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Efficacy of the Modified Tumescent Technique on Vascularization in Canine Mammary Tumors

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ABSTRACT

Background**:** Mammary tumors are among the most common neoplasms in dogs. As in various tumor types, angiogenesis plays a significant role in the development of mammary tumors. Since the nutritional requirements of newly formed tissues associated with malignancy increase, this situation is directly related to angiogenesis. The tumescent anesthesia technique, which is commonly used during this surgical procedure, involves the application of a sterile solution combined with a local anesthetic and vasoconstrictor. This approach aimed to determine the potential impact of the modified tumescent technique on tumor vascularization.

Materials, Methods & Results**:** The study included 28 dogs diagnosed with mammary tumors with medium breeds, aged over 8 to 13 years and weighing between 11-23 kg. For the preparation of the tumescent solution, 0.09% isotonic saline (Bioflex®, Turkey) was stored at 4°C for the cold group and kept at room temperature for the other group. After the prepared solution was dosed according to body weight, it was equally administered into the mammary tissue. Color Doppler was employed to assess the distribution of vascularization (peripheral, mixed), and diameter of the largest vessel at the pre- and posttumescent application. In colorflow mode, the frequency was set at 5 MHz, with a pulse repetition frequency (PRF) of 1 kHz and a gain of 38%. Finally, Pulsed Doppler was used to measure the PI and RI in both the pre- and post tumescent applications. After tumescent application, a significant reduction in VD and a significant increase in RI were observed in both temperature groups, indicating effective vasoconstriction. The decrease in VD was significantly greater in dogs receiving the cooled tumescent solution compared to those receiving the solution at room temperature. Statistical analyses were performed using SPSS version 23.0 (IBM Corp., USA). Normal distribution of data and homogeneity of variances were evaluated by Shaphiro wilk test and Levene's test, respectively. The comparisons of the ultrasonography parameters between the groups (+4°C vs +20°C) and prior/after tumescent application within groups were performed by Student's t-test and Mann-Whitney U test. When ultrasonographic changes were analyzed according to histological grade, statistically significant vasoconstriction was primarily observed in grade 1 tumors, whereas grade 2 and grade 3 tumors demonstrated a reduced vascular response.

Discussion**:** The findings of the present study demonstrate that the modified tumescent anesthesia technique induces a significant vasoconstrictive effect in canine mammary tumors, as evidenced by reduced vessel diameter and increased vascular resistance following application. Importantly, no postoperative complications such as delayed wound healing or wound dehiscence were observed, indicating that the modified formulation is safe and well tolerated. the consistent and significant vasoconstriction observed at different temperature settings (+4°C and +20°C) and across all histological grades clearly demonstrates the efficacy of the technique in reducing local vascular perfusion. Overall, the results suggest that cooled modified tumescent anesthesia, may be a reliable and effective approach for improving intraoperative vascular control and surgical field management during canine mastectomy procedures.

Keywords: dog, mammary tumor, tumescent, vascularization.

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INTRODUCTION

Mammary tumors are among the most common neoplasms in dogs [28]. As in various tumor types, angiogenesis plays a significant role in the development of mammary tumors. Since the nutritional requirements of newly formed tissues associated with malignancy increase, this situation is directly related to angiogenesis [16]. Ultrasonography is frequently used in canine mammary tumors and allows numerous measurements related to vascularization and hemodynamic characteristics [8,13]. Doppler ultrasonography enables quantification of multiple key hemodynamic parameters, such as RI, and PI [24]. Mastectomy is considered the gold standard in the treatment of mammary tumors, except in cases of inflammatory carcinoma and metastatic disease [22,25]. The tumescent anesthesia technique, which is commonly used during this surgical procedure, involves the application of a sterile solution combined with a local anesthetic and vasoconstrictor [1]. The infiltration of the tumescent solution is performed using a Klein cannula or an infusion pump [6,21]. This technique provides intraoperative and postoperative analgesia, reduces surgical bleeding, and facilitates tissue dissection [9,11].

The present study was designed to investigate the effectiveness of the tumescent solution stored at room temperature and at 4°C in canine mastectomy operations. For this purpose, ultrasonographic parameters, including vascularization patterns, tumor size, RI, PI, and vessel diameter (VD), were evaluated in the pre- and post- tumescent application. This approach aimed to determine the potential impact of the modified tumescent technique on tumor vascularization.

MATERIALS AND METHODS

Animals

The study included 28 dogs diagnosed with mammary tumors with medium breeds, aged over 8 to 13 years and weighing between 11-23 kg. Preoperative clinical, hematological and biochemical evaluationsalong with thoracic radiography and abdominal ultrasonography, were performed assess distant metastasis. Dogs with cardiomyopathy or renal disease were excluded.

Preparation of solution - Kırşan's modified tumescent technique

The tumescent solution was modified by I. Kırşan, and termed "Kırşan's modified tumescent technique". For preparation of the tumescent solution, 0.09% isotonic saline[1] was stored at 4°C for the cold group and at room temperature for the other group. Subsequently, 30 mL of lidocaine [40 mg/2 mL] and epinephrine [0.025 mg/2 mL] injectable solution[2] were added to 470 mL of isotonic saline. Mastectomy was initiated 15 min after tumescent administration.

Experimental design

Dogs were fasted for 8 h without water restriction before surgery. After premedication with atropine sulfate[3] [0.02 mg/kg, IM], anesthesia was induced with propofol[4] [6-8 mg/kg, IV] and maintained with isoflurane. Dogs were randomly assigned to 2 groups receiving tumescent solution at room temperature (Group RT) or at 4°C (Group C). Bitches were positioned in dorsal recumbency, and the tumescent solution was administered subcutaneously around the mammary glands using a Klein cannula at a standardized dose of 15 mL/kg. After the prepared solution was dosed according to body weight, it was equally administered into the mammary tissue. For the infiltration of the tumescent solution, a small incision was made through the subcutaneous tissue, infiltration into the mammary tissue was performed using a Klein cannula. Tumescent solution was administered according to the guidelines reported by Lapid *et al*. [23].

Ultrasonographic examination

Ultrasonographic evaluations were performed using a color Doppler ultrasound system[5] with a 5–8 MHz microconvex transducer. B-mode ultrasonography was conducted at 8 MHz with a depth of 6 cm and gain of 50% to assess mass size, echogenicity, and structure (solid, cystic, or lobular). Color Doppler ultrasonography was used to assess vascularization distribution (peripheral, mixed) and the diameter of the largest vessel before and after tumescent application. In color flow mode, frequency was set at 5 MHz, pulse repetition frequency (PRF) at 1 kHz, and gain at 38%. Finally, Pulsed Doppler was used to measure the PI and RI in both the pre- and post tumescent applications. For pulsed Doppler imaging, the frequency was also 5 MHz, with a PRF of 1.5 kHz and a gain of 58%.

Histopathological examination

Tumor samples from 28 dogs were fixed in 10% neutral buffered formalin for at least 48 h, then routinely processed, paraffin-embedded, and sectioned at 4-5 µm. Sections were stained with hematoxylin and eosin (H&E) and examined by light microscopy. Tumors were histopathologically classified and graded according to Goldschmidt *et al*. [18].

Statistical analysis

The required sample size was calculated a priori using G*Power version 3.1.9.26 A point-biserial correlation model within the t-test family was selected. Parameters were set at an effect size of r = 0.50, a one-tailed α error probability of 0.05, and a desired statistical power (1–β) of 0.90. Based on these criteria, the analysis indicated a minimum total sample size of 28 subjects (df = 26). Statistical analyses were performed using SPSS version 23.07 Normal distribution of data and homogeneity of variances were evaluated by Shaphiro-Wilk test and Levene's test, respectively. The comparisons of the ultrasonography parameters between the groups (+4°C vs +20°C) and prior/after tumescent application within groups were performed by Student's t-test and Mann-Whitney U test. Values were given as mean ± standard error of the mean (SEM). The significance level was accepted as $P < 0.05$.

RESULTS

Clinical findings

The groups consisted of medium-sized dogs with mean ages of 10.86 ± 0.69 and 11.26 ± 0.78 years, respectively. In cases with multiple masses, only the primary mass was considered for histopathological classification and grading. Primary mass locations were right cranio-thoracic (R1; n=2), caudo-thoracic (R2; n=3), cranio-abdominal (R3; n=2), caudo-abdominal (R4; n=4), inguinal (R5; n=6); left cranio-abdominal (L3; n=2), caudo-abdominal (L4; n=4), and inguinal (L5; n=5). Dogs with ulcerated and inflammatory masses were not included in the study.

Histopathological findings

All cases included in the study were of malignant epithelial mammary tumor. Histological subtypes of tumors were simple carcinoma (n=11), solid carcinoma (n=5), carcinoma complex type (n=3), carcinoma mixed type (n=2), ductal carcinoma (n=4) and intraductal papillary carcinoma (n=3). Furthermore, 8 tumors were classified as Grade 1, 9 as Grade 2, and 11 as Grade 3. According to clinical and histopathological evaluation, no ulceration and/or inflammation was observed in the tumors that interpreted in the study.

Ultrasonographic findings

B-mode ultrasonography identified solid (n=17) and lobular (n=11) masses; cystic tumors were excluded. Based on primary masses, tumor size was classified as T1 in 12 cases and T2 in 16 cases. Color Doppler ultrasonography revealed mixed vascularization in 17 cases and peripheral vascularization in 11 cases (Figure 1). Vessel diameter significantly decreased after tumescent infiltration compared with pre-application values. Ultrasonographic measurements were obtained before (Pre-T) and after (Post-T) tumescent application (Figures 2 & 3). Changes in ultrasonographic parameters, including primary tumor diameter, PI, RI, and widest intratumoral vessel diameter, are presented in Table 1. Comparision of ultrasonography parameters according to tumescent application at cold (+4°C) and room temperature (+20°C) were also evaluated (Table 2). Also, the differences in VD (Pre-T VD– Post-T VD) were evaluated in terms of temperature of tumescent solution (Table 2) and histological grading of tumor (Table 3). The evaluation according to grades was performed as a single group regardless of tumescent temperature. Ultrasonographic parameters according to tumor grade are presented in Table 3. Pre- and post-tumescent changes in PI, RI, and VD values and their statistical significance for each grade are shown in Table 4.

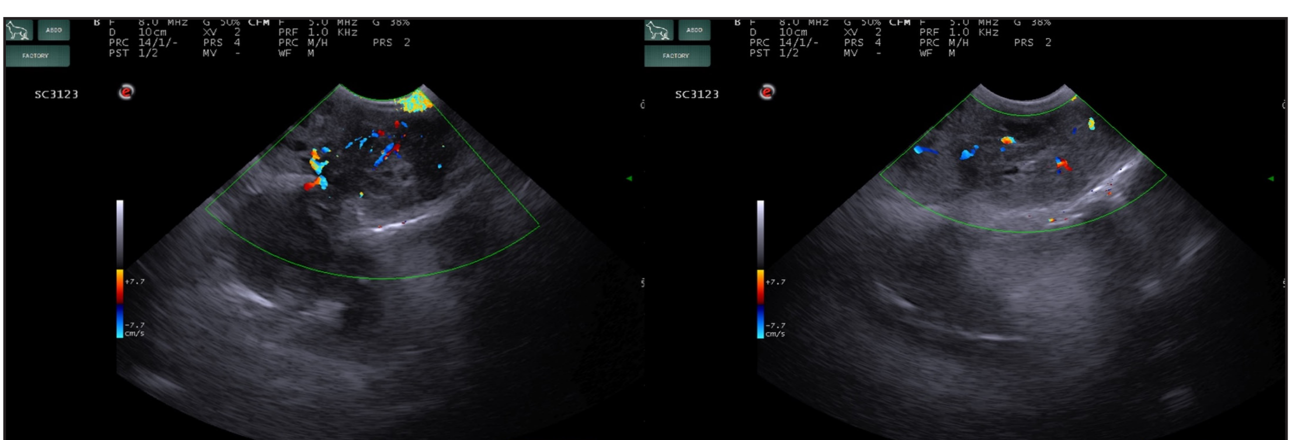

Figure 1. Color Doppler images of the primary mass obtained Pre-T and Post-T solution infiltration.

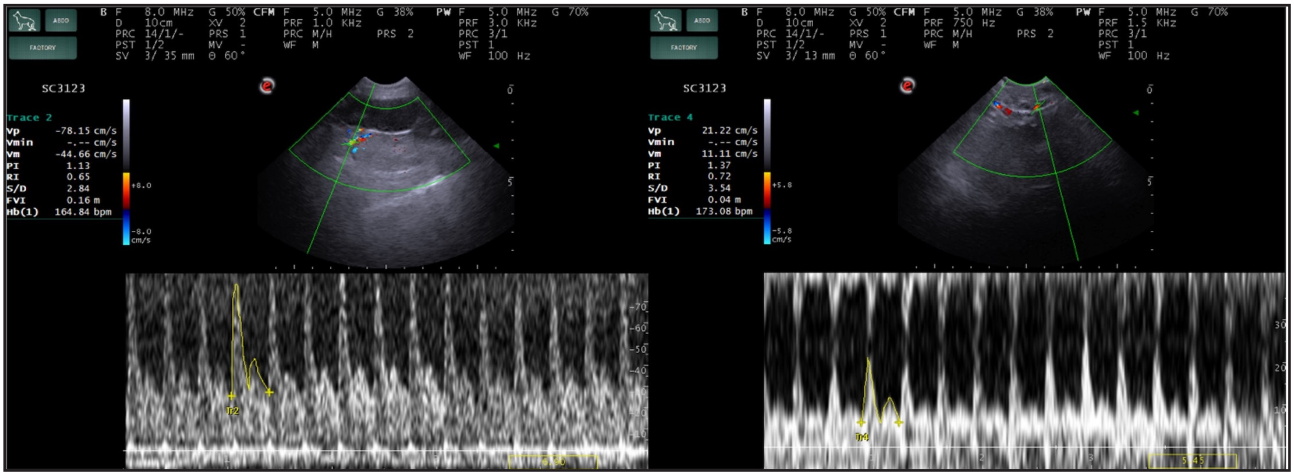

Figure 2. Color Doppler images of the primary mass at room temperature obtained Pre-T and Post-T solution infiltration, demonstrating changes in vascularization.

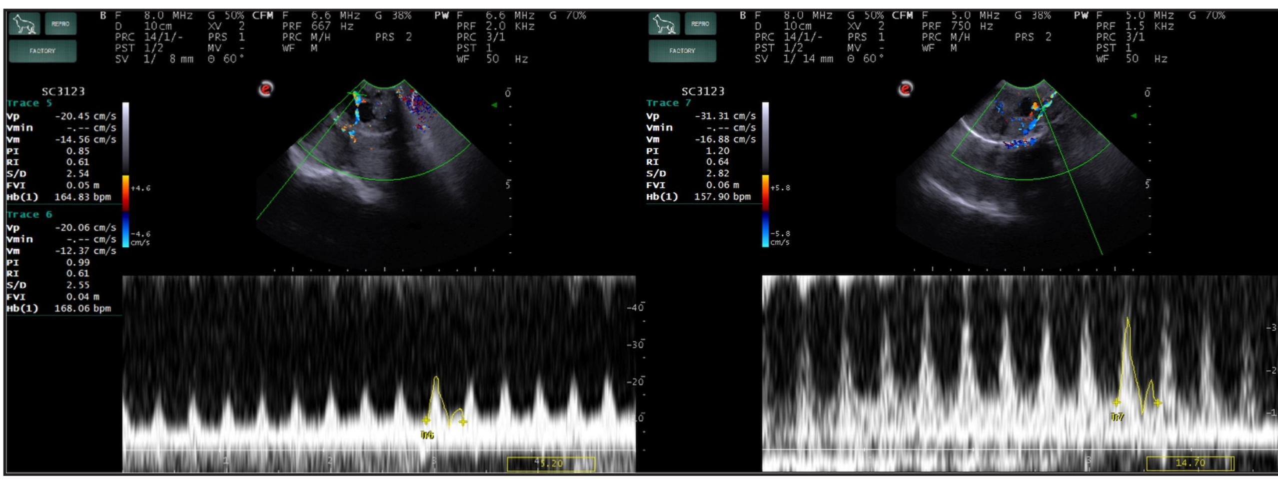

Figure 3. Color Doppler images of the primary mass in the cold group obtained Pre-T and Post-T solution infiltration, demonstrating changes in vascularization.

Table 1. Comparison of ultrasonography parameters within each group before (Pre-T) and after (Post-T) tumescent application.

Ultrasonography Parameters	Cold Tumescent (+4°C) (n=11)	Room temp. Tumescent (+20°C) (n=17)	Significance
Tumor size (cm)	3.96 ± 0.48	2.87 ± 0.29	ns
Pre-T. PI	1.67 ± 0.23	1.62 ± 0.15	ns
Pre-T. RI	0.75 ± 0.03[a]	0.76 ± 0.03[b]	< 0.001
Post-T. PI	1.67 ± 0.15[a]	1.90 ± 0.14[b]	< 0.001
Post-T. RI	0.81 ± 0.01[a]	0.80 ± 0.02[b]	< 0.001
Pre-T. Vessel diameter- (d2)	2.33 ± 0.11[a]	2.01 ± 0.12[b]	< 0.001
Post-T. Vessel diameter- (d1)	1.63 ± 0.11[a]	1.42 ± 0.09[b]	< 0.001
d2-d1	0.69 ± 0.05[a]	0.58 ± 0.07[b]	< 0.001

[a,b]Different letters in the same line indicate the significance. Pre-T: Pre-tumescent; Post-T: Post-tumescent; PI: Pulsatility index; RI: Resistance index; ns: $P > 0.05$.

Table 2. Comparison of ultrasonography parameters according to tumescent application at different temperatures.

Tumescent Temperatures	Pre-T PI	Post-T PI	Significance
Cold	1.67 ± 0.23	1.67 ± 0.15	ns
Room temperature	1.62 ± 0.15^{a}	1.90 ± 0.14^{b}	<0.001
	Pre-T RI	Post-T RI	Significance
Cold	0.75 ± 0.03^{a}	0.81 ± 0.01^{b}	<0.05
Room temperature	0.76 ± 0.03^{a}	0.80 ± 0.02^{b}	<0.001
	Pre-T Vessel Diameter	Post-T Vessel Diameter	Significance
Cold	2.33 ± 0.11^{a}	1.63 ± 0.11^{b}	<0.001
Room temperature	2.01 ± 0.12^{a}	1.42 ± 0.09^{b}	<0.001

^{a,b}Different letters in the same line indicate the significance. Pre-T: Pre-tumescent; Post-T: Post-tumescent; PI: Pulsatility index; RI: Resistance index; ns: $P > 0.05$.

Table 3. The comparison of ultrasonographic parameters according to tumor grades regardless of tumor tumescent temperature.

Histological Grades	Pre-T PI	Post-T PI	Significance
G1	1.73 ± 0.23	2.01 ± 0.17	ns
G2	1.62 ± 0.29	1.67 ± 0.18	ns
G3	1.59 ± 0.17	1.78 ± 0.19	ns
	Pre-T RI	Post-T RI	Significance
G1	0.69 ± 0.03^{a}	0.83 ± 0.02^{b}	< 0.001
G2	0.78 ± 0.04	0.75 ± 0.02	ns
G3	0.79 ± 0.02	0.82 ± 0.02	ns
	Pre-T VD	Post-T VD	Significance
G1	1.82 ± 0.17^{a}	1.29 ± 0.10^{b}	< 0.001
G2	2.22 ± 0.06^{a}	1.55 ± 0.09^{b}	< 0.001
G3	2.30 ± 0.17^{a}	1.64 ± 0.15^{b}	< 0.001

^{a,b}Different letters in the same line indicate the significance. Pre-T: Pre-tumescent; Post-T: Post-tumescent; PI: Pulsatility index; RI: Resistance index; ns: $P > 0.05$.

Table 4. Changes in PI, RI and VD values before and after tumescent application in each grade group and their significance.

Parameters	Grade 1 (G1; n=8)	Grade 2 (G2; n=9)	Grade 3 (G3; n=11)	Significance		
				G1xG2	G1xG3	G2xG3
Tumor size (cm)	1.98 ± 0.15^{a}	3.36 ± 0.57^{b}	4.22 ± 0.27^{b}	< 0.05	< 0.001	= 0.08
Pre-T / PI	1.73 ± 0.23	1.62 ± 0.29	1.59 ± 0.17	ns	ns	ns
Pre-T/ RI	0.69 ± 0.03^{a}	0.78 ± 0.0 ^{b}	0.79 ± 0.02^{b}	< 0.05	< 0.05	ns
Post-T/ PI	2.01 ± 0.17	1.67 ± 0.18	1.78 ± 0.19	ns	ns	ns
Post-T/ RI	0.83 ± 0.02^{a}	0.75 ± 0.02^{b}	0.82 ± 0.02^{a}	< 0.05	ns	< 0.05
Vessel diameter Pre-T (d2)	1.82 ± 0.17^{a}	2.22 ± 0.06^{b}	2.30 ± 0.17^{b}	< 0.05	< 0.05	ns
Vessel Diameter Post-T (d1)	1.29 ± 0.10^{a}	1.55 ± 0.09^{b}	1.64 ± 0.15^{b}	< 0.05	< 0.05	ns
d2-d1	0.53 ± 0.10	0.66 ± 0.08	0.66 ± 0.08	ns	ns	ns

^{a,b}Different letters in the same line indicate the significance. Pre-T: Pre-tumescent; Post-T: Post-tumescent; G: Grade; PI: Pulsatility index; RI: Resistance index; VD: Vessel Diameter; ns: $P > 0.05$.

DISCUSSION

The present study evaluated the impact of a modified tumescent anesthesia technique on local vascularization at the surgical site, comparing the effects of the solution administered at room temperature and cooled temperature. Unlike the dosage regimen reported by Rocha *et al*. [27] the same concentration was not used in the present study, as preliminary trials at that dose resulted in delayed wound healing and partial dehiscence along the suture line. The preparation used by Rocha *et al*. [27] contained 20 mg lidocaine and 15 µg adrenaline per mL, and 12.5 mL of this solution was mixed with an equal volume of lactated Ringer's, resulting in 25 mL containing 250 mg lidocaine and 0.18 mg adrenaline. In contrast, the formulation used in the present study consisted of 600 mg lidocaine and 0.75 mg adrenaline in 500 mL of isotonic solution. When standardized to a 25 mL volume for comparison, this corresponds to approximately 30 mg lidocaine and 0.0375 mg adrenaline, which represents only one-twentieth of the concentration [27]. Similarly, Vullo *et al*. [30] investigated tumescent anesthesia with a constant rate lidocaine infusion in dogs undergoing unilateral mastectomy and reported using a solution containing 250 mg lidocaine and 0.18 mg adrenaline per 25 mL. In the present study, even when administered at 15 mL/kg, the total dose remained below that concentration. Preliminary trials with higher doses resulted in delayed wound healing and partial wound edge separation, likely due to the local vasoconstrictive effects of adrenaline. Therefore, the lidocaine-adrenaline concentrations were reduced in the current study. Importantly, at this substantially lower dose, no complications such as wound dehiscence or delayed healing were observed in any of the treated dogs, suggesting that the modified formulation was both effective and well-tolerated for surgical site management in accordance with the recommendations of Bednarski *et al*. [5]. Lidocaine is a rapidly acting local anaesthetic with a relatively short duration of action; however, when used without a vasoconstrictor, its intrinsic local vasodilatory effect may increase tissue perfusion and predispose the surgical field to greater bleeding [20]. Epinephrine, a compound with both α- and β-adrenergic receptor agonist properties, produces vasoconstriction through α-receptor–mediated mechanisms [7]. Consequently, it reduces the systemic absorption of local anesthetic agents and minimizes surgery-related blood loss [15]. Therefore, the vasoconstrictive effect observed in the tumescent solution used in the present study is attributed to epinephrine rather than lidocaine. Previous reports have indicated that the 4th and 5th mammary glands are the most commonly affected sites in canine mammary tumors [19]. Consistent with these findings, 67% of the primary tumors in the present study were located in the 4th and 5th mammary glands. Tumor size was comparable between the groups, indicating that both groups had similar tumor dimensions and that tumor size did not influence the evaluated parameters. However, tumor size increased markedly with higher histological grades, supporting the well-established association between the degree of malignancy and tumor growth potential [17]. Angiogenesis has been shown to be more prominent in high-grade canine mammary tumors [10]. In present study, vasoconstriction was statistically significant only in grade 1 tumors, whereas no significant difference was observed in grades 2 and 3. This may be attributed to the increased angiogenic activity in higher-grade tumors, which could diminish the vasoconstrictive response. Although the increase in vascular resistance in grade 3 tumors did not reach statistical significance, a clear upward trend was observed, indicating a possible relationship between tumor grade, angiogenic activity, and vascular resistance. These results suggest that the vasoconstriction induced by the tumescent solution was more pronounced in low-grade tumors, whereas the enhanced angiogenesis and impaired vascular maturation in high-grade tumors may have limited this effect. The statistical evaluation according to tumor grade revealed significant differences between grades G1 and G2, and between G2 and G3. This difference may have contributed to the higher level of statistical significance observed in the Post-T RI values of the room-temperature group. B-mod ultrasonography could be an extremely useful tool for the preevaluation of canine mammary gland tumors, especially in relation to the evaluation of tumor size, shape, border irregularity, echotexture, internal echogenicity and acoustic transmission [4]. Also, they observed that the features most predictive of a malignant tumor diagnosis were spiculated or microlobulated margins (76% of masses with this feature were malignant), and irregular shape (78% were malignant) [4]. In harmony with the previous report, the features of canine mammary tumors were evaluated by B-mod ultrasonography and all malignant epithelial tumors in present study were solid (n=17) and lobular (n=11) structure. Both B-Mode ultrasonography and color Doppler sonography techniques were inadequate to diagnose tumor characteristics [13]. In literature, the use of B-mode ultrasonography to analyze 30 malignant, 8 atypical, and 11 benign mammary lesions in bitches concluded that the larger masses were correlated to high-grade malignancy and worse prognosis [26]. Although, all tumors had malignant character in this study, similar with the previous report, all Grade 3 tumors were in T2 (3-5 cm) size. Doppler mode ultrasound can give real-time information about vascularisation and haemodynamic aspects of blood flow [8]. Color Doppler and Power Doppler ultrasound qualitatively assess the presence or absence of vascularity, morphology, number, and distribution (central, peripheral, and penetrating blood vessels). Spectral Doppler ultrasound evaluates RI, PI, Peak Systolic Velocity (PSV), and end-diastolic velocity (EDV) quantitatively [4]. The color Doppler function can be employed to increase the sensitivity of the blood flow measurements and to transform the examination from angle-independent to insonation or incident angle [12]. In line with the previous reports, Color Doppler ultrasound was performed to assess the hemodynamic changes of the tumor vascularization by evaluating insonation angle-independent. Doppler indices before and after the tumescent application. Cancerous growths release vascular growth factors, which promote development of newer vessels. However, these vessels are tortuous with an abnormal structure, resulting in increased resistance to blood. Thus, Doppler ultrasound of malignant masses reveals a higher RI as compared to benign or normal tissues with normal or reduced number of vessels. Malignant lesions also have features such as central vascularity, hypervascularity or tortuous vessels while benign lesions are usually having normal or reduced number of vessels [4,29]. Similarly, the highest RI values were determined in G3 tumors in the measurements taken before tumescent application. This result supported by previous reports that indicate the increased RI observed in the increased malignancies. Previous studies evaluating vascularization have reported that RI is a more specific parameter than PI for assessing blood flow resistance [31]. Azhar *et al*. [3] indicated that increase in the value of the RI indicates an increase of risk of malignancy in breast cancer. Similarly, the present study supports this finding by demonstrating that PI values were not as specific as RI in reflecting vascular changes. Moreover, an increase in RI is known to be associated with elevated vascular resistance [2]. In the current study, the increases observed in both pre- and post-treatment RI values in each group suggest an elevation in vascular resistance, indicating the occurrence of vasoconstriction. When the pre- and post-treatment vessel diameters were compared, a significant reduction was observed in both groups. The reduction was greater in the cold group than in the room-temperature group. These findings indicate that the tumescent solution induced vasoconstriction under both temperature conditions; however, the effect was more pronounced in the cold application group, suggesting that lower temperature enhanced the vasoconstrictive response. Additionally, there are several limitations that should be considered when interpreting the findings of this study. Pulse or heart rate measurements were not recorded during the Doppler evaluations. Because the PI is affected by pulse pressure, the absence of concurrent pulse monitoring may have limited the interpretation of PI variations following tumescent application. Further studies incorporating pulse and hemodynamic monitoring are needed to better elucidate the relationship between vascular resistance and pulsatile flow dynamics.

CONCLUSIONS

The modified tumescent technique can be regarded as a reliable and effective alternative for use in mastectomy procedures, as it did not cause any postoperative complications such as delayed wound healing or wound dehiscence in the present study. The absence of these adverse effects indicates that the reduced lidocaine–adrenaline concentration and the use of isotonic saline provided a formulation that is both safe and biocompatible for surgical applications. Moreover, the consistent and significant vasoconstriction observed at different temperature settings (+4°C and +20°C) and across all histological grades clearly demonstrates the efficacy of the technique in reducing local vascular perfusion.

In conclusion these findings suggest that the modified tumescent formulation may contribute to improved intraoperative hemostasis and better visualization of the surgical field without compromising tissue viability. Further clinical evaluations could help to optimize this protocol for wider application in veterinary surgical practice.

MANUFACTURERS

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Ethical approval. All animal procedures were approved by the Local Ethical Committee of Istanbul University Cerrahpaşa (Approval ID: 2024/101).

Declaration of interest. The authors report no conflicts of interest. The authors alone are responsible for the content and writing of paper.

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