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# Can Peri-Surgical Electroacupuncture Relieve Immunity Suppression? A Pilot Study in Dogs

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**Simple Summary:** The immunosuppressive effect of surgery and anesthesia is a real concern due to its implications for patient's health. Evidence suggests that acupuncture can modulate the immune system response. The purpose of this study was to explore the potential ability of electroacupuncture (EAP) to counteract immunity suppression in dogs undergoing elective ovariectomy. These preliminary results suggest an improvement of leucocytes count, neutrophils, monocytes and T-cells due to EAP therapy that also seems to shorten the time for immune system restoration after surgery. On the contrary B-cells and cytotoxic T-cells decreased in dogs treated with EAP but not in control dogs while helper T-cells and immunoglobulins M and A did not show significant differences between treated and control group and over time. EAP has proven to be a non-pharmacological and non-invasive approach with promising potential to reduce immunosuppressive perioperative risk in dogs by activating or inhibiting the same mechanism relayed on the body's self-healing.

**Abstract:** General anesthesia and surgical stress can suppress the immunological response by acting both directly on the immune system and indirectly on the hypothalamic-pituitary-adrenal axis and the sympathetic nervous system. Disturbance of the immune system during the perioperative period can lead to complications such as wound-healing disorders and infections up to sepsis. Effectiveness of acupuncture in regulating the immune function by increasing leukocyte numbers and inhibiting inflammatory response has been proven. The aim of this study was to explore the impact of electroacupuncture (EAP) on the dynamic balance of the immune system and immune cell populations in dogs undergoing surgery. For this purpose, twelve healthy bitches scheduled for elective ovariectomy were divided into two groups according to whether (EAP, n=6) or not (CTR, n=6) a peri-operative electroacupuncture treatment was performed. Levels of leukocytes (neutrophils, monocytes, T- and B-cells) and immunoglobulins M (IgM) and A (IgA) were measured in blood samples collected before (T0), 1 hour (T1) and 2.5 hours (T2) after anesthesia induction. Leukocytes count decreased from T0 to T1 in both groups and restored within 1.5 hours in EAP group whereas remained significantly lower in CTR group ($P<0.02$). In particular, neutrophils and monocytes increased in dogs receiving EAP ($P<0.01$) while T-cells decreased in CTR group ($P<0.04$) at T2. B-cells and cytotoxic T-cells decreased in EAP dogs ($P<0.04$) at T2. No differences in helper T-cells, IgM and IgA levels were recorded between groups and over time. Our results suggest a modulatory effect of EAP on the immune system which is early expressed on neutrophils, monocytes and T-cells.

**Keywords:** immunoglobulins; leukocytes; lymphocytes

## 1. Introduction

The innate immunity is the first defense of the organism, it acts immediately after an aggression and involves both cellular and molecular defenses. In particular, phagocytic cells such as neutrophils, monocytes and macrophages play a crucial role, whereas complement, interferon, lysozyme and

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defensins are some examples of molecules [1]. The innate immunity uses primitive nonspecific recognition systems to bind, neutralize and destroy pathogens [2]. The adaptive or acquired immunity is responsible for a targeted response to antigens through the activation of B- and T-cells and the synthesis of proteins such as antibodies and cytokines [2–4]. Adaptive response comprises humoral and cellular components [4]. Humoral immunity is mediated by antibodies (immunoglobulins) secreted by plasma cells. In particular, immunoglobulins M (IgM) are produced in response to the first antigenic stimulation and are soon replaced by immunoglobulins G (IgG) or A (IgA) [1,5]. Cell-mediated immunity involves helper T-cells and cytotoxic T-cells. Helper T-cells play a central role in facilitating and driving the activity of other immune cells through the release of cytokines and chemokines, either improving antibody production or cell-mediated responses depending on the antigen nature and localization. Cytotoxic T-cells act by killing cells expressing non-self antigens, including tumor cells and virus-infected cells [1,2,5]. These cells have been reported to increase during the surgical trauma period [6].

Surgical tissue injury and exposure to anesthetic drugs during the perioperative period can affect the immune system [7]. The first pro-inflammatory response to surgical stress triggers the innate immune system eliciting an increase in monocytes, neutrophils and macrophages [7,8]. Since an excessive reaction may be harmful, a compensatory immunosuppressive response occurs, the extent and duration of which depends on the surgery magnitude [9]. It mainly involves cells of the adaptive immune system and mediators such as cytokines, chemokines and other molecules, leading to a decrease in circulating lymphocytes [7–9]. Apparently, T-cells are the most affected, whereas B-cells numbers change little [9]. A progressive suppression of the immune response during the first week after surgical trauma contributes to the development of sepsis and the multiorgan dysfunction syndrome [4].

Evidence suggests that acupuncture can regulate the immune system [10,11]. In humans, electroacupuncture (EAP), an application of electrical current on acupuncture needles, is reported to be effective in enhancing immune function by alleviating immunosuppression of patients during [4] and after surgery [4,11]. Similarly, in laboratory species, EAP resulted in a decrease in lymphocyte apoptosis induced by surgical trauma [12] and in a restoration of suppressed lymphocyte proliferation [4,13]. Electroacupuncture appears to reduce immunosuppression of both humoral and cellular components by contrasting the decrease in IgM and IgA levels as soon as 2 hours after anesthesia [4].

The present study aimed to explore the effect of EAP on the immune system and immune cell populations in healthy dogs undergoing ovariectomy. In particular, leukocytes count (WBC) and neutrophils, monocytes, T- (cytotoxic and helper) and B-cells, and IgM and IgA levels were evaluated at three time points within 2.5 hours from induction of anesthesia in dogs treated with peri-operatively EAP and compared with those of untreated dogs. Furthermore, a complete blood cell count (CBC) was performed at each time, also red cells and platelet parameters were evaluated.

We expected that EAP could restrain the immunosuppression that develops during the perioperative period by harmonizing cellular and humoral immune responses.

## 2. Materials and Methods

This study was approved by the Institutional Ethical Committee for Animal Care of Università degli Studi di Milano, Italy (OPBA_56_2023). Written consent acquisition by the owner was mandatory to participate to the research.

### *2.1. Animals*

Twelve healthy bitches (ASA status I–II) undergoing elective ovariectomy were randomly (www.randomizer.org) assigned either to the electroacupuncture (EAP, n=6) or to control (CTR, n=6) group. Bitches were both purebred dogs (American Staffordshire n=3; Bouledogue n=1; Hound n=1; Schnauzer n=1) and mongrels (n=6), aged from 9 months to 2 years (1.4±0.4), weighted 7.8 kg to 25 kg (18.2±5.5) and had body condition score (BCS) 2/5 to 3/5 (2.6±0.5).

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Dogs with ASA status > II, aged more than 3 years, that underwent previous anesthesia and surgery and that were administered pharmacological therapy within 6 months prior to ovariectomy, or that were otherwise unhealthy were excluded from the present study.

*2.2. Anesthetic Protocol*

All dogs were fasted for 8 hours, and water was withheld for 2 hours before the beginning of the study. Dogs were premedicated with intramuscular methadone (Semfortan, Dechra Veterinary Products, Italy) at 0.3 mg $kg^{-1}$. After 20 minutes, an intravenous (IV) catheter was aseptically placed into a cephalic vein and general anesthesia was induced with IV propofol (Proposure; Merial Italia S.p.A., Italy) at 2.5 mg $kg^{-1}$, in combination with dexmedetomidine (Dexdomitor, Vetoquinol S.r.l., Italy) administered IV at a dose of 2 mcg $kg^{-1}$, as co-inducer and supplementary analgesic drug. After orotracheal intubation, general anesthesia was maintained with isoflurane (Isoflo, Esteve S.p.A., Italy) in oxygen (100%) titrated to effect in order to obtain a plane of anesthesia that maintained a ventral eye globe position, absence of palpebral reflex and relaxed jaw tone. During anesthesia, IV lactated Ringer's solution (Ringer Lattato; Fresenius Kabi, Italy) was given at 5 ml $kg^{-1}$ $h^{-1}$ and IV cefazolin (Cefazolina, Teva, Italy) 25 mg $kg^{-1}$ was administered 20 minutes before surgery. During the anesthesiologic and surgical periods, the animals were continuously monitored for respiratory rate, heart rate, electrocardiogram, oxyhemoglobin saturation, end-tidal $CO_2$ concentration and non-invasive arterial blood pressure using a multiparameter monitor (Datex Ohmeda S5, GE Healthcare, Italy). Post-operative pain was further controlled by a non-steroidal anti-inflammatory drug, meloxicam (Metacam; Boehringer Ingelheim, Germany), administered subcutaneously at 0.2 mg $kg^{-1}$ upon awakening, according to standard practice. Surgical and anesthetic procedures were performed routinely by the same operators and their duration was recorded.

*2.3. Electroacupuncture Protocol*

In EAP group, acupoints stimulation began 20 minutes after general anesthesia induction, i.e. 15 minutes before the start of surgery, and lasted until the last skin suture was placed. An electronic acupunctoscope (WQ-6F(57–6F), Beijing Haidian, China) was used. Dogs in CTR group were kept inducted as long as those in the EAP group; for both groups surgery started 35 minutes after general anesthesia induction. Selected acupoints were *Kong Zui* or Lung 6 (LU6), *Hegu* or Large intestine 4 (LI4), *Zusanli* or Stomach 36 (ST36), *Xuan Zhong* or Gall bladder 39 (GB39), *Ge Shu* or Bladder 17 (BL17), and *Shenshu* or Bladder 23 (BL23).

Needles (0.30 x 50 mm; Hwato, GMT2000, Laveno, Italy) were inserted at a depth of 15-20 mm. A frequency of 16 Hz and 0.4 V was applied to all acupoints except BL17 and BL23 stimulated with 43 Hz and 0.1 V.

*2.4. Sampling and Analysis of Immune Function*

Three blood samples were taken in both groups at the time of anesthesia induction (T0), 1 h after T0 (T1), i.e. 40 min from the start of EAP and 25 min after the start of surgery, and 2.5 hours after T0 (T2), i.e. after the end of the surgery. The blood samples were divided into two aliquots of 1.5 mL each: one was put in EDTA-containing tubes for hematological and flow cytometric analysis, the other was centrifugated at 4 °C for 5 min and the serum used for immunoglobulin titration. For each sample, a complete blood cell count (CBC) was performed with an automated hematology analyzer equipped with the veterinary software (Sysmex XN-V, Sysmex corporation, Kobe, Japan) and blood smear was prepared to perform leukocyte differential and platelet estimation. In order to further quantify leukocyte subclasses, flow cytometry (FC) was performed on each sample. All samples were processed according to already published protocols [14], acquired with a Bricyte E6 flow cytometer (Mindray, Shenzhen, China) and analyzed with the specific software MRflow (Mindray) by a single experienced operator (VM). A panel of five antibodies was applied, including anti-CD11b (neutrophils and monocytes), anti-CD5 (T-cells), anti-CD8 (cytotoxic T-cells), anti-CD4 (Helper T-

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cells), and anti-CD21 (B-cells) [15]. Both CBC and FC were performed within few minutes from sampling.

Serum IgM and IgA levels were determined using specific ELISA kits (BT LAB Bioassay Technology Laboratory, Jiaxing, Zhejiang, China) based on the sandwich approach. Expressed as coefficient of variability (CV), the declared intra-assay precision was <8% and the declared inter-assay precision was <10%.

*2.5. Statistical Analysis*

Descriptive statistics are expressed as means (± sd). Data were analyzed using a commercial statistical software (IBM SPSS, 28.0) comparing analyzed different parameters in the experimental groups at each time point with non-parametric test U- Mann-Whitney, since data were not normally distributed (Shapiro-Wilk test). The effect of time in treated and control groups on different measured parameters was assessed with a non-parametric Friedman ANOVA test and pairwise comparison. Statistical significance was accepted at $P<0.05$.

## 3. Results

All surgeries and recoveries had a regular course. No peri-operative complications occurred in any dog. Dogs in the two groups had similar age (EAP: 1.6±0.5 years; CTR: 1.5±0.3), body weight (EAP: 17.8±3.1 kg; CTR:18.5±7.4) and BCS (EAP: 2.5±0.5; CTR: 2.6±0.5) as well as the same surgical (EAP: 40.8±11.9 min; CTR: 40.8±13.6 min) and anesthetic, i.e. from induction to extubation, (EAP: 86.7±4.5 min; CTR: 84.7±16) times. No statistical differences in these parameters were recorded between the groups.

Hematological parameters concerning erythrocytes (RBC) and platelets (PLT) showed no differences between groups and time points (Appendix A). Conversely, leukocytes count (WBC) was significantly lower in CTR than EAP group at all time points ($P\leq0.03$). They decreased soon after induction (at T1) in both groups but restored at T2 in EAP group while remained significantly lower than T0 in the CTR group (Figure 1; $P<0.02$).

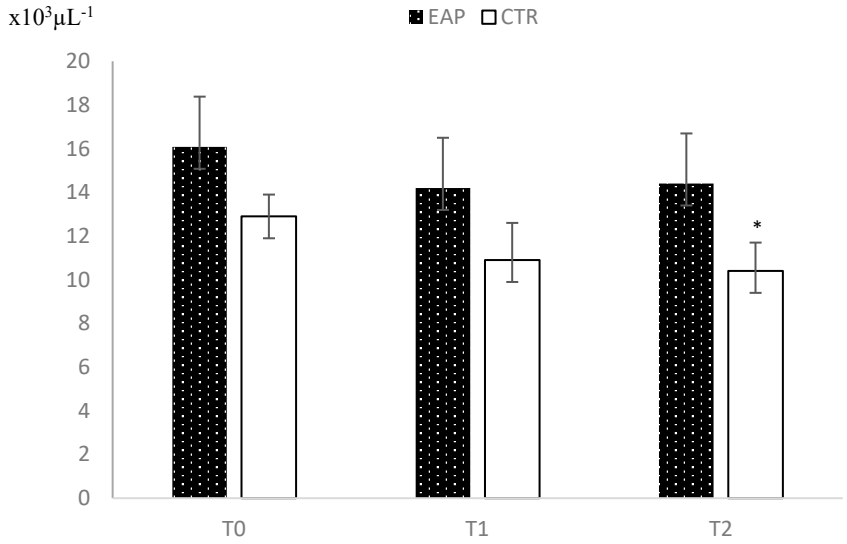


**Figure 1.** Leukocytes count in EAP and CTR groups over time. T0 represents the anesthesia induction time, T1 means 1 h after T0; T2 means 2.5 hours after T0. '*': $P<0.02$.

Myeloid cells (neutrophils and monocytes) increased at T2 compared to T0 in dogs undergoing EAP ($P=0.005$) but not in CTR group (Figure 2).

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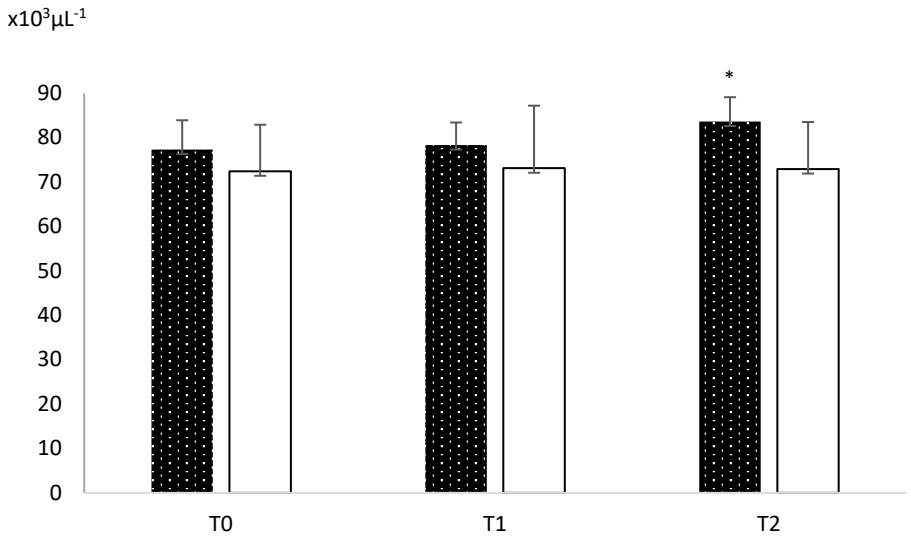


**Figure 2.** Percentage of myeloid cells in EAP and CTR groups over time. T0 represents the anesthesia induction time, T1 means 1 h after T0; T2 means 2.5 hours after T0. '*': P=0.005.

On the contrary, 2.5 hours after induction T-cells decreased in CTR group (P<0.04) but not in EAP group (Figure 3).

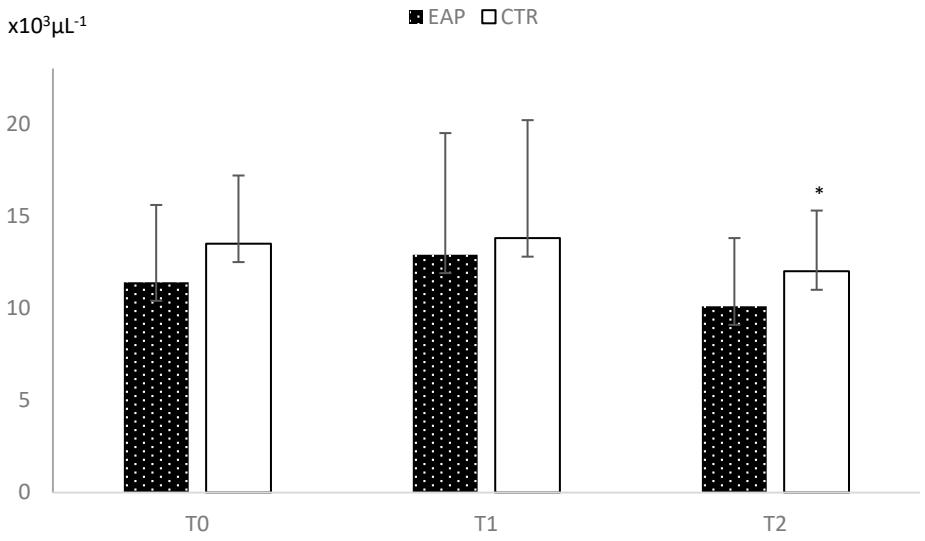


**Figure 3.** Concentrations of the T-cells in EAP and CTR groups over time. T0 represents the anesthesia induction time, T1 means 1 h after T0; T2 means 2.5 hours after T0. '*': P=0.038.

Cytotoxic T-cells (P=0.03) and B-cells decreased in EAP group (P<0.04) at T2 compared to T0 (Figure 4 and 5). Helper T-cells did not show variations between groups and over time.

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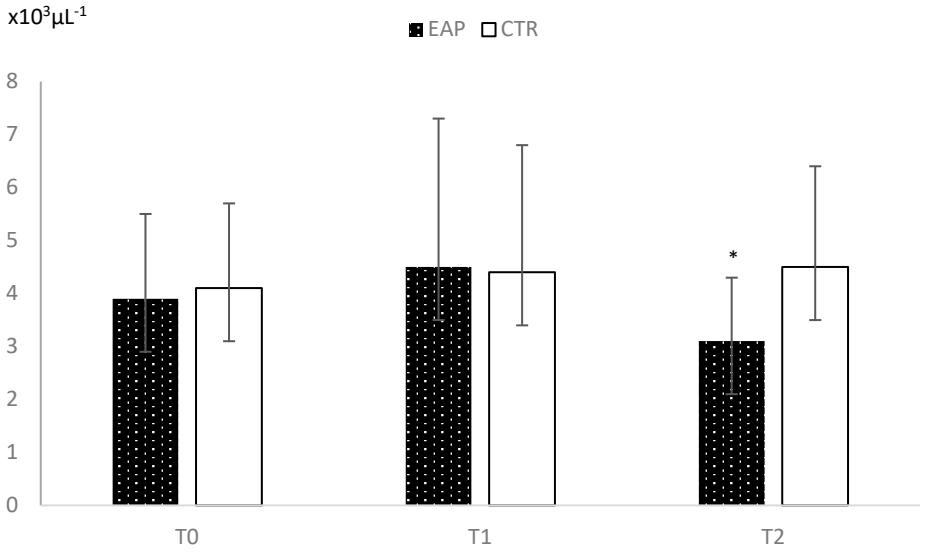


**Figure 4.** Concentrations of the cytotoxic T-cells in EAP and CTR groups over time. T0 represents the anesthesia induction time, T1 means 1 h after T0; T2 means 2.5 hours after T0. '*': P=0.03.

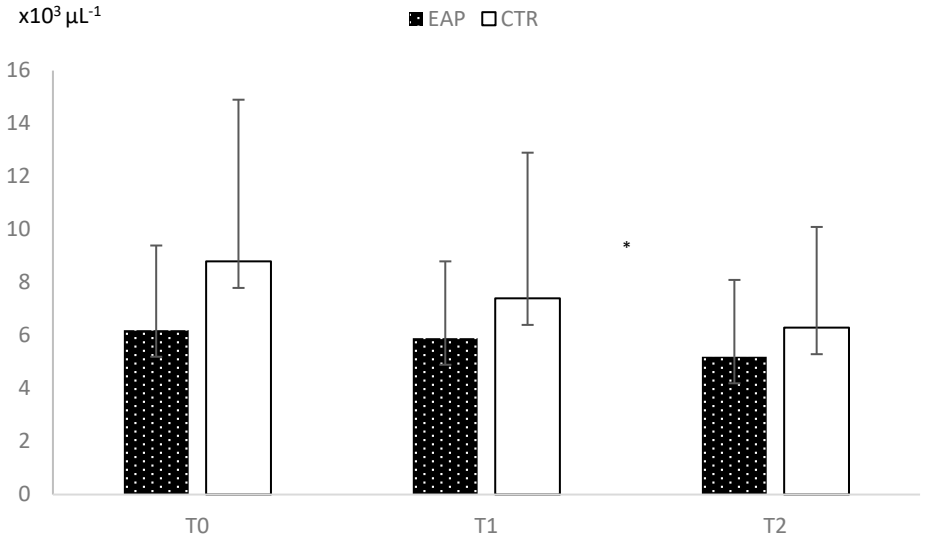


**Figure 5.** Concentrations of the B-cells in EAP and CTR groups over time. T0 represents the anesthesia induction time, T1 means 1 h after T0; T2 means 2.5 hours after T0. '*': P=0.036.

No significant differences in IgM and IgA concentrations were recorded between groups and over time. However, a decreasing trend of IgM levels was observed in EAP compared to CTR group (Figure 6).

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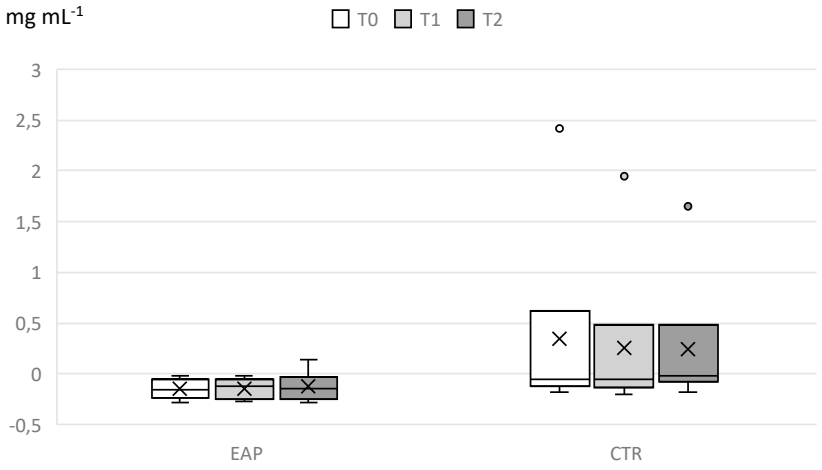


**Figure 6.** Concentrations of IgM in EAP and CTR groups over time. T0 represents the anesthesia induction time, T1 means 1 h after T0; T2 means 2.5 hours after T0.

On the contrary, EAP dogs tended to have higher IgA values than CTR dogs even without statistical significance (Figure 7).

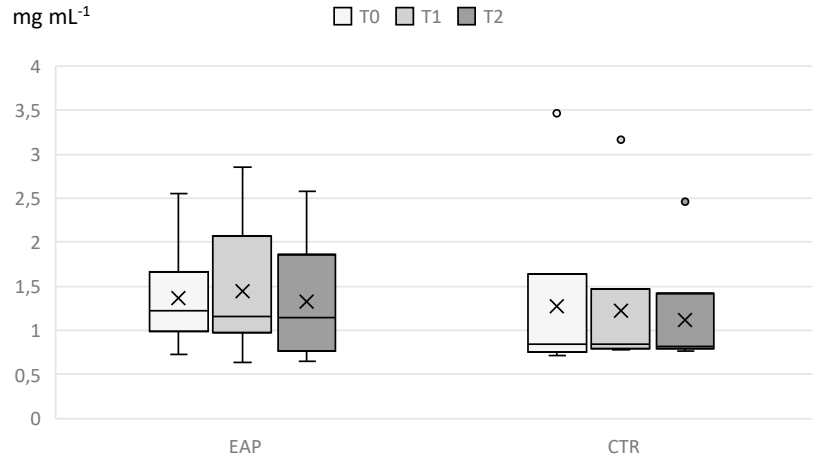


**Figure 7.** Concentrations of IgA in EAP and CTR groups over time. T0: time of anesthesia induction, T1: 1 h after T0; T2: 2.5 hours after T0.

## 4. Discussion

Immunosuppression due to surgery and anesthesia is a current and debated topic with many aspects still unknown in both human and veterinary medicine. Most publications report medium- and long-term effects on the immune system (days, weeks) [8,16,17], while few studies target the very early perioperative period [6,18]. The present study focused precisely on this period which may account for some unexpected results. We only included elective ovariectomy as a routine surgery performed on healthy dogs that allows for standardized clinical trials. In fact, the surgical technique together with its duration and extent are closely related to the degree of immunosuppression [9]. Moreover, inclusion of dogs with different diseases and undergoing different surgical procedures would have biased the results. In our study the two groups (EAP and CTR) were equivalent in terms of surgical and anesthesiologic procedures and exposition besides in size, age, body weight and BCS, making them suitable to compare and evaluate the effects of electroacupuncture.

To date, the detailed mechanisms of action of EAP are still unclear. Acupuncture is reported to be effective in regulating nonspecific immune function [19] as well as cellular and humoral immunity

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[20,21]. In general, acupuncture immunomodulation acts by stimulating the somatic-autonomic-immune reflexes that include the somatic-sympathetic-splenic reflex, the somatic-sympathetic-adrenal reflex, the somatic-vagal-splenic reflex and the somatic-vagal-adrenal reflex leading to a systemic involvement [22]. Furthermore, the stimulation of peripheral nerve due to the insertion of an acupuncture needle induces a traumatic inflammation also responsible for a local immunomodulation at the acupoint level [23].

The most intriguing aspect of acupuncture recently arose in the literature is its bidirectional effect aimed to maintain the homeostasis by balancing hyper- and hypo-functional states [24,25]. It refers to the ability of acupuncture to act by both activating and inhibiting the same mechanism that relies on the body's self-healing [22]. This is a unique aspect of acupuncture that no specific drug has achieved so far.

An initial pro-inflammatory effect is reported to occur as a result of surgical stress and tissue damage, which is followed by a compensatory immunosuppressive response aiming to facilitating the resolution of inflammation and protecting against excessive systemic consequences of the primary insult [18,26]. The decreasing trend of WBC observed in both EAP and CTR groups within 1 hour from general anesthesia induction can be interpreted as an early sign of immunosuppression. The latter appeared to persist until the end of monitoring, i.e. 2.5 hours after induction, in CTR ($P<0.02$) but not in EAP group, with the number of leucocytes significantly lower than pre-surgically. Furthermore, the percentage of neutrophils and monocytes increased in EAP dogs after 1.5 hours ($P=0.005$) while remained low in CTR dogs. Innate immunity is described to restore early during postoperative recovery taking 2 to 3 days for neutrophils and monocytes, respectively [18]. EAP seems to be able to shorten this time.

In accordance with a suppression of acquired immunity lasting several days postoperatively [18] and characterized by apoptotic reduction in the number of T-cells [26], we recorded a decrease in T-cells in the CTR group ($P<0.04$). The concentration of T-cells in the EAP group, however, remained unchanged over time, suggesting a possible counteracting action due to EAP treatment. On the other hand, B-cells and cytotoxic T-cells decreased in EAP dogs ($P<0.04$) but not in CTR group. Since acquired immune cells are reported not to recover until 5 days from surgical insults [18], it is possible that the effect of EAP may be noticeable after such period, not earlier. Moreover, a modulatory action of EAP on the type 1 (Th1) and type 2 (Th2) T helper cells is reported **[27–30]**. EAP can both stimulate and downregulate Th1 and Th2, that are responsible for pro-inflammatory and anti-inflammatory responses, respectively **[22,30–32]**. In the present study we have not performed a distinction between Th1 and Th2 T helper cells, therefore it is not possible to argue about them. Finally, EAP stimulation at *Zusanli* (ST36) acupoint in rats increases lymphocyte proliferation after surgery [13,33,34] while in mice suppresses Th2 cytokine IL-4 [31]. To the authors' knowledge, to date no studies in dogs have been published on this aspect, and a species-specific modality of EAP can only be speculated.

The few data in the literature on immunoglobulin are conflicting. Some authors reported no differences in IgM and IgA concentrations between groups and over time [35] while a study on the effect of EAP on postoperative immunoinflammatory response in human patients undergoing craniotomy showed that the blood IgA decreased significantly in control group 4 hours after induction of anesthesia and one day after surgery, but no significant differences were noted between control and treated groups [36]. The same research group soon after noted that electroacupuncture was able to alleviate intraoperative immunosuppression in the same type of human patients: in that case, both IgM and IgA decreased significantly in control group compared with treated groups 2 and 4 hours after induction of anesthesia, while no significant differences between groups were noted for IgG [4]. In our study, dogs treated with EAP showed a decreasing trend of IgM levels and an increasing trend of IgA levels compared to the CTR group. It should be noted that all dogs except one in the CTR group resulted below the expected values for IgM in canine species (0.7-2.7 mg/mL) [1]. On the contrary, IgA levels were always within the expected range (0.2-2.5 mg/mL) in both groups [1]. The degree of dogs immunization, and particularly few contacts with new antigens never encountered before (which would have resulted in the production of IgM), could have affected our results, but these are aspects that we did not take into account in this study.

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At last, even anesthetic drugs can have an impact on the immune function such as opioids which are mediated indirectly by activation of the HPA axis and sympathetic nervous system, and by a direct effect on many subtypes of immune cells [9]. Recent review studies underlined conflicting conclusions reporting immunosuppressive, immunostimulatory, or dual mechanisms for opioids [37]. Morphine is known to suppress a variety of immune functions including T lymphocytes proliferation [37]. The use of methadone to premedicate dogs in our study can justify the significant decrease in T-cells observed in the CRT group. Conversely in EAP group, endogenous endorphins releasing due to EAP stimulation may have acted as competitive agonists thus reducing the suppressive effect of methadone.

The small sample size of our caseload is a limitation that cannot be neglected before generalizing these findings. In addition, although we included a standardized population in order to avoid biases, it is possible that different anesthetic or surgical procedures might bring to different results. Moreover, we monitored hematological and immune parameters only for 2.5 hours after induction, and effects on longer periods could be quite different.

## 5. Conclusions

In conclusion, the results of this exploratory study suggest that EAP may influence the immune response in dogs undergoing elective ovariectomy. EAP appears to reduce the immunosuppression through a modulatory effect which is expressed early on neutrophils, monocytes and T-cells. EAP also seems to shorten the time of immune system restoration after surgery. EAP due to a non-pharmacological and non-invasive approach, is an attracting and promising therapy to reduce immunosuppressive perioperative risk in dogs.

In any case, a larger-scale randomized controlled trial also including a longer postoperative period is advisable to confirm these promising results.

**Author Contributions:** Conceptualization, V.R., F.A.B. and D.G.; methodology, V.R., G.R. and D.G.; formal analysis, V.B., V.M. and P.D.; investigation, A.P. and D.G.; data curation, V.R., V.B., V.M.; writing—original draft preparation, V.R., F.A.B.; and D.G.; writing—review and editing, all authors; supervision, G.R.; project administration, D.G. All authors have read and agreed to the published version of the manuscript.

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**Institutional Review Board Statement:** The study was conducted in accordance with the Declaration of Helsinki and approved by the Institutional Review Board of Università degli Studi di Milano (OPBA_56_2023).

**Informed Consent Statement:** Informed consent was obtained from all dog owners that were involved in the study.

**Data Availability Statement:** All the data that support the findings of this study are available from the corresponding author.

**Conflicts of Interest:** The authors declare no conflict of interest.

## Appendix A. Hematological Parameters in EAP and CTR Groups at Each Time Point

| | RBC | | | WBC | | | Hb | | | Ht | | | PLT | | | MCH | | | MCHC | | | MCV | | | RDW | | | MPV | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | $x10^6 \mu L^{-1}$ | | | $x10^3 \mu L^{-1}$ | | | g $dL^{-1}$ | | | % | | | $x10^3 \mu L^{-1}$ | | | pg | | | g $dL^{-1}$ | | | $\mu^3$ | | | % | | | $\mu^3$ | | |
| ID. | T0 | T1 | T2 | T0 | T1 | T2 | T0 | T1 | T2 | T0 | T1 | T2 | T0 | T1 | T2 | T0 | T1 | T2 | T0 | T1 | T2 | T0 | T1 | T2 | T0 | T1 | T2 | T0 | T1 | T2 |
| EAP1 | 5.8 | 6.4 | 6.8 | 14.8 | 10.4 | 18.5 | 12.9 | 14.2 | 15.2 | 37.6 | 41.9 | 44 | 385 | 243 | 372 | 22.1 | 22.2 | 22.2 | 34.3 | 33.9 | 34.5 | 64.3 | 65.4 | 64.2 | 16.9 | 18.4 | 18.7 | 10.3 | 11.5 | 10.1 |
| EAP 2 | 6.2 | 5.3 | 5.5 | 18.4 | 16.7 | 14.2 | 15.1 | 12.9 | 13.5 | 44.1 | 38.5 | 39.8 | 438 | 462 | 422 | 24.5 | 24.4 | 24.5 | 34.2 | 33.5 | 33.9 | 71.6 | 72.9 | 72.4 | 19 | 16.3 | 17.2 | 10.1 | 11.1 | 9.6 |
| EAP 3 | 7.5 | 6.1 | 6.5 | 13.5 | 13.7 | 12.6 | 18.3 | 14.8 | 15.7 | 50.6 | 44 | 45.6 | 213 | 236 | 233 | 24.4 | 24.1 | 24.1 | 36.2 | 33.6 | 34.4 | 67.4 | 71.8 | 70 | 20.3 | 17.9 | 18.5 | 11.7 | 12.4 | 12 |
| EAP 4 | 7.6 | 5.6 | 7 | 14.8 | 14.5 | 13.4 | 17.5 | 15.6 | 16.4 | 49.4 | 38.8 | 47.8 | 211 | 190 | 237 | 23 | 27.9 | 23.3 | 35.4 | 40.2 | 34.3 | 65 | 69.3 | 67.8 | 19.8 | 15.6 | 18.9 | 10.5 | 9.7 | 11.8 |
| EAP 5 | 7.2 | 6 | 6.1 | 19.5 | 16.2 | 15.2 | 16.4 | 14 | 14.1 | 46.1 | 41.6 | 41.7 | 201 | 192 | 184 | 22.9 | 23.2 | 23 | 35.6 | 33.7 | 33.8 | 64.4 | 68.9 | 68.1 | 20.2 | 18.2 | 18.1 | 11.6 | 11.4 | 11.6 |
| EAP 6 | 7.2 | 5.9 | 7.3 | 15.4 | 13.7 | 12.3 | 16.8 | 13.8 | 16.9 | 41.5 | 40.2 | 49.5 | 164 | 138 | 163 | 23.3 | 23.5 | 23.3 | 35.4 | 34.3 | 34.2 | 66 | 68.6 | 68.2 | 20.9 | 18.2 | 19.9 | 13.5 | 12.6 | 12.4 |
| CTR 1 | 7.9 | 6.9 | 7.7 | 13.1 | 10.2 | 11.9 | 19.6 | 16.9 | 18.8 | 53.5 | 47.9 | 52.4 | 311 | 172 | 278 | 24.6 | 24.6 | 24.5 | 36.6 | 35.3 | 35.9 | 67.1 | 69.7 | 67.3 | 20.1 | 18.7 | 20.1 | 11.3 | 11.9 | 11.3 |
| CTR 2 | 8 | 7 | 7.1 | 13.1 | 11.6 | 10.2 | 19.6 | 17.1 | 17.2 | 54.4 | 47.7 | 46.7 | 170 | 174 | 164 | 24.4 | 24.3 | 24.3 | 36 | 35.8 | 36.8 | 67.7 | 67.8 | 66 | 21.1 | 20.2 | 20.1 | 12.9 | 13 | 13.1 |
| CTR 3 | 6.7 | 5.4 | 5.7 | 11.4 | 8.2 | 8 | 16 | 13.1 | 13.2 | 47.9 | 39.4 | 40.1 | 230 | 212 | 139 | 23.8 | 24.1 | 23.3 | 33.4 | 33.2 | 32.9 | 71.2 | 72.6 | 70.7 | 19.1 | 16.3 | 17.8 | 12.3 | 12.6 | 12.3 |
| CTR 4 | 6.8 | 6.4 | 6.6 | 12.7 | 10.6 | 10.9 | 15.3 | 14.3 | 14.8 | 44 | 42.1 | 41.1 | 276 | 288 | 258 | 22.5 | 22.3 | 22.3 | 34.8 | 34 | 36 | 64.6 | 65.7 | 62 | 20 | 19.6 | 19.6 | 11.6 | 12 | 11.2 |
| CTR 5 | 7.7 | 6.8 | 6.7 | 12.7 | 13 | 10 | 18.3 | 16.2 | 16 | 52.7 | 45.9 | 45.6 | 193 | 192 | 199 | 23.7 | 23.9 | 23.7 | 34.7 | 35.3 | 35.1 | 68.4 | 67.6 | 67.7 | 19.9 | 18.8 | 18.3 | 12.8 | 13 | 13 |
| CTR 6 | 6.8 | 5.7 | 5.6 | 14.5 | 12.1 | 11.4 | 17.2 | 14.4 | 14.1 | 47.8 | 41.6 | 41.3 | 284 | 310 | 287 | 25.4 | 25.4 | 25.3 | 36 | 34.6 | 34.1 | 70.5 | 73.4 | 74.1 | 20.4 | 17.8 | 16.8 | 11.5 | 11.7 | 11.2 |

RBC: total red blood cell count, WBC: total white blood cell count, Hb: hemoglobin, Ht: hematocrit, PLT: platelet count, MCH: mean cell hemoglobin, MCHC: MCH concentration, MCV: mean cell volume, RDW: red cell distribution width, MPV: mean platelet volume. T0: at the time of induction of anesthesia, T1: 30 min after T0, T2: 2 hours after T0.



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