Closure of defects in a geometric figure pattern associated with tumescent anesthesia with lidocaine in rabbits (*Oryctolagus cuniculus*)

Fechamento de defeitos em padrão de figura geométrica associado ao emprego de anestesia por tumescência com lidocaína em coelhos (Oryctolagus cuniculus)

Eduardo Luís Serafim[1] , Josiane Morais Pazzini[1] , Michelle do Carmo Pereira Rocha[1] , Laís Calazans Menescal Linhares[1*] , Andrigo Barboza de Nardi[1] , Maria de Fátima Moutinho Gartner[2] , Irina Amorim[2] , Alexandra Rema[2] , Fátima Faria[2] , Ricardo Andres Ramirez Uscategui[1] , Vivian Tavares de Almeida[1] , Carlos Alfredo Calpaa[3] , Sabrina Gouveia Calazans[1]

[1]Universidade Estadual Paulista Júlio de Mesquita Filho (UNESP), Jaboticabal, São Paulo, Brazil
[2]Universidade do Porto (ICBAS-UP), Porto, Portugal.
[3]Universidade de Natño, Colombia.
*Correspondent: laiscmlinhares@gmail.com

Abstract
The use of tumescent anesthesia with lidocaine can provide better intra- and postoperative analgesia that would benefit extensive reconstructive surgery. However, lidocaine can interfere with the healing process. Therefore, this study aimed to assess the local interference of the healing of induced and closed skin defects in a geometric pattern associated with the use of tumescent anesthesia with lidocaine in rabbits. Furthermore, we assessed its influence on cardiorespiratory parameters and postoperative analgesia. This study included 27 rabbits divided into three groups: GC (without the use of tumescence), GS (use of tumescence with 0.9% NaCl solution), and GL (use of tumescent anesthesia with lidocaine). There was no statistically significant intergroup difference in any stage of the wound healing process on macroscopic evaluations, in the angiogenesis process, or in the process of collagenization and fibroblast deposition. There were significant differences in heart rate (lower in GL), respiratory rate (higher in GC), mean arterial pressure (higher in GL), and expired concentration of isoflurane (lower in GL). There was no significant intergroup difference in the von Frey filament test or the visual analog scale score used to evaluate postoperative analgesia. We concluded that tumescent anesthesia with lidocaine does not impair postoperative tissue repair. Its use features benefits such as reducing the volume of inhaled anesthetic, maintaining the anesthesia plan, stable heart and respiratory rates, and lower hypotension during the surgical procedure.
Keywords: Local anesthetic; Pain; Reconstructive surgery; Tissue repair

Resumo
O uso da anestesia por tumescência com lidocaína pode proporcionar melhor analgesia transoperatória e pós-operatória, o que seria um benefício em cirurgias reconstrutivas extensas. Entretanto, a lidocaína pode interferir na cicatrização. Diante disso, delineou-se um estudo a fim de avaliar a interferência local da cicatrização de defeitos cutâneos induzidos e fechados em padrão de figura geométrica, associado ao emprego de anestesia por tumescência com lidocaína em coelhos. Ademais, avaliar sua influência em parâmetros cardiorrespiratórios e na analgesia pós-operatória. Foi realizado um estudo com 27 coelhos, separados em três grupos, compreendidos em GC (sem o emprego de tumescência), GS (emprego de tumescência com solução NaCl 0.9%) e GL (emprego de anestesia por tumescência com lidocaína). Não houve diferença estatística em nenhuma etapa do processo cicatricial da ferida entre os grupos, tampouco nas avaliações macroscópicas, e no processo de angiogênese, ou ainda no processo de colagenização e deposição de fibroblastos. Houve diferença significativa na frequência cardíaca (menor no GL) e na respiratória (maior no GC), na pressão arterial média (maior no GL) e na concentração expirada de isoflurano (menor no GL). Não houve diferença significativa entre os grupos no teste de filamentos de von Frey e na Escala Visual Analógica, utilizados para avaliar a analgesia pós-operatória. Conclui-se que anestesia por tumescência com lidocaína não prejudica a regeneração tecidual após a cirurgia. O seu uso oferece benefícios como a diminuição do volume de anestésico inalatório, manutenção do plano anestésico, estabilidade das frequências cardíaca e respiratória, além de menor hipotensão durante o procedimento cirúrgico.
Palavras-chave: Anestésico local; Cirurgia reconstrutiva; Dor; Reparação tecidual

Introduction

Extensive lesions resulting from surgical tumor resection, congenital anomalies, and trauma are wounds for which reconstructive surgery techniques are indicated for their rapid recovery[1]. They are also indicated for patients in whom direct wound occlusion is not possible[2]. Among the available reconstructive techniques are those used to

close wounds in a geometric pattern, which can be used in the presence of wide margins and favor the achievement of good aesthetic results[1].

The great tissue manipulation that occurs with reconstructive surgical techniques implies a painful postoperative period[3]. In this context, the use of tumescent anesthesia provides better intraoperative[4] and postoperative[5,4] analgesia.

The technique of anesthesia by tumescence consists of injecting a large volume of diluted lidocaine and epinephrine in the subcutaneous plane, leaving the surgical area firm and tumescent and providing adequate local anesthesia[6,7.] However, despite being an effective and safe method with minimal complications[8], it is associated with a lower consumption of inhalational anesthetics and greater heart rate (HR) and respiratory rate (RR) stability[9]. Lidocaine used in the tumescent solution can negatively influence aspects related to tissue repair[10].

This study aimed to evaluate the healing process of induced cutaneous defects closed in a geometric pattern associated with the use of tumescent anesthesia with lidocaine in rabbits (*Oryctolagus cuniculus*). Furthermore, to evaluate the influence of the local action of tumescent anesthesia with lidocaine on cardiorespiratory parameters, monitored during the pre- and perioperative periods, as well as on postoperative analgesia.

Materials and methods

The present study was approved by the Ethics Committee on the Use of Animals (CEUA) of Universidade Estadual Paulista (UNESP), Campus de Jaboticabal (protocol no. 015013/4). This study included twenty-seven 60-day-old female New Zealand White rabbits (*Oryctolagus cuniculus*). The rabbits were kept in individual cages measuring 80 cm × 50 cm × 35 cm with commercial food and water *ad libitum*.

The animals were divided into three groups of nine animals each; all were submitted to the same reconstructive surgical technique of closing in a geometric pattern. The groups were the GC (control group; closed in a geometric pattern without the use of tumescence), GL (lidocaine group; closed in a geometric pattern associated with the use of tumescent anesthesia with lidocaine), and GS (solution group; closure in a geometric figure pattern associated with the use of tumescence with 0.9% NaCl solution).

The anesthetic protocol to which the animals were submitted consisted of midazolam 0.5 mg/kg by intramuscular (IM) and tramadol hydrochloride 5 mg/kg via the same route. With the animals tranquilized, extensive hair removal from the left lateral region of the thorax was performed, and venipuncture of the auricular vein was performed with a 24-gauge catheter for maintenance fluid therapy (0.9 NaCl solution 40 mL/kg/day). The anesthetic induction and maintenance were performed via a sealed face mask with isoflurane at a dose of 3% diluted in 100% oxygen to produce a surgical anesthesia plan.

The animals were placed in the right lateral decubitus position so that the previous demarcation of the 4 cm² defect was performed on the left side of the thorax. Starting 3 cm from the spine of the scapula in the caudal direction and 1 cm below the spinous process, demarcation of the defect was performed with the aid of a surgical pen and ruler from the 9th to the 11th intercostal space (Figure 1A). Subsequently, antisepsis was performed using chlorhexidine and a 70% alcohol solution. Soon after, the tumescent technique was used 10 min before the surgical incision after anesthetic induction and maintenance. With a 25 × 7 hypodermic needle positioned in the subcutaneous tissue connected to a 60 mL syringe (Figure 1B), the administration of the fan tumescent solution was started with a fixed infiltration volume of 15 mL/kg for all animals (adapted from Abimussi et al.)[11] and continued for 2 min (Figure 1C).

In the GL group, the solution was prepared at the time the tumescent procedure and consisted of 1000 mL of 0.9% NaCl solution, 50 mL of 1% lidocaine, 1 mL of 1:10000 epinephrine and 12.5 mL of 8.4% sodium bicarbonate according to Klein's tumescent solution recommended for humans[12] with a pH of 7.66.

In the GS group, the solution consisted exclusively of a sterile 0.9% solution with a pH of 7.63. The pH of each solution was measured using a pH meter. In the GC group, the defect was made under the same conditions as in the other two groups, but no solution was used to cause the tumescense.

After the tumescent technique was performed, definitive antisepsis was performed using chlorhexidine and 70% alcohol solution. A chest injury was then induced to initiate the reconstructive surgery procedure. With the aid of a number 15 scalpel blade, the 4 cm² skin fragment (2 cm long × 2 cm wide) was excised to form a square (Figure 2A), and the limit considered for the incision depth was the muscle trunk skin.

After removing the skin fragment from the solution of continuity arising from the induced lesion in the thorax, the defect was synthesized in a geometric pattern starting from the extremity toward the center of the lesion (Figure 2B). The surgical wound was synthesized with sutures in a separate simple pattern using nylon 4.0 thread for dermorrhaphy with closure in a geometric pattern in all groups (Figure 2C).
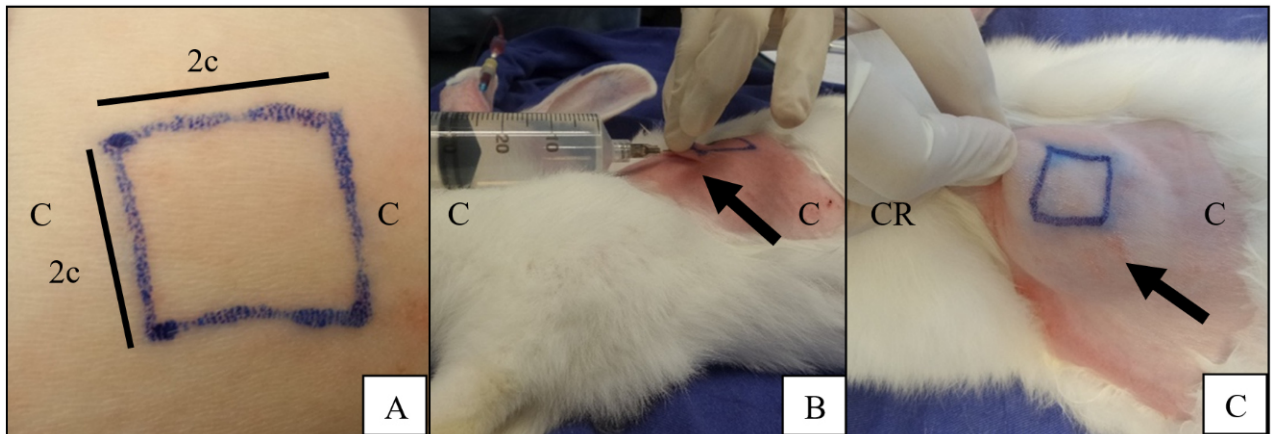
Figure 1. Photograph of the tumescent anesthesia with lidocaine performed in rabbits. A) A 4 cm² square was made with the aid of a surgical pen and ruler to create the defect in the thorax, where the tumescent solution was administered in the subcutaneous space. B) Tumescent technique performed with a 25 × 7 hypodermic needle positioned in the subcutaneous tissue and connected to a 60 mL syringe (arrow). C) Aspect of the region after subcutaneous infiltration of the tumescent solution (arrow). CD, flow; CR, cranial.
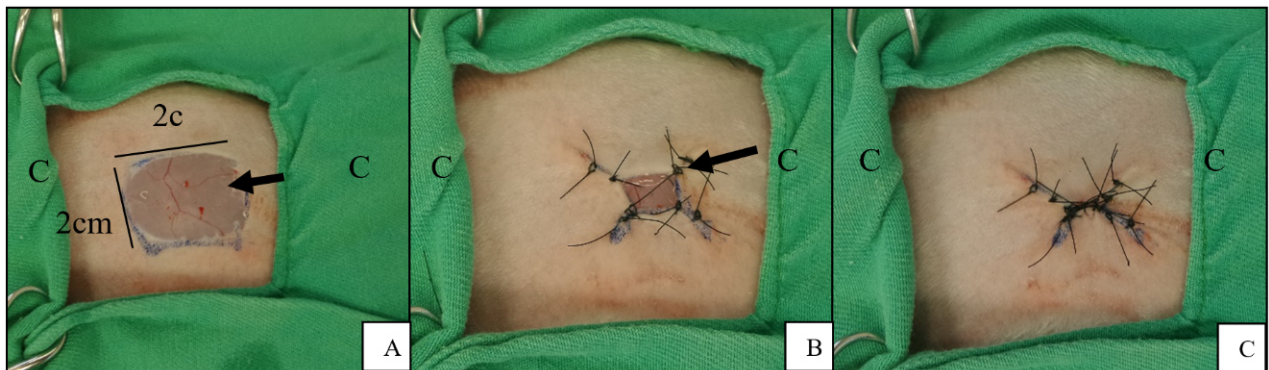

Figure 2. Photographs of the surgical wound. A) Square cutaneous defect (4 cm², 2 cm long × 2 cm wide) with the cutaneous muscle of the trunk as the limit for the incision depth (arrow). B) Synthesis of the defect in a geometric pattern pattern from the tip toward the center of the lesion (arrow). C) Final appearance of the surgical wound showing an X-shaped scar. CD, flow; CR, cranial.

At the end of the surgery, the wound was covered with gauze and tape. The parameters of HR, RR, mean arterial pressure (MAP), and end-tidal isoflurane concentration were monitored at seven different times between the preoperative and perioperative periods (continuous act, after administration of preoperative medication). Preanesthesia, anesthetic induction, administration of the tumescent solution, at the beginning of the surgery, at 5 min, 10 min, and at the end of surgery). The procedure time, from the administration of preanesthetic medication to the end of the surgery, was 30 min for all animals. In addition, the animals were randomly operated upon by the same surgeon.

The animals were returned to their respective cages for observation of the anesthetic recovery until complete normalization of the RR, the start of active movement, and the search for water and food. They were identified based on the date of the surgical procedure and the type of treatment they received. As an analgesia protocol, the animals received tramadol hydrochloride 4 mg/kg subcutaneously every 8 h for 7 days, antibiotic therapy with pentabiotic 0.06 mL/kg subcutaneously every 48 h for 5 days and anti-inflammatory meloxicam 0.2 mg/kg subcutaneously for 3 days on day 1 and 0.1 mg/kg every 24 h on days 2 and 3.

Evaluation of postoperative analgesia

To assess postoperative analgesia, the sensitivity of the peri-incisional region was evaluated using von Frey filaments (Electronic von Frey Anesthesiometer, model 1601; IITC Inc. Life Sciences, CA, USA) (Figure 3A). Two assessments were performed at 3-s intervals at two distinct previously marked points cranial to the surgical incision, one dorsal and one ventral, at 1 cm from the lesion (Figure 3B). The evaluators were blinded to the experimental groups.
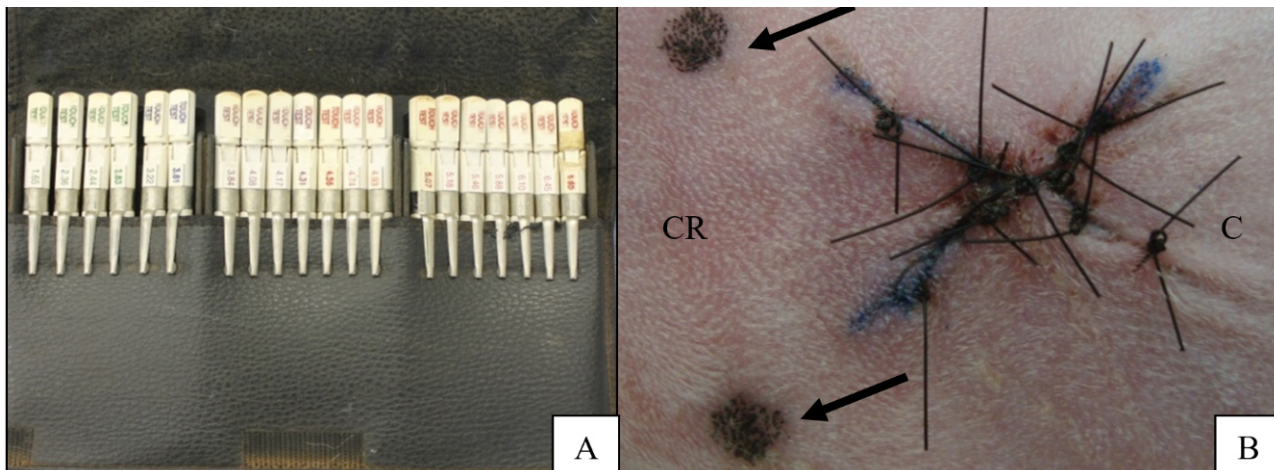
Figure 3. Photographic images of the analgesia analysis method. A) Set of von Frey filaments (Electronic von Frey Anesthesiometer, model 1601, IITC Inc. Life Science). B) Points previously marked with a pen to demarcate the site for the von Frey filaments to assess postoperative analgesia in rabbits undergoing surgical reconstruction (arrow). CD, flow; CR, cranial.

The absence of a response after filament use followed the evaluation with the next filament until an aversive response (escape attempt, vocalization) was obtained, which was recorded as the smaller diameter filament whose generated force produced a response. In cases in which the animal did not produce any response, even after evaluation with the filament with the largest diameter, the absence of pain sensitivity was recorded. The test was performed by two evaluators blinded to the group assignments before the surgical procedure to certify any response to the painful stimulus and 2, 8, 12, and 24 h after the end of the procedure.

Furthermore, for the evaluation of analgesia, the semi-objective visual analog scale (VAS) method was used. Using a straight 100-mm-long horizontal line with the ends representing antagonistic pain intensities (absence of pain and maximum pain possible)[13], two evaluators blinded to the group assignments visually evaluated each animal individually and noted the absence or presence of pain symptoms.

According to Rivera[14], the decrease in water and food consumption, looking at the back of the cage, limited movements, photosensitivity, and stoic behavior were recorded on a vertical line. The first evaluation was performed 1 h after the end of the surgery and repeated at 2, 4, 8, 12, and 24 h after the end of the surgical procedure. When necessary, these evaluators were instructed to reapply the analgesics (tramadol hydrochloride 4 mg/kg) in animals with a recorded value above 60 mm on the scale.

Macroscopic evaluation

The animals submitted to the surgical procedure were observed for 15 days for macroscopic evaluation of the lesion by the same blinded evaluator. All rabbits were examined daily for physiological characteristics, and macroscopic wound assessments were performed 3, 7,

and 14 days after the surgical procedure. The presence of exudate, coloration, and edema as well as the cosmetic aspect of the wound were observed and subsequently graded (Table 1).

Table 1. Analysis of the clinical classifications of the surgical wound (Adapted from Paim et al., 2002)[15]

Variables	Intensity			
Exudate	0 (absent)	1 (discreet)	2 (moderate)	3 (intense)
Coloring	0 (whitish)	1 (rose)	2 (reddish)	3 (blackened)
Edema	0 (absent)	1 (discreet)	2 (moderate)	3 (intense)
Cosmetic appearance	0 (excellent)	1 (good)	2 (regular)	3 (bad)

The data were recorded on a specific form for each rabbit for later analysis. The dressings were changed at 3, 7, and 14 days, as follows: 1) cleaning the wound with saline solution (NaCl 0.9%), 2) removal of exudate when necessary, 3) cover with gauze, and 4) fixation of the gauze with tape.

Euthanasia and material collection

Three, three, and three animals were euthanized in each group at 3, 7, and 15 days postoperative for the evaluations. Microscopic examinations of the surgical wound were performed in the three phases of healing, inflammatory, proliferation, and remodeling phases[16].

Each euthanasia was performed with propofol 10 mg/kg administered intravenously, followed by the infusion of potassium chloride according to the ethical principles in animal experimentation recommended by the National Council for the Control of Animal Experimentation.

Table 2. Classification and attribution of indexes to histological findings in sections stained by hematoxylin and eosin and immunostained by Ae1/Ae3 and Caveolin-1 antibodies (Adapted from Garros et al.[17])

Histological findings	Intensity			
	Absent	Discreet	Moderate	Accentuated
Vascular proliferation	0	1	2	3
Mononuclear cells	0	1	2	3
Polymorphonuclear cells	0	1	2	3
Fibroblast proliferation	0	1	2	3
Collagenization	0	1	2	3
Re-epithelialization	0	1	2	3
Bleeding	0	1	2	3

Histochemistry and immunohistochemistry

The sections were stained using a routine histochemical method (HE) for analysis under optical microscopy of the epidermis and dermis to identify mononuclear cells, polymorphonuclear cells, hemorrhage, collagenization, and fibroblast proliferation. The histological evaluation was performed at 400× magnification.

For the immunohistochemical study, the sections were spread on previously cleaned and degreased glass slides prepared with an organosilane-based adhesive (3-aminopropyltriethoxysilane; Sigma Chemical Co., USA).

The sections were immunostained with the monoclonal antibody Ae1/Ae3 for analysis by optical microscopy of re-epithelialization and the polyclonal antibody Caveolin-1 for analysis of vascular proliferation. The histological evaluation was performed at 200× magnification for the Ae1/Ae3 antibody and 400× for the Caveolin-1 antibody. The immunostaining was performed as described by the manufacturer (Table 3).

Table 3. Antibodies used for immunohistochemical analyses in rabbits at the Veterinary Pathology Laboratory of the Abel Salazar Institute of Biomedical Sciences – ICBAS, Porto, Portugal, 2015

Antibody	Clone	Company	Dilution	Antigen recovery	Incubation period	Detection system
Ae1/Ae3	Monoclonal, Ae1/Ae3	ImPath USA	0,3194444444	Novocastra™ Epitope Retrieval Solutions, Leica Biosystems, New Castle Ltd, UK, 30 min, Banhomaria	Overnight at 4ºC	Vectastain Elite ABC Kit, Vector Laboratories, USA
Caveolina- 1	Polyclonal SCBT – Sc 894	Dako	0,3888888889	Not performed by numerous tests	45 min	Novolink™ Polymer Detection Systems, Leica Biosystems, New Castle Ltd, UK

Finally, the slides were dehydrated in increasing dilutions of alcohol, diaphonized in xylene, and mounted for microscopic analysis.

The data obtained by the Ae1/Ae3 antibody were evaluated using photomicrographs obtained by optical microscopy at 2× magnification. The images were analyzed using ImageJ® software with the Threshold Color plug-in to obtain the percentage of the total area of cell differentiation through the analysis of automated

particles according to the selection and measurement of areas based on color (GOBI, 2013).

The angiogenic index for Caveolin-1 was determined using the microvascular counting technique recommended by Maeda et al. (1995). The areas with the highest number of vessels at the lesion depth were analyzed. Any positively stained endothelial cell or cell group separated from adjacent microvessels and other connective tissue elements was considered a unitary

vessel. Vessels were counted in five fields previously selected with high vascular density at 400× magnification using an optical light microscope to which a reticle was adapted for stereology to avoid recounting structures. The microvascular count was determined twice by a single evaluator (FG) at two different times and is expressed as the mean number of vessels in each case studied.

Statistical analysis

The statistical analysis was performed using R® software (R Foundation for Statistical Computing, Vienna, Austria). The experimental design corresponded to a completely randomized design with repeated measures over time. The comparison between groups (GC, GS, and GL) in relation to macroscopic and microscopic categorical variables were analyzed using Friedman's non-parametric test and Dunn's post-test. For cardiorespiratory parameters, after proving the normality of residues and homogeneity of variances, the real or transformed data were evaluated by analysis of variance; when the difference between treatments was significant, the means were compared using Tukey's test. To compare the scores over time of the VAS and the strength of von Frey filaments, Friedman's test and Dunn's post-test were used. Values of $p < 0.05$ were considered statistically significant.

Results and discussion

Macroscopic evaluation

The macroscopic variables studied included exudate, color, edema, and wound cosmetic aspect (Figure 4). In the descriptive analysis of the data, between the evaluation days, the presence of exudate occurred, especially on day 7, in a discreet form, in 83.3% of the animals in the GS, followed by the GC (66.7%) and GL (33.3%) groups, respectively. Moderate exudate occurred in the GS after 3 days of evaluation in 22.2% of the animals.
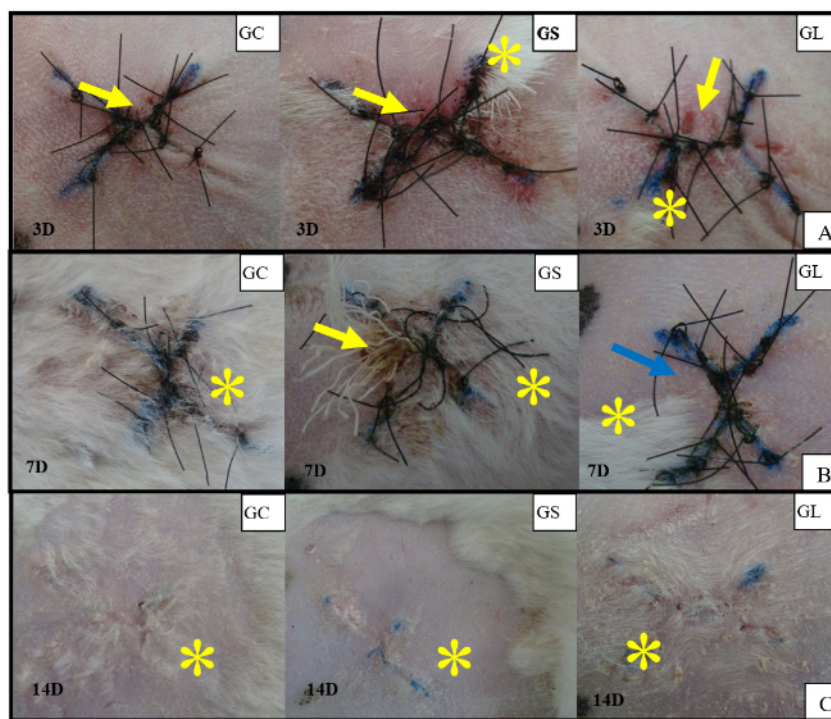
Figure 4. Photographs of macroscopic evaluations of the surgical wound of rabbits submitted to the closing of cutaneous defects in a geometric pattern. A) Presence of redness (arrow), hair growth (asterisk), and cosmetic appearance in the different groups 3 days after the surgical procedure. B) Presence of exudate (yellow arrow), hair growth (asterisk), and good cosmetic appearance of the surgical wound (blue arrow) at 7 days postoperative. C) Aspect of the wound at 14 days postoperative (asterisk). 3D, day 3; 7D, day 7; 14D, day 14; GC, control group; GL, lidocaine group; GS, solution group.

Regarding coloration, most animals were pink, with a reddish color (presence of redness) being observed mainly in GC and GS at 3 (11.1% in both groups) and 7 (16.7% in the two groups) days. As for edema, there was a slight presence in the three groups, occurring mainly at 3 days, especially in the GS group (22.2%). The cosmetic aspect ranged from excellent to regular, with most animals classified as having an excellent and good cosmetic appearance. A regular cosmetic appearance occurred mainly in the GS at 3 and 7 days (22.2% and 16.7%, respectively).

Among the results, no significant intergroup differences were noted at 3, 7, and 14 days (p>0.05).

The wound healing process can be divided into inflammatory, proliferation, and remodeling phases[16]. The characteristic signs of inflammation evaluated in the present study are related to the first phase of healing, and no intergroup differences were noted, which suggests that considering the macroscopic evaluation of the wound, lidocaine does not influence the first phase of healing. In addition, the fact that the color and cosmetic aspect of the surgical wound did not show intergroup differences, also suggests that the drug under the conditions of the present study did not impair the healing process. However, these results did not corroborate the study by Shekho et al. [18] in which lidocaine delayed the wound healing process in rabbits. Similarly, Ibrahim et al.[19] observed a delay in the healing process of wounds induced in donkeys when tumescense was performed with lidocaine, which was also used at higher concentrations. However, the associated use with epinephrine accelerated the healing process in the present study.

Microscopic evaluation

Discreet vascular proliferation was observed in 100% of the animals in the GC and GS on the third day of evaluation, while in the GL, in the same period, 66.7% of the animals were lacking vascular proliferation. Moderate vascular proliferation was observed at 7 days postoperative in 66.7% of the animals in the GC. At the time of evaluation, 66.7% of the animals in the GL and 66.7% of those in the GS showed discreet vascular proliferation. On day 14, the vascular proliferation varied from absent to moderate in the GC. In the GL, discreet vascular proliferation occurred in 66.7% of patients during the same evaluation period. In the GS group, 66.7% of the animals showed no vascular proliferation on day 14. However, there was no significant intergroup difference on the respective days (p>0.05).

Vessel proliferation in animals treated with lidocaine has been controversial in the literature. Drucker et al.[20] reported significantly less vascularization in guinea pigs subjected to infiltration with lidocaine, whereas Hanci et al.[21] reported that rats treated with lidocaine showed increased vascularization compared to those treated with saline. In the present study, the tumescent procedure with lidocaine did not influence angiogenesis. Drucker et al.[20] reported that, in addition to the concentration of lidocaine used being higher

than the concentration used in the present study, the route of administration differed, which may have interfered with angiogenesis, justifying the results found in the current study differing from theirs. Hanci et al.[21] used a higher concentration of lidocaine than that used here in addition to the different experimental models, which could explain the reported differences.

Mononuclear and polymorphonuclear cells were absent in the GL on the third day of healing, unlike in the GC, in which a slight presence of mononuclear cells was observed in 33.3% of the animals and polymorphonuclear cells in 66.7%. In the GS, a discreet presence of mononuclear cells was also observed in 66.7% of the animals and polymorphonuclear cells in 100% of the animals. On evaluation days 7 and 14, the animals of the three groups presented, above all, a slight presence of mononuclear cells, while polymorphonuclear cells varied from absent to moderate. However, no significant intergroup difference was observed on the respective days (p>0.05).

The absence of mononuclear and polymorphonuclear cells on evaluation day 3 in animals treated with lidocaine suggested a possible negative influence of lidocaine in the early stages of healing, when neutrophils and macrophages are attracted to the injured region. However, this was not confirmed by the statistical data analysis. These results are not in agreement with those of previous studies. Waite et al.[22] reported an increase in the number of neutrophils in rats treated with lidocaine, which influenced local inflammation. Hanci et al.[21] reported significant inflammation in rats treated with lidocaine compared to those treated with saline. These results, contrary to those found in the present study, are explained by the higher concentration of lidocaine used by these authors, which could be the cause of the greater inflammation.

Microscopic hemorrhage was observed within the three evaluated groups, ranging from absent to moderate, at different times of the evaluation. However, no significant intergroup difference was observed (p>0.05).

The pink color of the skin in the GL animals suggested a possible reduction in micro-hemorrhaging during the surgical procedure due to the use of epinephrine in the tumescent solution, which causes vasoconstriction. However, after the histological evaluations of the samples, microscopic hemorrhage was observed in the animals of the three groups ranging from absent to moderate at different times of the evaluation. The presence of microscopic hemorrhages in the animals at 3 days postoperative and the lack of significant intergroup differences contradicts the results that were expected in the GL versus the other groups. In the literature, the use of diluted epinephrine in the solution proposed for tumescense causes generalized and prolonged vasoconstriction[23,19], resulting in decreased intraoperative bleeding[24,25,5]. This result may have occurred because the surgical procedure was not very bloody, and significant macroscopic hemorrhages were not observed at

any time regardless of epinephrine status.

It can also be inferred that the presence of microscopic hemorrhages at 14 days postoperative is related to the process of angiogenesis, in which small-caliber newly formed capillaries rupture. Thus, the fact that there was no significant intergroup difference in hemorrhaging at evaluation day 14 is consistent with the result of no significant intergroup difference in vascular proliferation.

Moderate proliferation of fibroblasts and moderate collagenization were observed in 66.7% of the GC animals at evalution day 7. In the GL, moderate fibroblast proliferation and collagenization were observed only on day 14 in 66.7% of the animals. In the GS, both fibroblast proliferation and collagenization were absent in 66.7% of the animals at evaluation day 14. However, no significant intergroup difference was observed on the respective days (p>0.05).

Histological analysis of fibroblast proliferation and collagenization revealed possibly delayed healing in the GS and GL animals; however, this was not confirmed by the statistical analysis of the data, which was insufficient to create an unfavorable final result that could impede the surgical healing process. These results contradict those of Rodrigues et al.[10], Hanci et al.[21], and Shekho et al.[18], who reported an effect of lidocaine on collagenization in rats. The differences in the literature may have occurred due to

the higher concentration of lidocaine used by these authors, which could explain the negative influence of the anesthetic reported in these studies than in the current work.

In the study by Fedder et al.[26], the in vitro cytotoxic effect of lidocaine on the proliferation of human fibroblasts was dependent on the concentration of the anesthetic used. The results reported by Fedder et al.[26] corroborate the justification that the present study presents different results from those of Rodrigues et al.[10] and Hanci et al.[21] due to the different concentrations of lidocaine used. However, similar results were reported by Drucker et al.[20], in which no significant difference was observed in collagenization, and by Harris et al.[27], who did not find a cytotoxic effect or a significant reduction in proliferation in cultures of fibroblasts treated with lidocaine.

Moderate re-epithelialization was observed in 66.7% of the animals in the GC after 7 days of evaluation (Figure 5). However, in the GL, moderate re-epithelialization was observed in 66.7% of the animals after 14 days postoperative versus in 33.3% at 7 days. In the GS, at 7 and 14 days after surgery, 66.7% of the animals presented re-epithelialization, which was classified as absent. However, despite the intergroup differences in skin regeneration at the respective times, there was no overall significant difference between them (p>0.05).
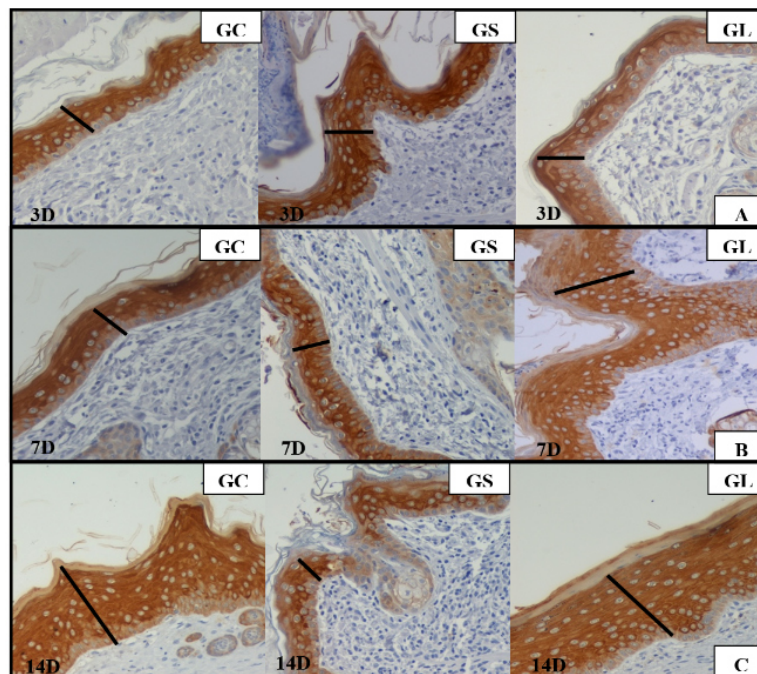

Figure 5. Photomicrographs of skin samples from rabbits submitted to the technique of closing skin defects in a geometric pattern pattern. A) Re-epithelialization at 3 days postoperative. B) Re-epithelialization at 7 days postoperative. C) Re-epithelialization at 14 days postoperative. Ae1/Ae3, immunostaining, streptoavidin-biotin complex method, DAB, 200× magnification. 3D, day 3; 7D, day 7; 14D, day 14; GC, control group; GL, lidocaine group; GS, solution group.

The re-epithelialization results were similar to those reported by Waite et al. (2010)(22) in lidocaine-treated rats. Although the concentrations used by those authors were higher than those used in the present study, there was no interference of lidocaine in the re-epithelialization of the surgical wound, suggesting that the drug did not influence it at the concentrations used. Harris et al. (2009)(27) also reported similar results in experimental models of the assessment of epithelial growth. The microscopic variable of re-epithelialization is shown in Figure 5, and the descriptive results of the microscopic evaluations are presented in Table 4.

Table 4. Microscopic evaluation findings of rabbits submitted to surgical reconstruction by study group on postoperative evaluation days 3, 7, and 14

	3D (n=3)			7D (n=3)			14D (n=3)		
	GC	GS	GL	GC	GS	GL	GC	GS	GL
Vascular proliferation (Caveolin-1)									
Absent	0	0	66,7	0	0	0	33.3	66.7	0
Discreet	100	100	33.3	33.3	66.7	66.7	33.3	33.3	66.7
Moderate	0	0	0	66.7	33.3	33.3	33.3	0	33.3
Increased	0	0	0	0	0	0	0	0	0
Mononuclear cells (HE)									
Absent	66.7	33.3	100	0	33.3	33.3	0	33.3	33.3
Discreet	33.3	66.7	0	100	66.7	66.7	100	66.7	66.7
Moderate	0	0	0	0	0	0	0	0	0
Increased	0	0	0	0	0	0	0	0	0
Polymorphonuclear cells (HE)									
Absent	33.3	0	100	0	33.3	33.3	0	33.3	0
Discreet	66.7	100	0	100	33.3	33.3	100	0	66.7
Moderate	0	0	0	0	33.3	33.3	0	66.7	33.3
Increased	0	0	0	0	0	0	0	0	0
Fibroblast proliferation (HE)									
Absent	100	33.3	100	33.3	33.3	33.3	0	66.7	33.3
Discreet	0	66.7	0	0	33.3	33.3	66.7	33.3	0
Moderate	0	0	0	66.7	33.3	33.3	33.3	0	66.7
Increased	0	0	0	0	0	0	0	0	0
Collagenization (HE)									
Absent	100	33.3	100	0	66,7	33.3	33.3	66.7	33.3
Discreet	0	66.7	0	33.3	33,3	33.3	33.3	33.3	0
Moderate	0	0	0	66.7	0	33.3	33.3	0	66.7
Increased	0	0	0	0	0	0	0	0	0
Re-epithelialization (Ae1/Ae3)									
Absent	0	0	33.3	0	66.7	0	33.3	66.7	33.3
Discreet	100	66.7	66.7	33.3	33.3	66.7	0	33.3	0
Moderate	0	33.3	0	66.7	0	33.3	66.7	0	66.7
Increased	0	0	0	0	0	0	0	0	0
Hemorrhage (HE)									
Absent	0	0	0	0	33.3	0	66.7	66.7	0
Discreet	66.7	33.3	33.3	0	33.3	66.7	33.3	0	66.7
Moderate	33.3	66.7	66.7	100	33.3	33.3	0	33.3	33.3
Increased	0	0	0	0	0	0	0	0	0

GC, control group; GL, lidocaine group; GS, solution group 3D, day 3; 7D, day 7; 14D, day 14; HE, hematoxylin and eosin

Cardiorespiratory parameters

The global mean value of cardiorespiratory parameters recorded at the seven proposed moments (shortly after the administration of preanesthetic medication; anesthesia induction; administration of the tumescent solution at the beginning of surgery, at 5 minutes, at 10 minutes, and at the end of surgery) was calculated and the following

results were found: HR and end-tidal isoflurane concentration was lower in the GL, RR was higher in the GC, and MAP was higher in the GL. The analyzed parameters are shown in (Figure 6).
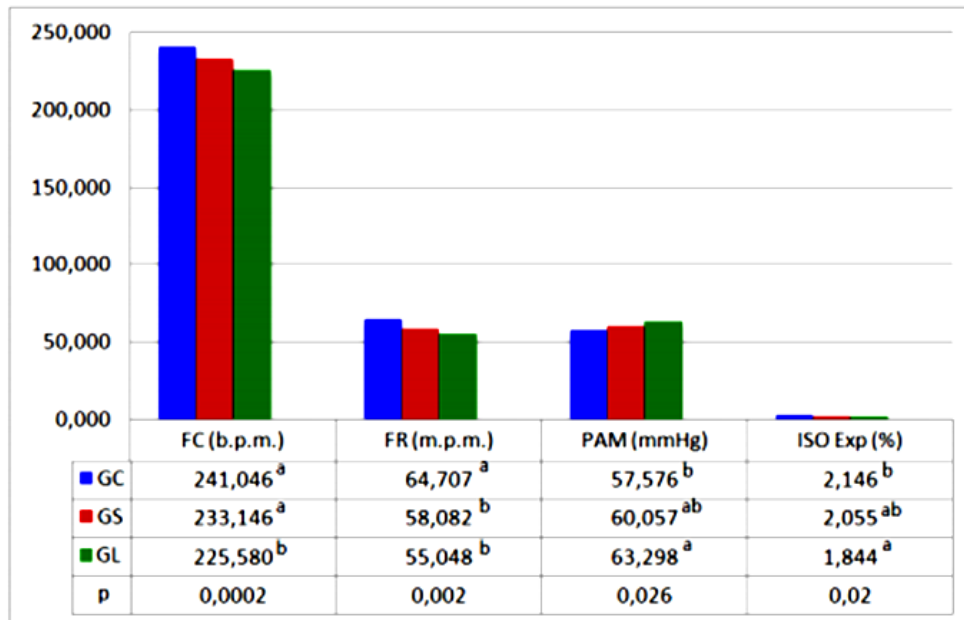

Figure 6. Graphic representation of the mean values of cardiorespiratory parameters in rabbits submitted to the technique of closing cutaneous defects in a geometric pattern. GC, control group; GL, lidocaine group; HR, heart rate; ISO Exp, expired isoflurane; MAP, mean arterial pressure; RR, respiratory rate
*Means with the same letter were not significant on Tukey's test (p<0.05).

The normal HR in rabbits is 160–300 bpm[28]; in the three study groups, the HR remained within the normal values. However, there was a significant difference in the values of the GL and those of the GC and GS (p=0.0002) during the procedure, in which the former remained below the other two at most of the evaluated moments. As for RR, the normal range is 30–60 m, and the rabbit has reduced lung capacity[29]. There was a significant difference in RR in the GC compared to the GS and GL (p=0.002) in which the former remained above the upper limit compared to the other groups.

The fact that the HR and RR were lower in the GL suggests less pain during the surgical procedure, a consequence of better intraoperative analgesia of these animals provided by the use of the tumescent anesthesia with lidocaine. According to Banchi et al.[30], pain and hypotension can increase HR and RR, which corroborates the findings of the present study. Guirro, Cunha, and Thomas[9] reported that the HR and RR of bitches submitted to mastectomy remained more stable when general anesthesia was associated with the use of tumescent anesthesia with lidocaine. These results corroborate those of the current study given the greater stability of the parameters over time, a consequence of the better intraoperative analgesia.

The physiological values for MAP in rabbits were 70–103 mmHg[31]. The statistical analysis of the data revealed a significant difference in the MAP between the GC and GL (p=0.026). The fact that the MAP was higher in the GL than in the GC suggests that in the animals in which the tumescent anesthesia with lidocaine was used, less hypotension was noted during the surgical procedure as a consequence of a lower consumption of inhalational anesthetic due to less intraoperative pain compared to animals in which inhalational anesthesia with isoflurane was used exclusively. The significant intergroup difference in MAP corroborates the significant difference found in HR, which remained lower as a reflection of a higher MAP.

There was a significant difference in expired isoflurane between the GC and GL groups (p=0.02). The results of the present study were in accordance with the literature. Guirro, Cunha, and Thomas[9] reported a reduction in isoflurane consumption in bitches undergoing mastectomy when general anesthesia was associated with the use of tumescent anesthesia with lidocaine. Furthermore, according to Moens[32], the use of locoregional anesthesia associated with general anesthesia allowed for more stable anesthesia with lower doses of general anesthetic required to obtain an adequate surgical plan, allowing a more

superficial surgical plan with less cardiovascular and respiratory depression.

Evaluation of postoperative analgesia

In terms of VAS scores, there was no significant intergroup difference in pain or between the evaluated and evaluator moments (p>0.05). Regarding the von Frey filament test, there was no significant difference in hyperalgesia between groups or time points (p>0.05). Furthermore, no animals required the reapplication of analgesics outside of the proposed times in the first 24 h postoperative, suggesting that the analgesic protocol was effective in all groups.

The von Frey filament test is an objective test that allows for cutaneous sensitivity. The VAS is characterized as a semi-objective scoring system with the purpose of quantifying pain intensity. Despite being tests that present totally different methodologies, the results were similar since there were no significant intergroup differences for either and both tests were adequate for evaluating analgesia under the conditions of the present study.

Pohl et al.[33] previously concluded that von Frey filaments were not very effective for assessing postoperative pain in bitches undergoing ovariohysterectomy, while the VAS was considered the best way to assess pain. The same author justified these differences because ovariohysterectomy promotes not only superficial pain; therefore, the von Frey filament test is inadequate. Unlike the work by Pohl et al.[33], the present study assessed only superficial pain, proving the usefulness of the VAS and the von Frey filament assessments and justifying the similar results obtained on both tests. On the other hand, in the study by Rocha et al.[34], von Frey filaments were effective for measuring postoperative hyperalgesia in bitches undergoing unilateral mastectomy with tumescent anesthesia.

According to Luna[35], it is necessary to know how to recognize pain to prevent and treat it. Thus, knowing the behavioral changes presented by the species under study and training the evaluators to recognize and identify them ensures that the VAS evaluation is adequate for the evaluation of postoperative pain, while the von Frey filament test can be adequate because the surgical procedure involved only superficial pain.

Results contrary to those observed in the present study were reported by Guirro, Cunha, and Thomas[9] in bitches submitted to a bilateral mastectomy procedure. Using the VAS method, the authors observed significant differences in pain between bitches treated with tumescent anesthesia with lidocaine and those not treated with tumescent anesthesia. Due to the scarcity of data and studies in the literature on the subject, the authors adapted the method used in female dogs. The different results between the work by Guirro, Cunha, and Thomas [9] and the present study can be explained by the different surgical techniques used as a surgical model for analgesia assessments. Guirro, Cunha, and Thomas [9] used bilateral mastectomy, whereas the present study used the technique of closing cutaneous

defects in a geometric figure pattern. According to Gakiya et al. [36], radical mastectomy is invasive and extensive and results in moderate and intense pain. Thus, the bilateral mastectomy surgery used by Guirro, Cunha, and Thomas[9] caused more postoperative pain than the reconstructive surgery of closure in a geometric figure pattern used in the present study, which involved only superficial pain. Therefore, the results show that the action of tumescent anesthesia in pain control in less invasive techniques does not interfere with the presence of postoperative pain, unlike what happened in the radical mastectomy study. It was also suggested that the analgesic protocol used in the current study in all animals adequately controlled postoperative pain and did not require the use of tumescent anesthesia.

Rosaeg et al.[37] also reported significant differences in women undergoing breast reduction in which reduced postoperative pain was observed using the VAS in patients who underwent tumescent anesthesia with lidocaine. Such differences can also be explained by the fact that the surgical procedures studied by other authors caused more postoperative pain than the surgical procedure used in the current study, which may demonstrate the action of tumescent anesthesia.

Conclusion

The results of the present study suggest that tumescent anesthesia with lidocaine does not influence the healing process or impair postoperative tissue repair. Its use offers benefits such as reduced consumption of inhalational anesthetic, which allows the surgical procedure to be performed in an appropriate anesthetic plane, maintains stable HR and RR, and ensures lower hypotension during the surgical procedure. Furthermore, we found no difference in postoperative analgesia between inhalational general anesthesia and tumescent anesthesia with lidocaine for non-radical procedures.

Conflict of interest

The authors declare no conflicts of interest.

Author Contributions

Conceptualization: E. L. Serafim, J. M. Pazzini, R. A., R. R. A. Uscategui; *Data curation:* E. L. Serafim, J. M. Pazzini; *Formal Analysis:* E. L. Serafim, J. M. Pazzini; *Funding acquisition:* E. L. Serafim, J. M. Pazzini; *Investigation:* E. L. Serafim, J. M. Pazzini, M. F. M. Gartner, I. Amorim, A. Rema, F. Faria, V. T. Almeida, C. A. Calpaa; *Methodology:* E. L. Serafim, J. M. Pazzini, M. F. M. Gartner, I. Amorim, A. Rema, F. Faria, V. T. Almeida, C. A. Calpaa; *Project administration:* E. L. Serafim, J. M. Pazzini; *Resources:* J. M. Pazzini; *Software:* R. R. A. Uscategui; *Supervision:* E. L. Serafim, J. M. Pazzini, A. B. De Nardi, S. G. Calazans; *Validation:* A. B. De Nardi, S. G. Calazans; *Visualization:* E. L. Serafim, J. M. Pazzini, A. B. De Nardi, S. G. Calazans; *Writing – original draft:* E. L. Serafim, J. M. Pazzini; M. C. P. Rocha; L. C. M. Linhares, M. F. M. Gartner, I. Amorim, A. Rema, F. Faria, V. T. Almeida, C. A. Calpaa;

References

1. Pazzini JM, De Nardi AB, Castro JLC, Huppes RR. Técnicas de fechamento geral e em padrão de figuras geométricas. In: Castro, JLC, Huppes RR, De Nardi AB, Pazzini JM. Princípios e Técnicas de Cirurgias Reconstrutivas da Pele de Cães e Gatos (Atlas Colorido). 1st ed. Curitiba: Medvep; 2015. p.77-88. Portuguese.

2. Trout NJ. Princípios da Cirurgia Plástica e Reconstrutiva. In: Slatter D. Manual de Cirurgia de Pequenos Animais. 3rd ed. Barueri: Manole; 2007. p. 274-292. Portuguese.

3. Uscategui, RAR; Silva, JAR. Anestesia e analgesia na cirurgia reconstrutiva. In: Castro JLC, Huppes RR, De Nardi AB, Pazzini JM. Princípios e Técnicas de Cirurgias Reconstrutivas da Pele de Cães e Gatos (Atlas Colorido). 1st ed. Curitiba: Medvep, 2015. p. 51-76. Portuguese.

4. García-Somacarrera E, Rodriguez-Sanjuán JC, Carceller-Malo JM, Gómez-Fleitas M. Anestesia local por tumescencia con solución de Klein modificada comparada con anestesia general en la intervención por sinus pilonidal. Cirugía Española. 2003; 74(2): 82-85. DOI: 10.1016/s0009-739x(03)72193-9.

5. Thomas J. Adjunctive tumescent technique in massive resections. Aesthetic Plastic Surgery. 2001; 25(5): 343-346. DOI: 10.1007/s002660010146

6. Holt NF. Tumescent anaesthesia: its applications and well tolerated use in the out-of-operating room setting. Current Opinion in Anesthesiology. 2017; 30(4): 518-524. DOI: 10.1097/ACO.0000000000000486.

7. Biben JA, Prasetyono TO, Tantri AR. Experimental study of tumescence injection to provide anesthesia without local anesthetics. Journal of Surgical Research. 2020; 245: 295-301. DOI: 10.1016/j.jss.2019.07.066.

8. Moreira CM, Oliveira RL, Costa, GA, Corgozinho KB, Luna SP, Souza HJ. Evaluation of tumescent local anesthesia in cats undergoing unilateral mastectomy. Veterinary Anaesthesia and Analgesia. 2021; 48(1): 134-141. DOI: 10.1016/j.vaa.2020.08.006.

9. Guirro ECB. P, Cunha O, Thomas LD. Efeito antinociceptivo da anestesia local por tumescência em cadelas submetidas à mastectomia completa bilateral. Veterinária em Foco. 2013; 11(1): 12-21. (http://www.periodicos.ulbra.br/index.php/veterinaria/article/view/1363/1115).

10. Rodrigues FV, Hochman B, Wood VT, Simões MJ, Juliano Y, Ferreira LM. Effects of lidocaine with epinephrine or with buffer on wound healing in rat skin. Wound Repair and Regeneration. 2011; 19(2): 223-228. DOI: 10.1111/j.1524-475X.2010.00654.x.

11. Abimussi CJX, Menegheti TM, Wagatsuma JT, Floriano BP, Arruda AMM, Santos PSP, Oliva VNLS. Tumescent local anesthesia with ropivacaine in different concentrations in bitches undergoing mastectomy: plasma concentration and post-operative analgesia. Veterinary Anaesthesia and Analgesia.

2014; 41(5):516-525. DOI: 10.1111/vaa.12178.S

12. Uttamani RR, Venkataram A, Venkataram J, Mysore V. Tumescent anesthesia for dermatosurgical procedures other than liposuction. Journal of Cutaneous and Aesthetic Surgery. 2020; 13(4): 275. DOI: 10.4103/JCAS.JCAS_192_19.

13. Hellyer PW. Pain Identification. In: Ettinger S, Feldman C. Textbook of Veterinary Internal Medicine. St. Louis: Elsevier Inc, 2005. v. 1, p. 16- 21. English.

14. Rivera EAB. Analgesia em Animais de Experimentação. In: Andrade A, Pinto SC, Oliveira RS. Animais de laboratório: criação e experimentação. Rio de Janeiro: Fiocruz, 2002. p.247-253. Portuguese. DOI: https://doi.org/10.7476/9788575413869.

15. Paim CBV, Raiser AG, Cardoso E, Beck C. Enxerto autólogo de pele, em malha, com espessura completa, na reparação de feridas carpometacarpianas de cães. Resposta a irradiação a laser AsGa. Ciência Rural. 2002; 32(3): 451-457. (https://www.researchgate.net/publication/277166945)

16. Tottoli EM, Dorati R, Genta I, Chiesa E, Pisani S, Conti B. Skin wound healing process and new emerging technologies for skin wound care and regeneration. Pharmaceutics. 2020; 12(8): 735. DOI: 10.3390/pharmaceutics12080735.

17. Garros IC, Campos ACL, Tâmbara EM, Tenório SB. Torres O. JM, Agulham MA, Araújo ACF, Santis-Isolan PMB, Oliveira, RM, Arruda ECM. Extrato de *Passiflora edulis* na cicatrização de feridas cutâneas abertas em ratos: estudo morfológico e histológico. Acta Cirúrgica Brasile**ira**. 2006; 21: 55-65. DOI: 10.1590/S0102-86502006000900009.

18. Shekho HA, Wadeeb SA, Al-bayatic HHK, Ismaeald, MA. Evaluation the effect and efficacy of lidocaine on healing of skin wound in rabbits. *Systematic Reviews in Pharmacy*. 2020; *11*(12), 1368-1372.(https://www.sysrevpharm.org/articles/evaluation-the-effect-and-efficacy-of-lidocaine-on-healing-of-skin-wound-in-rabbits.pdf).

19. Ibrahim A, Ali M, Khafar SA. The Effect of Lidocaine with/without Epinephrine on Healing of Cutaneous Incised Wounds in Donkeys: An Experimental Study. J Anesth Clin Res. 2015; 6(533), 2. DOI: 10.4172/2155-6148.1000533.

20. Drucker M, Cardenas E, Arizti P, Valenzuela A, Gamboa A. Experimental studies on the effect of lidocaine on wound healing. World Journal of Surgery. 1998; 22(4): 394-397. DOI: 10.1007/s002689900403.

21. Hanci V, Hakimoğlu S, Özaçmak H, Bektaş S, Özaçmak HS, Özdamar ŞO, Yurtlu S, Turan IÖ. Comparação dos Efeitos da Infiltração com Bupivacaína, Lidocaína e Tramadol na Cicatrização de Feridas em Ratos. Revista Brasileira de Anestesiologia. 2012; 62(6): 799-810. DOI: 10.1590/S0034-70942012000600005.

22. Waite A, Gilliver SC, Masterson GR, Hardman MJ, Ashcroft GS. Clinically relevant doses of lidocaine and bupivacaine do not impair cutaneous wound healing in mice. British Journal of Anaesthesia. 2010; 104(6): 768-773. DOI: 10.1093/bja/aeq093.

23. Klein JA. Tumescent liposuction and improved postoperative care using Tumescent Liposuction Garments. Dermatologic Clinics. 1995; 13(2): 329-338. DOI: 10.1016/S0733-8635(18)30085-8.

24. Kim H, Hwang K, Yun SM, Kim DJ. Usage of epinephrine mixed with lidocaine in plastic surgery. Journal of Craniofacial Surgery. 2020; 31(3), 791-793. DOI: 10.1097/SCS.0000000000006156.

25. Uslu AB. Effect of tumescent lidocaine and epinephrine infiltration on blood loss in inferior pedicle wise-pattern breast reduction: a prospective randomized study. Aesthetic Plastic

Surgery. 2021; 45(2): 442-450. DOI: 10.1007/s00266-020-01859-z.

26.Fedder C, Beck-schimmer B, Aguirre J, Hasler M, Rothz'graggen B, Urner M, Kalberer S, Schlicker A, Votta-velis G, Bonvini JM, Graetz K, Borgeat A. In vitro exposure of human fibroblasts to local anaesthetics impairs cell growth. Clinical & Experimental Immunology. 2010; 162(2): 280-288. DOI: 10.1111/j.1365-2249.2010.04252.x.

27.Harris KL, Bainbridge NJ, Jordan NR, Sharpe JR. The effect of topical analgesics on ex vivo skin growth and human keratinocyte and fibroblast behavior. Wound Repair and Regeneration. 2009; 17(3): 340-346. DOI: 10.1111/j.1524-475X.2009.00488.x.

28.Fonseca NM, Goldenberg S, Gomes PO, Lima CAP. Anestesia em coelhos. Acta Cirúrgica Brasileira. 1996; 11(2): 82-104. (https://pesquisa.bvsalud.org/portal/resource/pt/lil-177652).

29.Gillet CS. Selected drug dosages and clinical reference data. In: Manning PJ, Ringler DH, Newcomer CE. The biology of the laboratory rabbit. 2. ed. London: Academic Press, 1994. p. 468-472. English.

30.Banchi P, Quaranta G, Ricci A, Mauthe von Degerfeld M. Reliability and construct validity of a composite pain scale for rabbit (CANCRS) in a clinical environment. *PloS one*. 2020; *15*(4): e0221377. DOI: 10.1371/journal.pone.0221377.

31.Flecknell PA. Anaesthesia of animals for biomedical research. British Journal of Anaesthesia. 1993; 71(6): 885-894. DOI: 10.1093/bja/71.6.885.

32.Moens Y. Local Anaesthetics for Perioperative Pain Management: Underestimated Potential? World Small Animal Veterinary Association World Congress Proceedings, 2004. (https://www.vin.com/apputil/content/defaultadv1.aspx?id=3852117&pid=11181&)

33.Pohl VH, Carregaro AB, Lopes C, Garlet C, Marques JS. Correlação entre as escalas visual analógica, de Melbourne e filamentos de Von Frey na avaliação da dor pós-operatória em cadelas submetidas à ovariossalpingohisterectomia. Ciência Rural. 2011; 41(1): 154-159. DOI: 10.1590/S0103-84782011000100025.

34.Rocha FDL, Nunes N, dos Santos PCD, Ido CK, Santo Silva PDE, Aidar ESA, da Silva RA, Prada TC. Postoperative Analgesia Time in Dogs Submitted to Mastectomy and Anesthetized with Tumescent Solutions of Lidocaine or Ropivacaine. Acta Scientiae Veterinariae. 2020; 48: 1747. DOI: 10.22456/1679-9216.102649.

35.Luna SPL. Dor, senciência e bem-estar em animais: senciência e dor. Ciência Veterinária nos Trópicos. 2008; 11(1): 17-21. (https://docplayer.com.br/7331585-Dor-senciencia-e-bem-estar-em-animais-senciencia-e-dor-stelio-pacca-loureiro-luna-1.html)

36.Gakiya HH, Silva DA, Gomes J, Stevanin H, Cassu RN. Electroacupuncture versus morphine for the postoperative control pain in dogs. Acta Cirúrgica Brasileira. 2011; 26(5): 346-351. DOI: 10.1590/S0102-86502011000500004.

37.Rosaeg OP, Bell M, Cicutti NJ, Dennehy KC, Lui AC, Krepski B. Pre-incision infiltration with lidocaine reduces pain and opioid consumption after reduction mammoplasty. Regional Anesthesia and Pain Medicine. 1998; 23(6): 575-579. DOI: 0.1016/s1098-7339(98)90084-0.