International Journal of Multiphysics
Volume 18, No. 4, 2024
ISSN: 1750-9548

Ultrasonographic study of uterus and fallopian tubes in domestic short-haired cats after administration of Levovist® contrast agent

Farzad Yazdi[1], Farrokhreza Kabir[1*], Ahmad Asghari[1], Ghasem Akbari[1]

[1]Department of Clinical Sciences, Faculty of Veterinary Medicine, Science and Research Branch, Islamic Azad University, Tehran, Iran.

Abstract

This study investigated the effects of the Levovist® contrast agent on ultrasonography in domestic short-haired (DSH) cats, focusing on the uterus and fallopian tubes. The main question was whether Levovist® could enhance visualization of these small structures. Twelve female DSH cats underwent a complete blood count (CBC) before and after receiving 5 microliters of Levovist® via a Foley catheter. Ultrasound examinations were performed using a linear probe, and statistical analysis was conducted using ANOVA ($p<0.05$). Results showed that prior to Levovist injection, differentiation between the endometrium and myometrium was not possible. Post-injection, the average thickness was 0.95 ± 0.1 mm for the endometrium and 0.81 ± 0.12 mm for the myometrium, indicating significant improvement in imaging. However, effective evaluation of the fallopian tubes was not achieved. In conclusion, Levovist significantly enhanced ultrasound visualization of uterine structures in DSH cats, highlighting its potential in veterinary reproductive diagnostics and improving imaging techniques for small anatomical structures.
Keywords: Ultrasonography, Levovist® contrast agent, Cats, Uterus

1. INTRODUCTION

Ultrasound, also known as echography or sonography, is a highly valued diagnostic tool in the medical and veterinary medicine field.[1] One of the most essential uses of ultrasound in veterinary medicine is diagnosing pregnancy and fetal age in animals.[2] Therefore, veterinarians must have sufficient knowledge and experience regarding the anatomy of the reproductive system in small animals and how to perform an ultrasound of the reproductive organs.[3] This non-invasive imaging technique is widely used in dogs and cats to examine subcutaneous tissues, including muscles, joints, tendons, and internal organs, to detect any lesions or abnormalities.[4]

Contrast material, also known as contrast agent, allows the radiologist to examine the internal structure in greater detail and clarity.[5] Contrast-enhanced imaging provides better scans of organs, ligaments, tendons, blood vessels, bones, or nerves.[6,7] Contrast material is often used during diagnostic imaging to provide more detailed information about a patient's condition.[6,7] Iodinated contrast agents, barium sulfate, and gadolinium are commonly used in human and animal radiography, computed tomography, and magnetic resonance imaging scans.[5] Although some side effects may be associated with using contrast agents, the benefits usually outweigh the risks.[8,9] Levovist® contrast material, increases the appearance of tissues not well seen in ultrasound.

Several studies have been published on contrast-enhanced ultrasound in diagnosing and investigating the characteristics of focal lesions of the pancreas, liver, lymph nodes, and subepithelial tumors, and investigating the cardiovascular system, as well as the principles of image texture description.[10-12] Also, ease of use of this contrast material, short half-life, high quality of the resulting image, and lack of tissue irritation are other positive points of this material compared to other contrast materials.[13-15] Ultrasound is used to examine the reproductive system in female cats. However, assessing domestic short-haired (DSH) cats is challenging due to their small uterine size and narrow uterine horns. This study investigates the use of Levovist® contrast material to improve ultrasonography of the uterus and fallopian tubes in DSH cats. By using this contrast agent, we aim to enhance visualization and improve diagnostic accuracy.

2. MATERIALS AND METHODS

2.1. Levovist® Contrast Agent

2.1.1. Composition and Properties

Levovist® (galactose/palmitic acid) is a contrast agent used in medical imaging to enhance ultrasound echoes. It consists of 99.9% galactose and 0.1% palmitic acid. When mixed with water, it forms a milky suspension of micro-bubbles with a median diameter of 2 µm, with approximately 95% of these micro-bubbles smaller than 8 µm.

2.1.2. Preparation Method

The Levovist® product was procured from Schering AG in Germany as white granules or powder and was mixed with sterile water to create a white suspension. Once injected intravenously, the micro-bubbles can pass through lung capillaries into systemic circulation, acting as effective back-scatters for ultrasound and resulting in a stronger returning signal.

2.2. Animal Care and Ethical Considerations

2.2.1. Ethical Approval

Animal care and experimental procedures adhered to the guidelines set by the Medical Sciences Ethics Committee of Islamic Azad University, Tehran, Iran (IR.IAU.SRB.REC.1401.432).

2.2.2. Animal Housing Conditions

Twelve female domestic short-haired (DSH) cats of varying ages and weights were referred to the Central Veterinary Hospital and Sharq Veterinary Hospital in Tehran, Iran. Each cat was housed in an animal cage with unrestricted access to food and water for one week.

2.3. Study Design

2.3.1. Patient Selection

The cats were treated with the antiparasitic Zipyran Plus, and their health was verified by a veterinary specialist prior to the study.

2.3.2. Treatment Protocol

Five microliters of Levovist® contrast material were injected into the uterus of each DSH cat via a Foley catheter. Investigations were conducted in two stages: before and after the injection.

2.4. Blood Sampling Procedures

2.4.1. Blood Collection Method

Blood samples were collected from DSH cats through the cephalic vein under suitable conditions.

2.4.2. Parameters Measured

Complete blood counts were performed before and after administering Levovist®. The following parameters were measured: white blood cells, lymphocytes, monocytes, neutrophils, eosinophils, basophils, hematocrit, red blood cells, hemoglobin, average volume of red blood cells, globule hemoglobin, average hemoglobin concentration of red blood cells, nucleated red blood cells, and platelet count using the Exigo Veterinary Tuberculosis Device (Sweden).

2.5. Imaging Procedures

2.5.1. Radiography Protocol

Radiography of the abdominal region was performed on DSH cats using a Konica Minolta radiography device in both ventrodorsal and lateral views before the injection of Levovist®.

2.5.2. Ultrasound Protocol

After Levovist® injection, ultrasound examinations were conducted using a SonoScape E2V model device. The cats were placed supine on an examination table, and ultrasound gel was applied to the skin for optimal imaging absorption for 2-3 minutes. A linear probe (8-12 MHz) and a GE 10C-SC curved array probe (6-8.7 MHz) were used to examine the uterus between the bladder and descending colon.

2.6. Statistical Analysis

Data are presented as mean ± SD, with graphs plotted using GraphPad Prism version 8. Statistical analysis was performed using analysis of variance (ANOVA) followed by Tukey's post hoc test; a p-value less than 0.05 was considered statistically significant.

3. RESULTS

3.1. Complete Blood Count Before and After Injection of Levovist® in DSH cat

The results of the complete blood count (CBC) tests conducted before and after administering Levovist® contrast material are summarized in **Tables 1** and **2** and a comparison is done in **Chart 1**. Analysis revealed that all measured parameters—such as white blood cells, lymphocytes, monocytes, neutrophils, eosinophils, hematocrit, red blood cells, hemoglobin, average red blood cell volume, globule hemoglobin, average hemoglobin concentration, nucleated red blood cells, and platelets—remained within the normal range for Domestic Short-Haired (DSH) cats. This indicates that Levovist® is safe for use in these animals during diagnostic procedures.

Parameters	Result	Normal Range	Unit	Parameters	Result	Normal Range	Unit
White blood cells	7.4±0.5	5.19-50.5	10^3/µL	Hematocrit	43.9±8.43	24-45	%
Lymphocytes	23±1	20-55	%	Red blood cells	10±1	5-10	10^3/µL
Monocytes	3±1	1-4	%	Hemoglobin	13.7±2.23	8-15	g/dL
Neutrophils	70±2	35-75	%	Average volume of red blood cells	42.9±8.12	39-55	fL
Eosinophils	1±0.0	2-12	%	Globule hemoglobin	13.2±2.01	31-37	g/dL
Mature neutrophils	-	35-80	%	Average hemoglobin concentration of red blood cell	13.2±1.23	9-16	g/dL
Young neutrophils	3±1	0-31	%	Nucleated red blood cells,	-	-	%
Basophils	-	0-1	%	Platelets	379±12	150-550	10^3/µL

Table 1. The results of the complete blood count test obtained before the injection of Levovist® contrast agent

International Journal of Multiphysics
Volume 18, No. 4, 2024
ISSN: 1750-9548

Parameters	**Result**	**Normal Range**	**Unit**	**Parameters**	**Result**	**Normal Range**	**Unit**
White blood cells	5.5±1.23	5.19-50.5	$10^3/\mu L$	Hematocrit	24.1±5.76	24-45	%
Lymphocytes	15±1	20-55	%	Red blood cells	5.57±1.34	5-10	$10^3/\mu L$
Monocytes	1±0.0	1-4	%	Hemoglobin	8±2	8-15	g/dL
Neutrophils	79±7	35-75	%	Average volume of red blood cells	43.3±6.65	39-55	fL
Eosinophils	1±0.0	2-12	%	Globule hemoglobin	12.7±2.23	31-37	g/dL
Mature neutrophils	-	35-80	%	Average hemoglobin concentration of red blood cell	31±2	9-16	g/dL
Young neutrophils	4±1	0-31	%	Nucleated red blood cells,	-	-	%
Basophils	-	0-1	%	Platelets	238±12	150-550	$10^3/\mu L$

Table 2. The results of the complete blood count test obtained after the injection of Levovist® contrast agent.

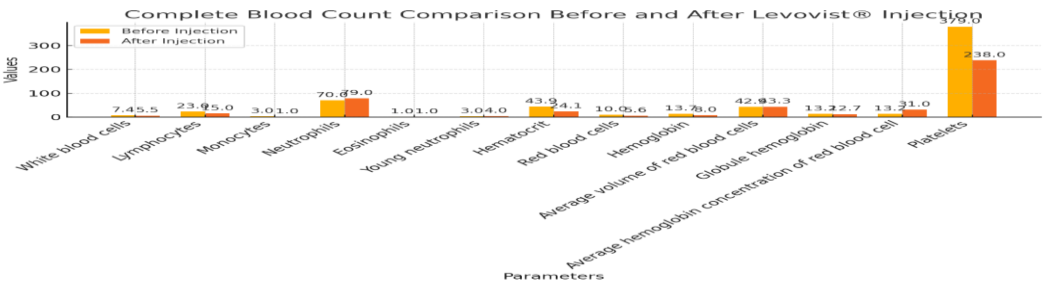

Chart 1. Comparison of the complete blood count results before and after the administration of Levovist®.

3.2. Radiography Before Injection of Levovist® in DSH Cat

Abdominal radiography was conducted on Domestic Short-Haired (DSH) cats before the injection of Levovist® contrast agent. X-rays do not clearly show the reproductive organs, such as the uterus, fallopian tubes, and ovaries, in healthy female cats. The initial radiograph confirmed the absence of pregnancy and any visible diseases in the uterus. However, due to the small size of a cat's uterus, ultrasound alone is insufficient for detailed examination, as the fallopian tubes are not visible even with ultrasound. To obtain more information about the uterus and fallopian tubes, including wall thickness and lumen details, we proceeded with the injection of Levovist® contrast material.

3.3. Ultrasound Scanning of Uterus After Injection of Levovist® in DSH Cat

After administering the Levovist® contrast agent to Domestic Short-Haired (DSH) cats, ultrasonography was performed to assess uterine changes. The qualitative results from eight DSH cats are illustrated in **Figures 1 and 2**.

3.3.1. Measurement Outcomes of Uterine Ultrasound Post-Levovist® Injection

This study aimed to measure the internal diameter of the uterus, the diameter of the uterine horns, and the thickness of the uterine walls both before and after the injection. In total, 12 DSH cats were evaluated. Before injection, the average inner diameter of the uterus was 3.48 ± 0.7 mm, and the uterine horns measured 3.43 ± 0.2 mm. After injection, these measurements significantly increased to 5.90 ± 0.6 mm for the uterus and 5.40 ± 0.21 mm for the uterine horns ($P<0.0001$). Additionally, the thickness of the uterine wall decreased from 2.21 ± 0.61 mm to 1.75 ± 0.3 mm ($P<0.0001$).The study also assessed endometrial and myometrial thicknesses before and after injection. Initially, these layers could not be distinguished; however, post-injection measurements revealed average thicknesses of 0.95 ± 0.1 mm for the endometrium and 0.81 ± 0.12 mm for the myometrium. It is important to note that the study did not evaluate the fallopian tubes before or after the injection of Levovist® contrast agent in DSH cats.

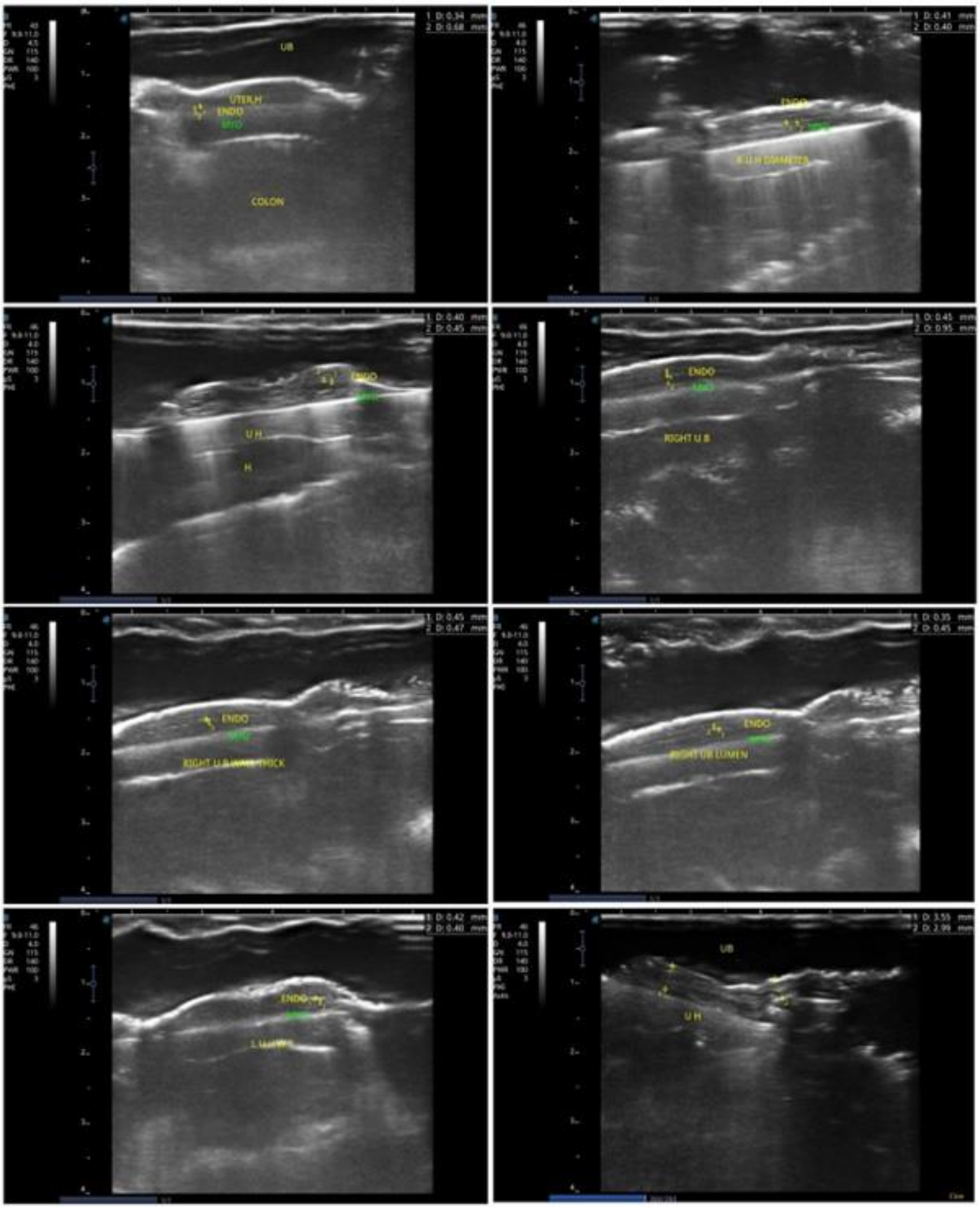

Figure 1. Ultrasonography images of the uterus in DSH cat No. 2 after injection of Levovist®.

International Journal of Multiphysics
Volume 18, No. 4, 2024
ISSN: 1750-9548

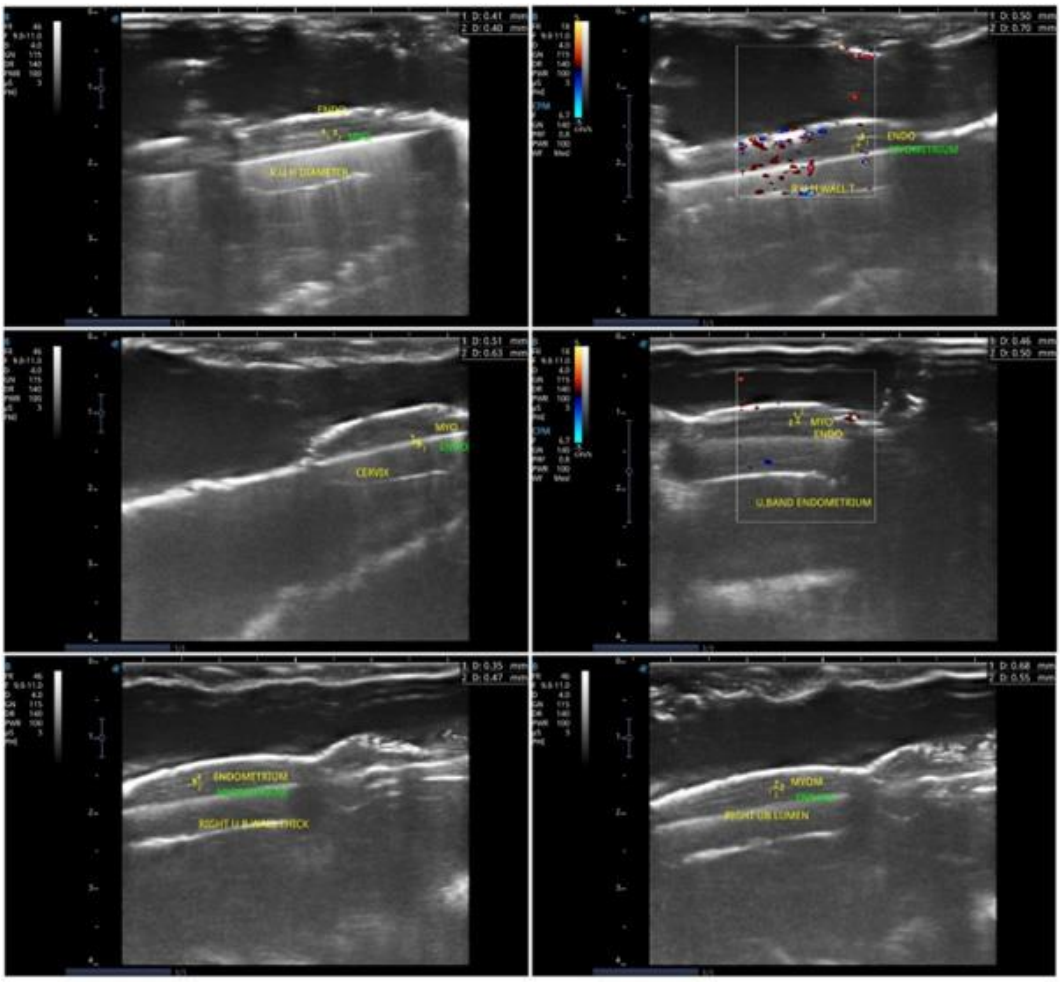

Figure 2. Ultrasonography images of the uterus in DSH cat No. 3 after injection of Levovist®

4. DISCUSSION

This study explored the use of Levovist® contrast agent in enhancing ultrasound diagnostics of the female reproductive system in Domestic Short-Haired (DSH) cats. Given the challenges posed by the small size of their uterine structures, contrast-enhanced ultrasound offers a valuable, non-invasive assessment method. Key findings indicate that Levovist is safe, with no significant adverse effects on blood parameters or histological tissue damage. Notably, the contrast agent significantly improved the visualization of the uterus and fallopian tubes, demonstrating its effectiveness in enhancing diagnostic capabilities for reproductive health evaluations in DSH cats.

There are several studies conducted on the use of ultrasound technology in medical and veterinary medicine. In the study by Sinagra et al. (2023), the authors aimed to analyze the utility of contrast-enhanced ultrasound (CEUS) in characterizing canine reproductive physiology and pathologies. A comprehensive review of articles from 1990 to 2022 was conducted, focusing on CEUS applications in various reproductive organs. The findings indicated that CEUS effectively differentiated between normal and abnormal conditions in the uterus, ovaries, and mammary glands, while highlighting its safety in pregnant dogs. However, CEUS lacked specificity for distinguishing neoplastic from non-neoplastic masses, emphasizing its role as a reliable diagnostic tool in veterinary reproductive imaging.[16]

In the study by Nyman et al. (2024), the authors aimed to evaluate the efficacy of contrast-enhanced ultrasound (CEUS) for diagnosing uterine conditions in female dogs. The study involved a cohort of dogs presenting with suspected uterine pathologies, where CEUS was employed to assess blood flow and tissue characteristics. Results demonstrated that CEUS significantly improved the detection of abnormalities compared to conventional ultrasound, allowing for better differentiation between types of uterine diseases. The findings suggest that CEUS is a valuable tool in veterinary diagnostics, enhancing the accuracy of uterine evaluations in canine patients.[17]

In the study by Koo et al. (2013), the authors aimed to assess the safety of contrast-enhanced ultrasonography (CEUS) in dogs and cats by evaluating the incidence of adverse events within 24 hours post-imaging. A retrospective case-control study involved 750 animals, comparing those that underwent CEUS with those that had standard ultrasonography without contrast. The results showed that among 411 case dogs, only four experienced adverse events, while no adverse events were noted in case cats. Additionally, the risk of death within 24 hours was similar between both groups, indicating that CEUS is a safe procedure for veterinary patients.[18]

In the study by Stoelinga et al. (2021), the aim was to evaluate the utility of contrast-enhanced ultrasound (CEUS) in diagnosing myometrial and endometrial disorders. A systematic review was conducted, analyzing 34 studies published between 2007 and 2021, focusing on CEUS characteristics and its effectiveness in differentiating benign from malignant lesions. The findings indicated that CEUS provides valuable insights into microvascular patterns, enhancing diagnostic accuracy for conditions like fibroids and endometrial carcinoma, and may also monitor the efficacy of minimally invasive therapies. The authors concluded that CEUS holds significant potential for gynecological applications, warranting further research.[19]

In the study by Stoelinga et al. (2018), the authors aimed to evaluate the feasibility of contrast-enhanced ultrasound (CEUS) for visualizing the microvasculature of uterine fibroids. Four women with diagnosed fibroids underwent a series of imaging techniques, including conventional gray-scale ultrasound and CEUS. The results indicated that CEUS effectively revealed initial perfusion in the fibroid's peripheral rim and subsequent enhancement throughout the lesion, allowing for clear delineation and identification of areas lacking vascularization. The findings suggest that CEUS offers valuable diagnostic information beyond conventional ultrasound, enhancing the assessment of uterine fibroids.[20]

The results achieved in our study regarding the use of the Levovist® contrast agent in Domestic Short-Haired (DSH) cats reveal several significant findings that warrant comparison with previously mentioned studies. The present study indicates that Levovist® is safe for use in DSH cats, as no adverse effects on blood parameters or histological factors were observed. This finding is consistent with research by Koo et al. (2013), which reported minimal adverse effects associated with contrast-enhanced ultrasound (CEUS) in both dogs and cats. Additionally, Levovist® significantly enhanced the visibility of uterine structures, thereby improving diagnostic accuracy. This aligns with findings from Nyman et al. (2024), which demonstrated that CEUS facilitates better detection of uterine abnormalities compared to conventional ultrasound methods. Both studies underscore the effectiveness of contrast agents in enhancing reproductive imaging in veterinary medicine. While Levovist® improved the visualization of uterine structures, it exhibited limitations in effectively assessing fallopian tubes. Other studies, such as those by Sinagra et al. (2023), noted that CEUS can differentiate between normal and abnormal conditions but may struggle with specificity when distinguishing neoplastic from non-neoplastic masses. This shared recognition of limitations highlights the necessity for further research to optimize these diagnostic tools within veterinary practice.

ACKNOWLEDGEMENTS

The authors would like to express their gratitude to the directorate and respected staff of Sharq and Central Veterinary Hospitals of Tehran for their cooperation in boarding, laboratory, radiology and surgery departments who helped us during the implementation of this project.

ABBREVIATION

CBC: Complete Blood Count

REFERENCES

1. Schlief R, Alhassan A, Wiggins J, *et al*. Safety of the galactose–based ultrasound contrast agent Levovist. Acad. Radiol. 2002; 9(1):S240-S2.
2. Crilly J, Politis A, Hamer K. Use of ultrasonographic examination in sheep veterinary practice. Small Rumin. Res. 2017; 152:166-73.
3. Chen Z, Wang Z, Du M, *et al*. Artificial intelligence in the assessment of female reproductive function using ultrasound: A review. J. Med. Ultrasound. 2022; 41(6):1343-53.
4. Occelli LM, Pirie CG, Petersen-Jones SM. Non-invasive optical coherence tomography angiography: A comparison with fluorescein and indocyanine green angiography in normal adult dogs and cats. Vet. Ophthalmol. 2022; 25(S1):164-78.
5. Messina C, Banfi G, Aliprandi A, *et al.* Ultrasound guidance to perform intra-articular injection of gadolinium-based contrast material for magnetic resonance arthrography as an alternative to fluoroscopy: the time is now. Eur. Radiol. 2016; 26:1221-5.
6. de Bournonville S, Vangrunderbeeck S, Kerckhofs G. Contrast-enhanced microCT for virtual 3D anatomical pathology of biological tissues: a literature review. Contrast Media Mol Imaging. 2019.
7. Entrekin RR, Porter BA, Sillesen HH, *et al.* Real-time spatial compound imaging: application to breast, vascular, and musculoskeletal ultrasound. Semin Ultrasound CT MR. 2001; 22(1):50-64.
8. Klibanov AL. Ultrasound contrast materials in cardiovascular medicine: from perfusion assessment to molecular imaging. J. Cardiovasc. Transl. Res. 2013; 6(5):729-739.
9. Ignee A, Atkinson NS, Schuessler G, *et al*. Ultrasound contrast agents. Endosc. Ultrasound. 2016; 5(6):355-362.
10. Tedesco G, Sarno A, Rizzo G, *et al.* Clinical use of contrast-enhanced ultrasound beyond the liver: a focus on renal, splenic, and pancreatic applications. Ultrasonography. 2019; 38(4):278-288.
11. Beckmann S, Simanowski JH. Update in contrast-enhanced ultrasound. Visc. Med. 2020; 36(6):476-486.
12. Dietrich CF, Sharma M, Hocke M. Contrast-enhanced endoscopic ultrasound. Endosc. Ultrasound. 2017; 1(3):130-6.
13. Caschera L, Lazzara A, Piergallini L, *et al.* Contrast agents in diagnostic imaging: Present and future. Pharmacol. Res. 2016; 110:65-75.
14. Lusic H, Grinstaff MW. X-ray-computed tomography contrast agents. Chem. Rev. 2013; 113(3):1641-66.
15. Schima W, Mukerjee A, Saini S. Contrast-enhanced MR imaging. Clin Radiol. 1996; 51(4):235-44.
16. Sinagra, L., Orlandi, R., Caspanello, T., Troisi, A., Iannelli, N. M., Vallesi, E., Pettina, G., Bargellini, P., De Majo, M., Boiti, C., Cristarella, S., Quartuccio, M., & Polisca, A. (2023). Contrast-Enhanced Ultrasonography (CEUS) in Imaging of the Reproductive System in Dogs: A Literature Review. *Animals : an open access journal from MDPI*, *13*(10), 1615. https://doi.org/10.3390/ani13101615
17. Chhoey, S., Kim, S., Kim, E., Lee, D., Kang, K., Keo, S., Acorda, J. A., Yoon, J., & Choi, J. (2024). Intraluminal Contrast-Enhanced Ultrasonography Application in Dogs and Cats. *Veterinary sciences*, *11*(9), 443. https://doi.org/10.3390/vetsci11090443
18. Seiler, G. S., Brown, J. C., Reetz, J. A., Taeymans, O., Bucknoff, M., Rossi, F., Ohlerth, S., Alder, D., Rademacher, N., Drost, W. T., Pollard, R. E., Travetti, O., Pey, P., Saunders, J. H., Shanaman, M. M., Oliveira, C. R., O'Brien, R. T., & Gaschen, L. (2013). Safety of contrast-enhanced ultrasonography in dogs and cats: 488 cases (2002-2011). *Journal of the American Veterinary Medical Association*, *242*(9), 1255–1259. https://doi.org/10.2460/javma.242.9.1255
19. Stoelinga B, Juffermans L, Dooper A, de Lange M, Hehenkamp W, Van den Bosch T, Huirne J. Contrast-Enhanced Ultrasound Imaging of Uterine Disorders: A Systematic Review. Ultrason Imaging. 2021 Sep;43(5):239-252. doi: 10.1177/01617346211017462. Epub 2021 May 26. PMID: 34036872; PMCID: PMC8299780.
20. Stoelinga, B., Dooper, A. M. C., Juffermans, L. J. M., Postema, A. W., Wijkstra, H., Brölmann, H. A. M., & Huirne, J. A. F. (2018). Use of Contrast-Enhanced Ultrasound in the Assessment of Uterine Fibroids: A Feasibility Study. *Ultrasound in medicine & biology*, *44*(8), 1901–1909. https://doi.org/10.1016/j.ultrasmedbio.2018.03.030