J) Control group	Sig.
	30	0	0.033*
Reticulocytes	20	0	0.003*
	10	0	0.668
	30	0	0.145
WBC	20	0	.200
	10	0	0.431
	30	0	.297
RBC	20	0	.163
	10	0	.336
	30	0	.168
HGB	20	0	.433
	10	0	.878
	30	0	.050*
HCT	20	0	.062
	10	0	0.273
	30	0	.055
MCV	20	0	.055
	10	0	.103
	30	0	.248
MCH	20	0	.924
	10	0	.565
	30	0	.0300*
PLT	20	0	.800
	10	0	.800

Appendix 2. Dunnett post-hoc (other days vs day 0) for PCV (%)

Comparison	Coefficient	P-value
D1 vs 0	-2.25	0.042*
D2 vs 0	-3.32	0.003*
D3 vs 0	-4.13	<0.001**
D4 vs 0	-4.88	<0.001**
D5 vs 0	-4.62	<0.001**

Appendix 3. Dunnett post-hoc (other days vs day 0) for Hgb (g/dL)

Comparison	Coefficient	P-value
D1 vs 0	-0.55	0.073
D2 vs 0	-1.18	<0.001**
D3 vs 0	-1.33	<0.001**
D4 vs 0	-1.36	<0.001**
D5 vs 0	-1.06	<0.001**

Appendix 4. Dunnett post-hoc (other days vs day 0) for RBC ($\times 10^{12}$/L)

Comparison	Coefficient	P-value
D1 vs 0	-0.29	0.106
D2 vs 0	-0.56	0.002*
D3 vs 0	-0.70	<0.001**
D4 vs 0	-0.75	<0.001**
D5 vs 0	-0.72	<0.001**

Appendix 5. Dunnett post-hoc (other days vs day 0) for PLT ($\times 10^9$/L)

Comparison	Coefficient	P-value
D1 vs 0	+195.2	0.019*
D2 vs 0	+258.9	0.002*
D3 vs 0	+109.1	0.189
D4 vs 0	+75.6	0.363
D5 vs 0	+159.4	0.055

Appendix 6. Table showing changes in erythrocytic parameters (Dunnett post hoc test) from day 0 (Baseline) (Group*Day)

Group	Parameter	D1	D2	D3	D4	D5
	PCV	-1.8	-4.5	-7.3 (0.012) *	-7.4 (0.011) *	-11.2 (0.001) *
Group 3 (30 mins)	Hgb	-0.6	-2.0 (0.054)	-2.6 (0.013) *	-2.4 (0.017) *	-2.6 (0.013) *
	RBC	-0.3	-0.9 (0.066)	-1.3 (0.009) *	-1.2 (0.010) *	-1.9 (0.001) *
	PCV	-3.8	-5.5 (0.032) *	-6.7 (0.010) *	-9.4 (0.001) *	-6.7 (0.009) *
Group 2 (20 mins)	Hgb	-0.2	-1.1	-1.6 (0.019) *	-2.0 (0.005) *	-1.4 (0.043) *
	RBC	-0.1	-0.6	-0.9	-1.2 (0.042) *	-0.6
	PCV	-3.3	-3.2	-2.3	-2.4	-0.2
Group 1 (10 mins)	Hgb	-1.4 (0.083)	-1.6 (0.049)	-1.2	-1.0	-0.3
	RBC	-0.7	-0.7	-0.5	-0.5	-0.2

Appendix 7. Table showing Dunnett post hoc test for leucocytic parameters.

Parameters	(I) Group	(J) Group	Sig.
	3	0	>0.05
Heterophils	2	0	0.077
	1	0	>0.05
	3	0	0.003*
Monocyte	2	0	0.086
	1	0	>0.05
	3	0	0.544
Basophil	2	0	0.813
	1	0	0.07
	3	0	0.189
Eosinophil	2	0	0.032*
	1	0	0.246
	3	0	>0.05
Lymphocyte	2	0	<0.001*
	1	0	>0.05

Appendix 8. Table showing day interaction for Heterophils

Parameter	Comparison	p-value
	D1 vs 0	0.196
	D2 vs 0	0.750
Heterophils	D3 vs 0	0.663
	D4 vs 0	0.291
	D5 vs 0	0.015*

Appendix 9. Summary of group effects and Dunnett-adjusted comparisons with the control (n = 3 per group). Values are p-values; n.s., not significant. The final column gives the direction of the 30-min group relative to its own baseline.

Variable	Group effect	G1 (10 min)	G2 (20 min)	G3 (30 min)	G3 against its own baseline
PCV	0.124	0.273	0.062	0.050	Lower (days 3–5)
Haemoglobin	0.371	n.s.	n.s.	n.s.	Lower (days 3–5)
RBC count	0.416	n.s.	n.s.	n.s.	Lower (days 3–5)
MCV	0.051	n.s.	n.s.	0.055	Unchanged
MCH	0.441	n.s.	n.s.	n.s.	Little change
Reticulocytes	0.04	0.668	0.003	0.033	Negligible change
Platelets	0.024	0.800	0.800	0.030	Higher
Total WBC	0.344	n.s.	n.s.	n.s.	Higher, n.s.
Heterophils	0.056	> 0.05	0.077	> 0.05	Higher (day 5)
Lymphocytes	0.001	> 0.05	< 0.001	> 0.05	Lower, n.s.
Basophils	0.134	0.07	0.813	0.544	Lower, n.s.
Eosinophils	0.008	0.246	0.032	0.189	Higher, n.s.
Monocytes	0.027	> 0.05	0.086	0.003	Higher

Appendix 10. Table showing sampling timeline

Day	Sample collection
Day 0	Baseline blood collection (2ml)
Day 1	Ultrasound session for each rabbit according to the duration assigned to its group, followed immediately by blood collection (2ml)
Day 2	Ultrasound session for each rabbit according to the duration assigned to its group, followed immediately by blood collection (2ml)
Day 3	Ultrasound session for each rabbit according to the duration assigned to its group, followed immediately by blood collection (2ml)
Day 4	Ultrasound session for each rabbit according to the duration assigned to its group, followed immediately by blood collection (2ml)
Day 5	Ultrasound session for each rabbit according to the duration assigned to its group, followed immediately by blood collection (2ml)

Appendix 11. Table showing comparison of baseline values across groups

Parameter	p-value	Result
PCV	0.906	No sig. difference
Hgb	0.530	No sig. difference
RBC	0.701	No sig. difference
MCV	0.190	No sig. difference
MCH	0.211	No sig. difference
PLT	0.202	No sig. difference
WBC	0.615	No sig. difference
Lymphocytes	0.736	No sig. difference
Monocytes	0.316	No sig. difference
Basophils	0.453	No sig. difference
Eosinophils	0.160	No sig. difference
Heterophils	0.518	No sig. difference