Ciência Animal Brasileira
Brazilian Animal Science

DOI: 10.1590/1809-6891v25e-79416E

e-ISSN 1809-6891
Veterinary Medicine | Research article

# Acute-phase proteins in rabbits undergoing laparoscopic cholecystectomy: LigaSure device versus electrosurgery

## Proteínas de fase aguda em coelhos submetidos à colecistectomia laparoscópica – LigaSure versus eletrocirúrgico

**Ana Clara Silva Machado*[1], Mônica Carolina Nery Wittmaack[1], Maria Camila Maldonado Vera[1], Daniela Gomes da Silva[1], Guillherme Sembenelli[1], Gabriel Luiz Montanhim[1], Cléber Kazuo Ido[1], Juliana de Oliveira Ribeiro[1], Mareliza Possa de Menezes[1], Paola Castro Moraes[1]**

[1]Universidade Estadual Paulista "Júlio de Mesquita Filho" (Unesp), Jaboticabal, São Paulo, Brazil
*corresponding author: acmachado237@hotmail.com

**Abstract:** Studies have demonstrated that the LigaSure device causes less tissue damage than bipolar electrosurgery. Increases and decreases in protein and immunoglobulin concentrations after laparoscopic cholecystectomy are expected and transient. This study aimed to compare serum values of acute-phase proteins and immunoglobulins in rabbits undergoing gallbladder dissection using bipolar electrosurgery (Maryland forceps) and vessel sealing device (VSD) LigaSure. The objective was to determine which method resulted in less inflammatory change. Twenty rabbits were divided into two groups of ten each. Group 1 underwent laparoscopic cholecystectomy with bipolar electrosurgical forceps for dissection and LigaSure for sealing the cystic duct. Group 2 underwent dissection and cystic duct sealing using VSD–LigaSure only. Acute-phase proteins and immunoglobulins were evaluated on postoperative days three, seven, and fifteen. Serum concentrations of fibrinogen, transferrin, IgG, α1-acid glycoprotein, PM 23000 Da, and C-reactive protein (CRP) did not differ significantly between groups. However, significant differences were observed between evaluation days within the same group. IgA, ceruloplasmin, and haptoglobin were not statistically analyzed for either group or day comparisons. Only albumin levels differed between groups, with group 1 showing a lower protein concentration on day 15. Both methods caused changes in acute-phase proteins, indicating no significant advantage for using the LigaSure device.

**Keywords:** dissection; gallbladder; inflammation; video surgery

**Resumo:** Estudos demonstram que o dispositivo selante de vasos DSV-LigaSure promove menor dano tecidual que o eletrocirúrgico bipolar e o aumento e diminuição das concentrações de proteínas e imunoglobulinas após a colecistectomia laparoscópica é esperado e transitório. Objetivou-se comparar os valores séricos das proteínas de fase aguda e imunoglobulinas de coelhos submetidos à dissecção da vesícula biliar utilizando eletrocirúrgico bipolar (pinça Maryland) e DSV-LigaSure para determinar qual dos dissectores apresentou menor alteração inflamatória. Vinte coelhos foram distribuídos em dois grupos com dez animais cada, sendo o Grupo 1 submetido à técnica de colecistectomia laparoscópica utilizando o eletrocirúrgico bipolar para dissecção e DSV-LigaSure para selar o ducto cístico e o Grupo 2, submetido à dissecção da vesícula biliar e selagem do ducto cístico utilizando o DSV-LigaSure. As proteínas de fase aguda e imunoglobulinas foram avaliadas nos dias três, sete e 15 do período

Received: May 21, 2024. Accepted: June 25, 2024. Published: September 24, 2024.

pós-operatório. As concentrações séricas de fibrinogênio, transferrina, IgG, α1- glicoproteína ácida, PM 23000 Da e proteína C reativa não apresentaram diferenças significativas entre os grupos, apenas diferenças significativas entre os dias de avaliação no mesmo grupo. IgA, ceruloplasmina e haptoglobina não apresentaram diferenças estatísticas entre grupos e nem entre dias de avaliação. Apenas albumina apresentou diferença entre grupos, onde o Grupo 1 demonstrou menor concentração da proteína após 15 dias. Ambos dissectores apresentaram alterações nas proteínas de fase aguda, demonstrando que não houve superioridade na técnica utilizando o DSV-LigaSure.

**Palavras-chave:** dissecção; inflamação; videocirurgia; vesícula biliar

## 1. Introduction

The use of minimally invasive surgical techniques in veterinary medicine has intensified in recent years. These techniques are associated with less tissue damage, faster recovery times, reduced pain, and fewer postoperative complications than traditional open surgery [1]. Surgical trauma triggers inflammatory processes through the release of cytokines. However, minimally invasive techniques like laparoscopy appear to induce a less pronounced inflammatory response by reducing surgical trauma [2].

Laparoscopic cholecystectomy (LC) is a surgical procedure indicated for gallbladder abnormalities such as cholecystitis, mucocele, neoplasms, and ruptures. Studies in humans compare different laparoscopic techniques and their suitability based on the severity of the condition [2]. Several studies have investigated the use of vessel sealing devices (VSDs) like LigaSure and harmonic devices in laparoscopic surgery. These devices employ bipolar or ultrasonic energy for sealing and cutting tissues. High frequency is known to promote tissue heating and protein denaturation, creating a template of denatured collagen and elastin for a biological seal [3, 4].

A study in pigs evaluated the ability of different laparoscopic devices to cause postoperative adhesion formation. This study compared monopolar electrocautery and LigaSure VSD. The group treated with LigaSure VSD did not develop adhesions, while the monopolar electrocautery group had a higher adhesion score [5]. Another study concluded that VSD-LigaSure performed well during canine splenectomy procedures, achieving good hemostasis without requiring surgical dissection before vessel sealing. It also resulted in minimal postoperative complications [6].

Acute-phase proteins (APPs) are blood proteins whose concentrations change in response to surgical injury, infection, inflammation, and stress. These changes are linked to the severity of disorders and the extent of tissue damage, aiding in diagnosis and prognosis. After an injury, an inflammatory response, known as an acute-phase response, is activated. This response releases cytokines, such as interleukins, which stimulate the release of leukocytes, fibroblasts, endothelial cells, and the synthesis of APPs in hepatocytes. APPs can be classified as positive or negative based on their concentration changes. While the body increases the production of some proteins, others decrease due to inhibited synthesis. During injury and inflammation, serum concentrations of positive proteins like C-reactive protein, haptoglobin,

α1-acid glycoprotein, ceruloplasmin, and fibrinogen increase, while negative proteins like albumin and transferrin decrease [7, 8, 9].

This study investigated which laparoscopic technique, gallbladder dissection using VSD-LigaSure or Maryland bipolar electrosurgical forceps, promotes less inflammatory change in rabbits by analyzing the behavior of acute-phase proteins and immunoglobulins.

## 2. Materials and methods

The present study was approved by the Animal Use Ethics Committee (CEUA) of the College of Agricultural and Veterinary Sciences – FCAV/UNESP – Campus of Jaboticabal (protocol nº016539/17). Twenty New Zealand White rabbits were used, weighing between 3.0 and 4.0 kg, all males and aged between eight and 12 months. The 20 rabbits were divided into two groups: Group 1 (Bipolar electrosurgery – Maryland) and Group 2 (Vessel sealing device [VSD] LigaSure), with 10 animals in each group. Before the surgical procedure, all rabbits underwent physical and hematological evaluation, including blood count, total protein, albumin, GGT, ALP, ALT, AST, total, and direct bilirubin. Ultrasound examination of the abdomen was performed on all animals to check the bile ducts and ensure the absence of any disorders.

### 2.1 Anesthesia protocol

The rabbits were not subjected to food and water fasting as emesis during anesthetic-surgical procedures is rare. For pre-anesthetic medication, morphine (1 mg/kg) and acepromazine (0.05 mg/kg) were administered intramuscularly. After 20 minutes, induction was performed with an anesthetic mask using isoflurane, followed by the administration of 10% lidocaine spray in the oral cavity. Orotracheal intubation was then performed, and the rabbits were maintained on spontaneous ventilation. Intubation was confirmed using a capnograph.

### 2.2 Surgical technique and postoperative period

After induction of anesthesia, rabbits were placed in dorsal decubitus, and the ventral and lateral portions of the abdomen were trichotomized. The animals were positioned in reverse Trendelenburg with the right side up, allowing the stomach and small intestine to move caudally. Antisepsis was performed with 2% chlorhexidine and 70% alcohol before and after the procedure, followed by the placement of surgical drapes. A 0.5 cm skin incision was made in the ventral abdominal midline, one-centimeter caudal to the umbilical scar. After subcutaneous dissection, a small 3-4 mm incision was made in the linea alba, penetrating the abdominal cavity. A 5-mm trocar was then inserted, through which a 5-mm laparoscope, coupled to a micro-camera and light source, was placed. The insufflator was attached to the cannula, inflating the peritoneal cavity with carbon dioxide ($CO_2$) at a rate of 2.0 L/min. Two 5-mm access portals were inserted in the cranial quadrant of the right abdomen, located 3 and 5 cm lateral to the midline and 3 and 4 cm cranial to the umbilical scar, positioned

to triangulate around the location of the gallbladder. Through these two portals, Babcock's atraumatic grasping forceps were inserted to elevate the hepatic lobe and manipulate the gallbladder. A fourth 5 mm port was inserted in the left abdominal quadrant (near the costal arch) and was used to introduce bipolar electrosurgery (Maryland forceps) or LigaSure (VSD – LigaSure) (Figure 1).

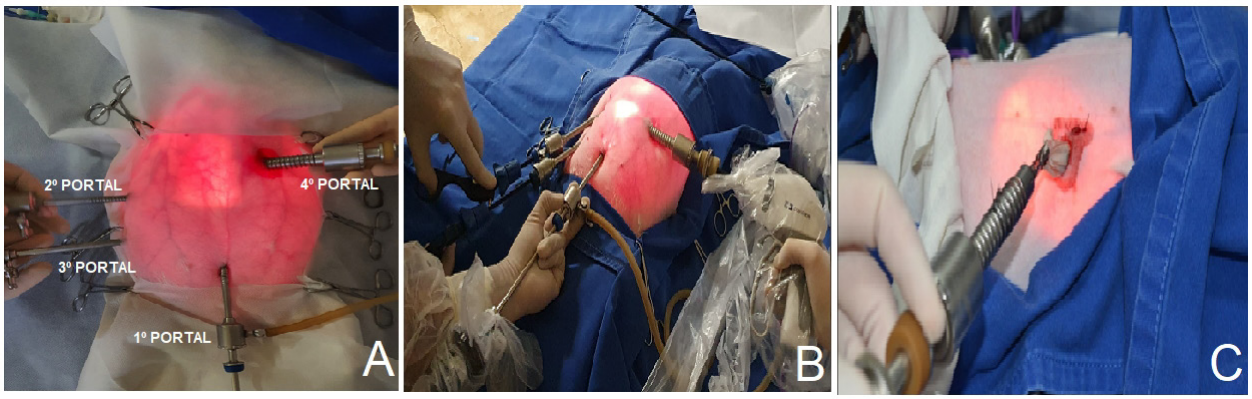


**Figure 1** Surgical technique for cholecystectomy in rabbits. (A) Access location for the first, second, third, and fourth portals. (B) Place of insertion of the four access portals and LigaSure device. (C) Removal of the gallbladder within the recovery bag.

After identifying the gallbladder and the cystic duct, the gallbladder was grasped with atraumatic forceps, capturing and retracting the gallbladder fundus in the cranial and right lateral direction over the hepatic dome. The infundibulum was identified and seized, then retracted laterally towards the right lower quadrant with another atraumatic forceps, exposing Calot's triangle, which was dissected to expose the cystic duct, cystic artery, and lymph node. After identifying these structures, the cystic artery was sealed with LigaSure (VSD – LigaSure) in both groups.

In Group 1 animals (n=10), dissection of the gallbladder began using Calot's triangle with bipolar electrosurgery (Maryland forceps) until it was determined that the only remaining structure connected to the gallbladder was the cystic duct. Its sealing was performed with LigaSure (VSD – LigaSure™), where two occlusions around the duct were made. After occlusion and transection of the cystic duct, the peritoneal insertions between the gallbladder and its hepatic bed were dissected with bipolar electrosurgery (Maryland forceps) (Figure 2).

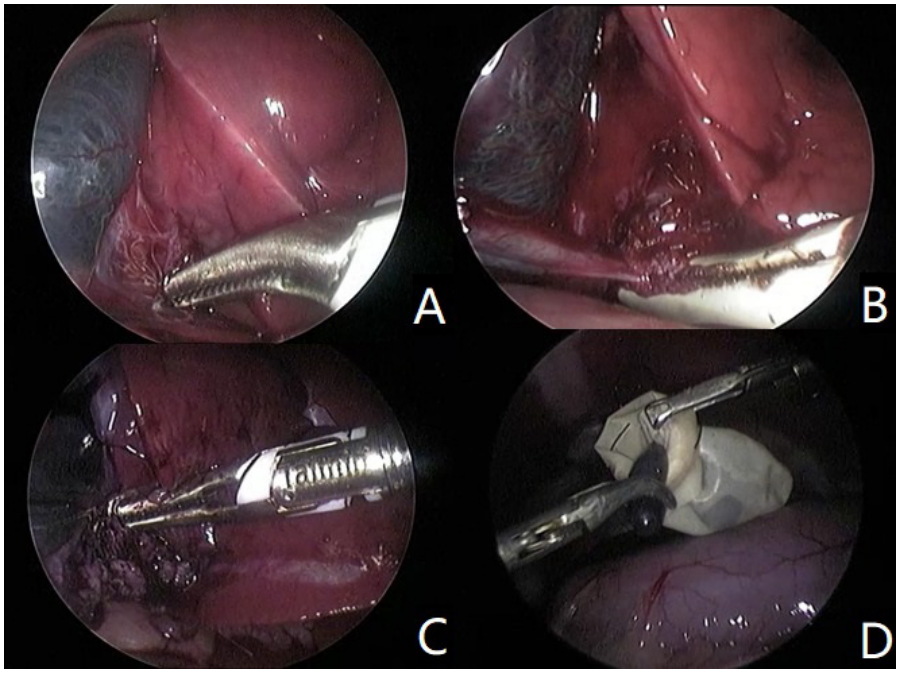


**Figure 2** Group 1 - bipolar electrosurgical device (Maryland forceps) (A) Dissection of Calot's triangle using Maryland bipolar forceps. (B) Occlusion of the cystic duct using the LigaSure device (DSV - LigaSure). (C) Dissection of the peritoneal insertions between the gallbladder and liver bed using Maryland bipolar forceps. (D) Gallbladder placed in the recovery bag for removal.

Group 2 animals (n=10) had their gallbladders dissected using Calot's triangle, with their cystic ducts being occluded and transected using VSD-LigaSure™ (Figure 3).

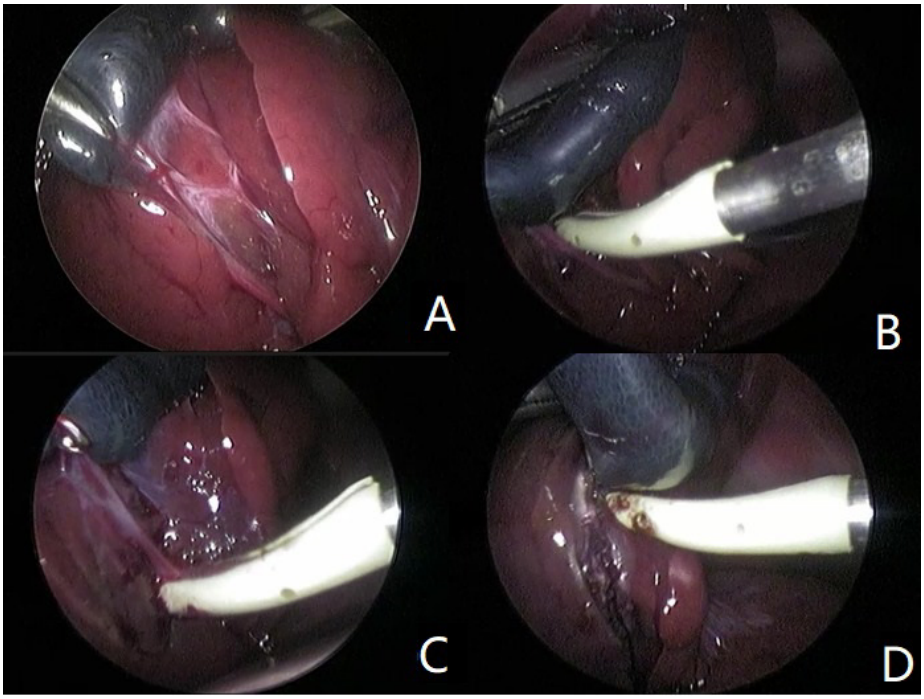


**Figure 3** Group 2 – LigaSure (VSD – LigaSure) (A) Exposure of Calot's triangle, cystic duct, and cystic artery. (B) Dissection of Calot's triangle using LigaSure (VSD – LigaSure). (C) Occlusion and transection of the cystic duct. (D) Dissection of the peritoneal insertions between the gallbladder and liver bed using LigaSure (VSD – LigaSure).

After the complete dissection of the gallbladder and section of the cystic duct, the organ was placed in the recovery bag, which was removed by enlarging the right portal incision. Before endoscopic removal, the hepatic fossa was assessed to verify the absence of hemorrhage and bile leakage. Gas was removed from the abdominal cavity, and the cannula and endoscope were removed. Myorrhaphy was performed with a 2-0 Poliglecaprone thread using the Sultan suture pattern, and dermorrhaphy was performed with a 2-0 nylon thread using a simple interrupted suture pattern.

In the immediate postoperative period, tramadol hydrochloride (4 mg/kg), meloxicam (0.1 mg/kg), and enrofloxacin (5 mg/kg) were administered subcutaneously. Postoperatively, tramadol hydrochloride (4 mg/kg) was administered subcutaneously every eight hours for three days, meloxicam (0.1 mg/kg) every 24 hours for two days, and enrofloxacin (5 mg/kg) every 12 hours for seven days.

### 2.3 Collection of venous blood samples

Venous blood samples were obtained through jugular venipuncture at zero (D0) preoperative period, and three (D3), seven (D7), and 15 (D15) days after surgery. Samples of 2 mL of blood were collected in vials containing sodium citrate (Trombostab™ ref.45 – Labtest™) to determine the plasma concentration of fibrinogen, and samples of 2 mL of blood were collected in vials without anticoagulant to perform serum proteinogram and determine the serum concentrations of total proteins and C-reactive protein. The plasma was separated and frozen at -20ºC after centrifuging the samples at 1,500 x g for 15 minutes. After clot retraction, the tubes without anticoagulant were also centrifuged at 1,500 x g for 15 minutes to obtain blood serum, and the samples were frozen at -20ºC.

Plasma fibrinogen concentrations were determined in a coagulometer (Clot Quick Timer II, Drake™) using a commercial kit (Fibrinogen, Ref. 506-4/2 – Labtest Diagnóstica S.A.), at most, 15 days after freezing the samples.

### 2.4 Serum protein concentration

Protein was fractionated using polyacrylamide gel electrophoresis containing sodium dodecyl sulfate (SDS-PAGE), as proposed by Laemmli (1970). After fractionation, the gel was stained for two hours in a 0.2% Coomassie blue solution and subsequently bleached in a methanol and acetic acid solution to remove excess dye until the protein fractions were clear. The concentrations of these proteins were determined using a computerized densitometer (Shimadzu CS-9301 PC, Shimadzu). As a reference, a marker solution (Sigma Maker™, S8445-wide range, mol wt. 6,500- 200,000 Da, Sigma) with different molecular weights (200, 116, 97, 66, 55, 45, 36, 29, 24, 20 kDa) was used. The proteins evaluated were IgA, ceruloplasmin, transferrin, albumin, IgG, haptoglobin, α1-acid glycoprotein, and PM 23000 Da.

The serum concentration of C-reactive protein was determined by the immunoturbidimetry method in a semi-automatic spectrophotometer (LabQuest, Labtest Diagnóstica S.A), using a commercial kit (PCR Turbiquest Max, Ref 3002, Labtest Diagnóstica S.A). The serum

concentration of total proteins was determined by the biuret method in an automatic spectrophotometer (LabQuest, Labtest Diagnóstica S.A) using a commercial kit (Proteínas Totais, Ref 99-1/250, Labtest Diagnóstica S.A).

### 2.5 Statistical analysis

Statistical analyses were performed using R (version 4.4.1) and GraphPad Prism (version 6.01) software. The effects of both groups (Group 1 vs. Group 2) and evaluation days (D0, D3, D7, and D15) concerning the variables were evaluated using the Friedman test. The potential interaction between groups and evaluation days was evaluated using the Kruskal-Wallis and Dunn test. Results for each variable are presented as median ± interquartile range (IQR). In all tests, significance was declared at $p \leq 0.05$.

## 3. Results

Table 1 and Figure 4 present the results of protein and immunoglobulin concentrations in rabbits undergoing laparoscopic cholecystectomy using bipolar electrosurgery (Group 1) and a vessel sealing device (Group 2).

**Table 1** Median ± interquartile range of concentrations of acute-phase proteins and immunoglobulins in rabbits undergoing laparoscopic cholecystectomy using bipolar electrosurgery (Group 1) and vessel sealing device (Group 2).

| Proteins | Evaluation Days | | | |
|---|---|---|---|---|
| | D0 | D3 | D7 | D15 |
| **Fibrinogen (g/dL)** | | | | |
| Group 1 | 0.20 ± 0.20 $^{Aa}$ | 0.60 ± 0.28 $^{Ab}$ | 0.30 ± 0.35 $^{Aab}$ | 0.40 ± 0.20 $^{Aab}$ |
| Group 2 | 0.25 ± 0.18 $^{Aa}$ | 0.70 ± 0.35 $^{Ab}$ | 0.40 ± 0.20 $^{Aab}$ | 0.20 ± 0.20 $^{Aa}$ |
| **IgA (mg/dL)** | | | | |
| Group 1 | 10.5 ± 2.79 $^{Aa}$ | 14.4 ± 4.97 $^{Aa}$ | 11.4 ± 7.25 $^{Aa}$ | 12.8 ± 13.1 $^{Aa}$ |
| Group 2 | 12.9 ± 4.44 $^{Aa}$ | 13.0 ± 3.48 $^{Aa}$ | 10.6 ± 5.71 $^{Aa}$ | 10.5 ± 2.29 $^{Aa}$ |
| **Ceruloplasmin(mg/dL)** | | | | |
| Group 1 | 16.5 ±5.40 $^{Aa}$ | 22.1 ± 8.55 $^{Aa}$ | 18.4 ± 6.50 $^{Aa}$ | 19.1 ±7.57 $^{Aa}$ |
| Group 2 | 17.4 ± 3.05 $^{Aa}$ | 19.9 ± 4.82 $^{Aa}$ | 14.7 ± 8.71 $^{Aa}$ | 14.4 ± 11.0 $^{Aa}$ |
| **Transferrin (mg/dL)** | | | | |
| Group 1 | 290 ± 41.3 $^{Aa}$ | 405 ± 90.5 $^{Ab}$ | 370 ± 51.8 $^{Aab}$ | 300 ± 89.3 $^{Aa}$ |
| Group 2 | 325 ± 74.9 $^{Aa}$ | 390 ± 44.8 $^{Ab}$ | 359 ± 115 $^{Aab}$ | 367 ± 79.5 $^{Aab}$ |
| **Albumin (mg/dL)** | | | | |
| Group 1 | 5,261 ± 290 $^{Aab}$ | 5,383 ± 547 $^{Aa}$ | 4,549 ± 649 $^{Abc}$ | 4,295 ± 939 $^{Ac}$ |
| Group 2 | 5,684 ± 607 $^{Aa}$ | 5,568 ± 354 $^{Aab}$ | 5,089 ± 588 $^{Ab}$ | 5,106 ± 376 $^{Bb}$ |
| **Haptoglobin (mg/dL)** | | | | |
| Group 1 | 19.8 ± 5.96 $^{Aa}$ | 32.2 ± 21.4 $^{Aa}$ | 21.6 ± 9.00 $^{Aa}$ | 23.8 ± 5.81 $^{Aa}$ |
| Group 2 | 23.2 ± 3.21 $^{Aa}$ | 23.7 ± 12.4 $^{Aa}$ | 20.2 ± 16.1 $^{Aa}$ | 29.1 ± 9.87 $^{Aa}$ |
| **IgG (mg/dL)** | | | | |
| Group 1 | 256 ± 48.3 $^{Aab}$ | 211 ± 76.4 $^{Aa}$ | 187 ± 79.2 $^{Aa}$ | 289 ± 170 $^{Ab}$ |

| | | | | |
|---|---|---|---|---|
| Group 2 | 281 ± 47.5 $^{Aa}$ | 231 ± 43.3 $^{Aab}$ | 174 ± 24.4 $^{Ab}$ | 409 ± 249 $^{Aa}$ |
| **$\alpha_1$-acid glycoprotein (mg/dL)** | | | | |
| Group 1 | 43.6 ± 8.85 $^{Aa}$ | 43.2 ± 21.5 $^{Aa}$ | 29.1 ± 14.9 $^{Aa}$ | 38.0 ± 18.9 $^{Aa}$ |
| Group 2 | 46.7 ± 17.2 $^{Aa}$ | 41.0 ± 19.3 $^{Aab}$ | 31.0 ± 10.4 $^{Ab}$ | 40.0 ± 10.8 $^{Aab}$ |
| **PM 23.000 Da (mg/dL)** | | | | |
| Group 1 | 57.2 ± 24.6 $^{Aa}$ | 44.8 ± 13.7 $^{Ab}$ | 44.0 ± 27.7 $^{Aab}$ | 67.9 ± 29.5 $^{Aa}$ |
| Group 2 | 62.0 ± 11.5 $^{Aac}$ | 36.9 ± 15.2 $^{Ab}$ | 48.0 ± 15.4 $^{Aab}$ | 90.4 ± 29.7 $^{Ac}$ |
| **CRP (mg/dL)** | | | | |
| Group 1 | 1.50 ± 0.70 $^{Aab}$ | 5.65 ± 11.3 $^{Aa}$ | 1.00 ± 3.00 $^{Ab}$ | 0.90 ± 2.40 $^{Ab}$ |
| Group 2 | 2.28 ± 1.90 $^{Aa}$ | 6.00 ± 7.60 $^{Ab}$ | 4.10 ± 5.95 $^{Aab}$ | 2.55 ± 2.21 $^{Aa}$ |

*Medians followed by the same uppercase letters within columns and lowercase letters within rows do not differ from each other by Dunn's test (p>0.05).*

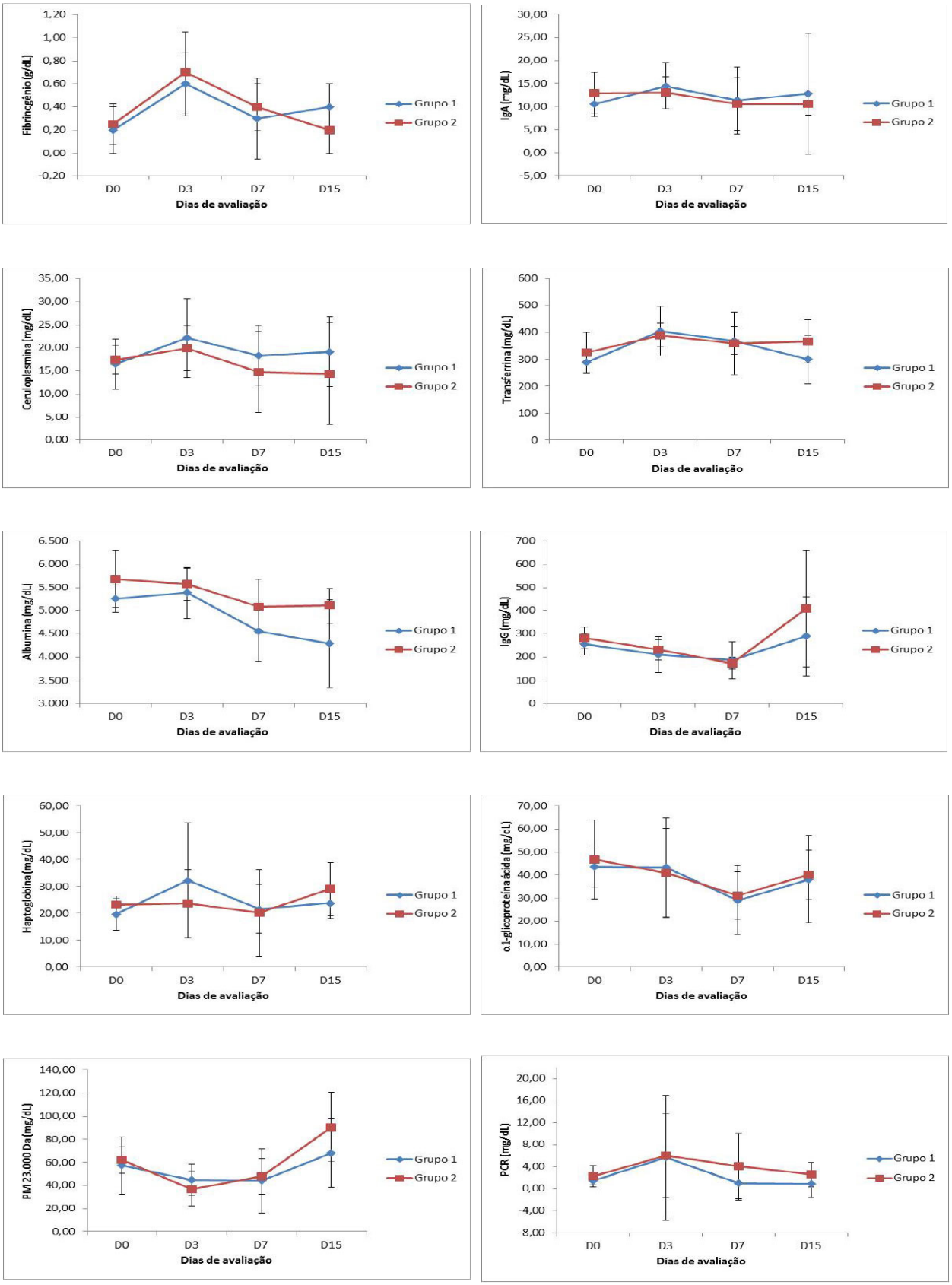


**Figure 4** Diagram of median ± interquartile range of acute-phase proteins and immunoglobulins in rabbits after laparoscopic cholecystectomy using bipolar electrosurgery (Group 1) and vessel sealing device (Group 2).

Fibrinogen plasma concentrations and transferrin, IgG, α1-acid glycoprotein, PM 23,000 Da, and C-reactive protein (CRP) serum concentrations did not show significant differences between the groups ($p>0.05$), but they did show differences over time within the same group ($p<0.05$). On the other hand, serum concentrations of IgA, ceruloplasmin, and haptoglobin showed no statistical difference between groups or evaluation days within the same group ($p>0.05$). Only serum albumin concentration showed a difference between groups and evaluation days within the same group ($p<0.05$).

Regarding plasma fibrinogen concentrations, a difference was observed between the baseline value (D0) and D3 in Group 1 ($p<0.05$), where the concentration was higher on D3 (0.60 g/dL). In Group 2, differences were found between D0 and D3, and D3 and D15, with higher plasma fibrinogen concentrations on D3 (0.70 g/dL).

A difference in serum transferrin concentrations was noted in Group 1 between days D0 and D3 ($p<0.05$), with higher concentrations on D3 (405 mg/dL), and between D3 and D15 ($p<0.05$), with D15 having the lowest value (300 mg/dL). Group 2 showed a difference only between D0 (325 mg/dL) and D3 (390 mg/dL) ($p<0.05$).

There was a significant difference in serum albumin concentrations between the two experimental groups on D15 ($p<0.05$), with lower protein concentrations in Group 1 compared to Group 2 (4,295 g/dL and 5,106 g/dL, respectively). Additionally, a difference was found between days D0 and D15, D3 and D7, and D3 and D15 in Group 1 ($p<0.05$), with the lowest concentrations on D7 (4,295 mg/dL). In Group 2, significant differences were noted between D0 and D7, and between D0 and D15 ($p<0.05$), with the lowest concentrations on D7 (5,089 mg/dL). Group 2 had higher serum albumin concentrations on all evaluation days compared to Group 1.

Although no differences were observed between serum IgG concentrations between the groups ($p>0.05$), significant differences were found in Group 1 between D3 and D15, and between D7 and D15 ($p<0.05$), with higher values on D15 (289 mg/dL). In Group 2, significant differences were noted between D0 and D7, and between D7 and D15 ($p<0.05$), with higher values on D15 (409 mg/dL).

No differences were observed in serum concentrations of α1-acid glycoprotein in Group 1 across evaluation days ($p>0.05$). In Group 2, differences were observed between D0 (46.7 mg/dL) and D7 (31.0 mg/dL) ($p<0.05$). Both groups had the lowest protein concentrations on D7 (29.1 mg/dL and 31.0 mg/dL, respectively).

Differences in serum concentrations of PM 23,000 Da protein were found in Group 1 between days D0 and D3, and D3 and D15 ($p<0.05$), and in Group 2 between days D0 and D3, D3 and D15, and D7 and D15 ($p<0.05$). In both groups, the highest protein concentrations were noted on D15 (67.9 and 90.4 mg/dL, respectively).

Although no differences were observed between serum CRP concentrations between Groups 1 and 2 ($p>0.05$), differences were observed in Group 1 between D3 and D7, and between D3 and D15 ($p<0.05$), with the highest values on D3 (5.65 mg/dL). In Group 2,

significant differences were noted between D0 and D3, and between D3 and D15 ($p<0.05$), with the highest values on D3 (6.00 mg/dL).

The results presented in Table 2 demonstrate that the majority of significant correlations between the protein and immunoglobulin variables were weak, with some moderate (transferrin and haptoglobin, haptoglobin and α1-acid glycoprotein, PM 23,000 Da and fibrinogen, PM 23,000 Da and CRP, CRP and fibrinogen) and no strong correlations. Regarding the p-value (<0.05), CRP had a positive correlation with fibrinogen, ceruloplasmin, transferrin, α1-acid glycoprotein, and PM 23,000 Da.

**Table 2** Spearman correlation coefficient and p-value between concentrations of acute-phase proteins and immunoglobulins in rabbits undergoing laparoscopic cholecystectomy using vessel sealing and bipolar electrosurgical devices for gallbladder dissection.

| Parameters | IgA | Cerul | Transf | Alb | IgG | Hapto | α1-acid | PM 23.000 | CRP |
|---|---|---|---|---|---|---|---|---|---|
| **Fib** | rho = 0.13<br>p = 0.2337 | rho = 0.11<br>p = 0.3369 | rho = 0.25<br>p = 0.0269 | rho = 0.13<br>p = 0.2694 | rho = -0.24<br>p = 0.0295 | rho = -0.07<br>p = 0.5513 | rho = -0.07<br>p = 0.5087 | rho = -0.41<br>p = 0.0002 | rho = 0.40<br>p = 0.0010 |
| | **IgA** | rho = 0.25<br>p = 0.0267 | rho = 0.25<br>p = 0.0280 | rho = 0.20<br>p = 0.0756 | rho = -0.04<br>p = 0.7035 | rho = 0.09<br>p = 0.4436 | rho = 0.16<br>p = 0.1628 | rho = -0.11<br>p = 0.3103 | rho = 0.03<br>p = 0.7949 |
| | | **Cerul** | rho = -0.03<br>p = 0.8110 | rho = 0.004<br>p = 0.9701 | rho = 0.17<br>p = 0.1375 | rho = 0.16<br>p = 0.1479 | rho = 0.26<br>p = 0.0177 | rho = -0.26<br>p = 0.0182 | rho = 0.31<br>p = 0.0127 |
| | | | **Transf** | rho = 0.33<br>p = 0.0030 | rho = -0.15<br>p = 0.1744 | rho = 0.41<br>p = 0.0002 | rho = 0.22<br>p = 0.0508 | rho = -0.17<br>p = 0.1304 | rho = 0.30<br>p = 0.0145 |
| | | | | **Alb** | rho = -0.06<br>p = 0.5812 | rho = 0.29<br>p = 0.0102 | rho = 0.39<br>p = 0.0005 | rho = -0.10<br>p = 0.3894 | rho = 0.09<br>p = 0.4761 |
| | | | | | **IgG** | rho = 0.11<br>p = 0.3177 | rho = 0.35<br>p = 0.0016 | rho= 0.39<br>p = 0.0003 | rho = -0.10<br>p = 0.4353 |
| | | | | | | **Hapto** | rho = 0.45<br>p < 0.0001 | rho = 0.04<br>p = 0.7341 | rho = 0.22<br>p = 0.0790 |
| | | | | | | | α1-**acid** | rho = 0.10<br>p = 0.3671 | rho = 0.35<br>p = 0.0042 |
| | | | | | | | | **PM 23.000** | rho = -0.41<br>p = 0.0008 |
| | | | | | | | | | **CRP** |

*Weak (0.01≤ r ≤0.39), moderate (0.40≤ r ≤0.69), and strong (0.70≤ r ≤1.00) scores were considered to classify correlation levels between variables. Fib – Fibrinogen, Cerul – Ceruloplasmin, Transf – Transferrin, Alb – Albumin, Hapto – Haptoglobin, α1-acid – α1- acid glycoprotein, CRP – C-reactive protein.*

## 4. Discussion

Despite the evolution of less invasive surgical techniques, laparoscopic cholecystectomy is not widely used in routine veterinary medicine. Compared to conventional techniques involving an open cavity, laparoscopic methods offer faster postoperative recovery, higher diagnostic accuracy, reduced infection rates, and other advantages. However, surgical trauma

induces metabolic, immunological, and neuroendocrine changes, triggering inflammatory and anti-inflammatory responses to repair the damage. This process releases cytokines into the bloodstream, potentially leading to inflammatory response syndromes, organ failure, and sepsis [1,10].

In a study evaluating hemostasis and thermal damage in mesenteric vessels of goats using bipolar electrosurgery, LigaSure, and an ultrasonic scalpel, bipolar electrosurgery caused greater thermal damage than LigaSure, which also provided better vessel hemostasis [11]. In rabbits undergoing ovariohysterectomy, LigaSure shortened surgical time compared to conventional ligatures [12]. When sealing the renal artery and vein in pigs, LigaSure was successful in all animals, whereas bipolar electrosurgery failed in two cases and caused tissue thermal damage, complicating the occlusion process [13]. In the present study, both dissectors increased basal levels of acute-phase proteins (APPs) and C-reactive protein (CRP) on different days within the same group; however, between groups, only albumin showed a difference between groups on D15.

In humans, the impact on liver parenchyma during transection using ultrasonic energy and a vessel sealing device showed no significant difference in CRP values between the groups, suggesting both methods are viable [14]. Another human study comparing vessel sealing and monopolar electrosurgical devices in thoracoscopic lobectomy and pulmonary lymphadenectomy found no difference in CRP values between the two [15]. Current research indicates greater changes in APP concentrations may be linked to the use of electrocautery [16]. Other studies have shown that the plasma half-life of CRP in rabbits is four to six hours, and peak serum concentration can reach three days post-injury [17,18]. In the present study, CRP levels in both groups increased on the third postoperative day, and in group 2 on the seventh postoperative day, without differences between groups. CRP values resumed to normal within 15 days postoperatively in both groups. Overall, human studies have also demonstrated increases in CRP levels and other inflammatory factors, even in minimally invasive cholecystectomy techniques [16].

IgG production responds to infectious and toxic agents, increasing in infectious diseases, liver diseases, and connective tissue diseases, among others [19]. Surgical trauma can suppress the immune system. A study on immunological function post-laparoscopic cholecystectomy in humans, measuring immunoglobulins such as IgG and IgA, found minimal changes, with values resuming to baseline six days post-procedure [20]. Another study noted IgG decreased on the first postoperative day but returned to baseline by day three [21]. In the present study, IgA and IgG values showed no difference between groups, though serum IgG concentration varied over time within the same group, with higher values on D15.

Haptoglobin levels showed no changes between groups or evaluation days. Fibrinogen levels peaked on day three in both groups. Increased plasma fibrinogen concentration has been reported post-laparoscopic and open cholecystectomy in humans [22]. A study using laparoscopic ovariectomy in bitches also observed increased haptoglobin concentrations 72 hours post-surgery, common in hemorrhage or hemolysis situations [23]. These changes were not observed in the present study.

Human studies have emphasized the importance of pre- and postoperative serum albumin concentration in laparoscopic cholecystectomy, as it can influence post-surgical mortality and morbidity [24]. Increased albumin concentration has been reported in dogs within 48 hours post-laparoscopic cholecystectomy [25]. In another study, dogs that died postoperatively showed lower albumin concentrations [26]. In rabbits, the half-life of albumin is five days. Albumin release half-time can range from two days in mice to 19 days in horses. As negative acute-phase proteins, albumin and transferrin levels decrease after injuries [27,28]. Despite this, albumin is considered a chronic injury protein, with limited literature on its relationship to surgical damage [23]. Fifteen days post-surgery, Group 1 showed significantly lower albumin concentration compared to Group 2.

## 5. Conclusion

Both bipolar electrosurgical dissectors (Maryland forceps) and LigaSure (VSD – LigaSure™) show minimal differences in serum concentrations of fibrinogen, IgA, ceruloplasmin, transferrin, albumin, haptoglobin, IgG, α1-acid glycoprotein, PM 23000 Da, and CRP on days three, seven, and 15 post-procedure. This indicates that both dissectors are suitable for performing laparoscopic cholecystectomy.

### Declaration of conflict of interest

The authors declare that there is no conflict of interest.

### Author's contributions

*Conceptualization:* M.C.N Wittmaack, J.O Ribeiro, C.K Ido, M.P de Menezes e P.C. Moraes. *Formal analysis:* D.G. da Silva. *Acquisition of financing:* M.C.N Wittmaack e P.C Moraes. *Investigation*: A.C.S Machado, M.C.N Wittmaack, C.K Ido, J.O Ribeiro, M.P de Menezes e P.C Moraes. *Methodology:* A.C.S Machado, M.C.M Vera, G. Sembenelli, G.L. Montanhim, C.K Ido. *Supervision:* P.C Moraes. *Resources:* D.G da Silva. *Preview:* A.C.S Machado. *Writing (original draft):* A.C.S Machado. *Writing (review and editing):* M.C.N Wittmaack, D.G. da Silva e P.C. Moraes.

### Data availability

Data will be available upon request.

### Acknowledgment

The authors would like to thank *Fundação de Amparo à Pesquisa do Estado de São Paulo* (FAPESP) (process 2017/23402-8), *Coordenação de Aperfeiçoamento de Pessoal de Nível Superior* (CAPES) (process 88887.529086/2020-00), and *Conselho Nacional de Desenvolvimento Científico e Tecnológico* (CNPq) for the scholarship and financial assistance that enabled full dedication to the graduate program and operationalization of the study.

### References

1. Benevides MPA. Colecistectomia por Laparoscopia em cães. PubVet. 2021 Jul; 15:208. https://doi.org/10.31533/pubvet.v15n07a868.1-14

2. Aspinen S, Kinnunen M, Harju J, Juvonen P, Selander T, Holopainen A, et al. Inflammatory response to surgical trauma in patients with minilaparotomy cholecystectomy versus laparoscopic cholecystectomy: a randomised multicentre study. Scand J Gastroenterol 2016 Jan; 51(6):739-744. https://doi.org/10.3109/00365521.2015.1129436

3. Zaidi N, Glover AR, Sidhu SB. The Covidien LigaSure Maryland Jaw Device. Expert Rev Med Devices. 2015 Nov; 12(2):151-155. https://doi.org/10.1586/17434440.2015.985650

4. Gardeweg S, Bockstahler B, Duprè G. Effect of multiple use and sterilization on sealing performance of bipolar vessel sealing devices. PLoS ONE. 2019 Aug; 14(8):e0221488. https://doi.org/10.1371/journal.pone.0221488

5. Hirota Y, Tsukada K, Nishio E, Yoshida M, Tada S, Udagawa Y. Postoperative adhesion formation after laparoscopic uterine horn resection in a porcine model: comparison of five instruments. J Laparoendosc Adv Surg Tech A. 2005 Dec; 15(6):581-585. https://doi.org/10.1089/lap.2005.15.581

6. Rivier P, Monnet E. Use of vessel sealant device for splenectomy in dogs. Veterinary Surgery. 2011 Jan; 40(1): 102-105. https://doi.org/10.1111/j.1532-950X.2010.00757.x

7. Murata H, Shimada N, Yoshioka M. Current research on acute phase proteins in veterinary diagnosis: an overview. Vet J 2004 Jul ;168(1): 28-40. https://doi.org/10.1016/S1090-0233(03)00119-9

8. Rubio CP, Schmidt SEM. Proteínas de fase aguda em cães: Possíveis aplicações em cirurgia. Vet. e Zootec 2014 Dez; 21(4):492-502. Disponível em: https://rvz.emnuvens.com.br/rvz/article/view/969

9. Gulhar R, Ashraf MA, Jialal I. Physiology, Acute Phase Reactants. 2023 Apr 24. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2024 Jan–. PMID: 30137854.

10. Caetano Júnior EM, Vieira JP, Franco RMAMM, et al. Evaluation of systemic inflammatory responses in cholecystectomy by means of access. Single-port umbilical incision, transvaginal NOTES, laparoscopy and laparotomy. Acta Cir Bras 2015 Oct; 30:691-703. https://doi.org/10.1590/S0102-86502015010000000.

11. Liang J, Xing H, Chang Y. Thermal damage width and hemostatic effect of bipolar electrocoagulation, LigaSure, and Ultracision techniques on goat mesenteric vessels and optimal power for bipolar electrocoagulation. BMC Surg. 2019 Oct; 19(1):147. https://doi.org/10.1186/s12893-019-0615-4

12. Dumartinet C, Matres-Lorenzo L, Linsart A, Bernardé A, Bernard F. Comparison of conventional ligatures and a vessel sealing device for haemostasis during open ovariohysterectomy in rabbits. New Zealand Veterinary Journal, 2022 May; 70(5), 256–262. https://doi.org/10.1080/00480169.2022.2079571

13. Landman J, Kerbl K, Rehman J, Andreoni C, Humphrey PA, Collyer W, et al. Evaluation of a Vessel Sealing System, Bipolar Electrosurgery, Harmonic Scalpel, Titanium Clips, Endoscopic Gastrointestinal Anastomosis Vascular Staples and Sutures for Arterial and Venous Ligation in a Porcine Model. Journal of Urology [Internet]. 2003 Feb; 169(2):697–700. https://doi.org/10.1016/S0022-5347(05)63995-X

14. Gotohda N, Yamanaka T, Saiura A, Uesaka K, Hashimoto M, Konishi M, et al. Impact of energy devices during liver parenchymal transection: a multicenter randomized controlled trial. World J Surg 2015 Jun; 39,1543–1549. https://doi.org/10.1007/s00268-015-2967-y

15. Gabryel P, Kasprzyk M, Roszak M, Campasi A, Smoliński S, Zieliński P, et al. Comparison of the LigaSure™ bipolar vessel sealer to monopolar electrocoagulation for thoracoscopic lobectomy and lymphadenectomy: a prospective randomized controlled trial. Surg Endosc 2023 Jun; 37(6):4449-4457. https://doi.org/10.1007/s00464-023-09892-0

16. Sari R, Sevinc A. The effects of laparascopic cholecystectomy operation on C-reactive protein, hormones, and cytokines. J Endocrinol Invest 2004 Feb; 27:106-110. https://doi.org/10.1007/BF03346253

17. Chelladurai M, Macintyre SS, Kushner I. In vivo studies of serum C-reactive protein turnover in rabbits. The Journal of Clinical Investigation, 1983 Mar; 71(3), 604-610. https://doi.org/10.1172/JCI110806

18. Ishigaki,Y., Satoh, T. Experimental studies of a mandibular bone infection model in the rabbit. Japanese Journal of Oral and Maxillofacial Surgery, 1998 Apr; 42(4), 413-423. https://doi.org/10.5794/jjoms.42.413

19. Eckersall PD, Conner JG, Harvie J. An immunoturbidimetric assay for canine C-reactive protein. Vet Res Commun 1991;15:17-24. https://doi.org/10.1007/BF00497786

20. Puglisi F, De Fazio M, Capuano P, Verzillo F, Martines G. Funzioni immunitarie dopo colecistectomia laparoscopica [Immunologic functions after laparoscopic cholecystectomy]. Chir Ital. 2001 Sep-Oct;53(5):659-63. Italian. PMID: 11723897.

21. Li CG. Effect of surgical methods of cholecystectomy on immunity and stress reaction in patients with gallstones. J Hainan Med Univ 2016;22(21);36-39. Disponível em: https://web.archive.org/web/20201125152838id_/http://www.hnykdxxb.com/PDF/201621/10.pdf

22. Schietroma M, Giuliani A, Agnifili A, Lely L, Carlei F, Pescosolido A, Amicucci G. Colecistectomia tradizionale vs laparoscopica. Modificazioni del profilo coagulativo [Changes in blood coagulation, fibrinolysis and cytokine profile during laparoscopic and open cholecystectomy]. Chir Ital. 2008 Mar-Apr;60(2):179-88. Italian. PMID: 18689165.

23. Del Romero A, Cuervo B, Peláez P, Miguel L, Torres M, Yeste M, et al. Changes in acute phase proteins in bitches after laparoscopic, midline, and flank ovariectomy using the same method for hemostasis. Animals 2020 Nov;10(12):2223. https://doi.org/10.3390/ani10122223

24. Rudasill SE, Morales RR, Sanaiha Y, Sareh S, Antonios JW, Khoury H, et al. Predicting morbidity and mortality in laparoscopic cholecystectomy: preoperative serum albumin still matters. Am J Surg 2020 Aug; 220(2): 432-437. https://doi.org/10.1016/j.amjsurg.2019.12.005

25. Malek S, Sinclair E, Hosgood G, Moens NM, Baily T, Boston SE. Clinical findings and prognostic factors for dogs undergoing cholecystectomy for gall bladder mucocele. Vet Surg 2013 Jan; 42(2):418-426. https://doi.org/10.1111/j.1532-950X.2012.01072.x

26. Youn G, Waschak MJ, Kunkel KA, Gerard PD. Outcome of elective cholecystectomy for the treatment of gallbladder disease in dogs. J Am Vet Med Assoc 2018 Apr; 252(8):970-975. https://doi.org/10.2460/javma.252.8.970

27. Dixon FJ, Maurer PH, Deichmiller MP. Half-Lives of Homologous Serum Albumins in Several Species. Proceedings of the Society for Experimental Biology and Medicine. 1953 Jun; 83(2):287-288. https://doi.org/10.3181/00379727-83-20336

28. Jain S, Gautam V, Naseem S. Acute-phase proteins: As diagnostic tool. Journal of Pharmacy and Bioallied Sciences 2011 Jan-Mar; 3(1): 118-127. https://doi.org/10.4103%2F0975-7406.76489