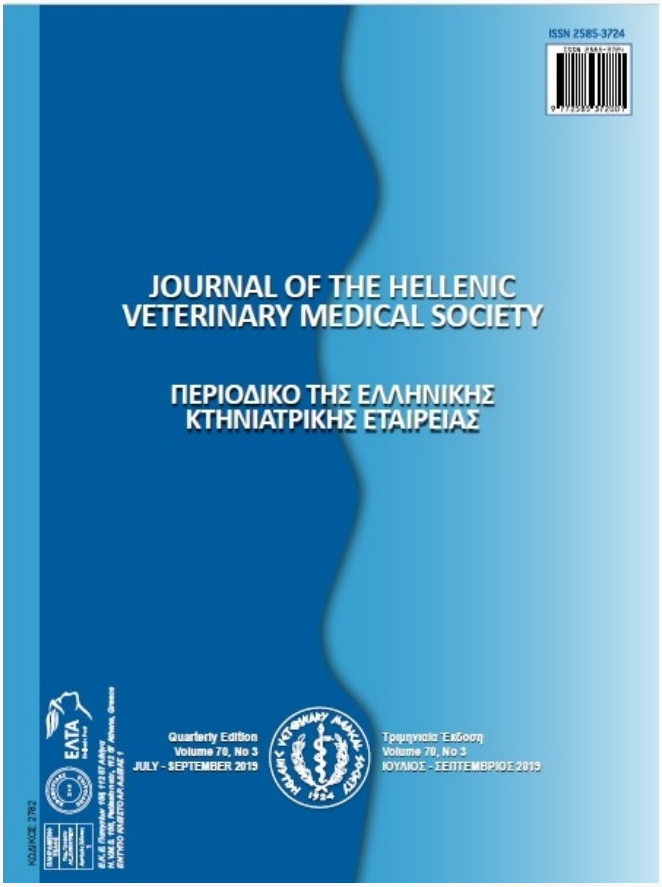
Περιοδικό της Ελληνικής Κτηνιατρικής Εταιρείας

Τόμ. 70, Αρ. 3 (2019)



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doi: 10.12681/jhvms.21798

Βιβλιογραφική αναφορά:

KOSTAKIS, C., ANAGNOSTOU, T., SAVVAS, I., VERVERIDIS, C., & KOURELIS, A. (2019). The effect of progesterone on the anesthetic and analgesic requirements for ovariohysterectomy in the dog. *Περιοδικό της Ελληνικής Κτηνιατρικής Εταιρείας*, *70*(3), 1717–1726. https://doi.org/10.12681/jhvms.21798

J HELLENIC VET MED SOC 2019, 70(3): 1717-1726
ΠΕΚΕ 2019, 70(3): 1717-1726

Research article
Ερευνητικό άρθρο

The effect of progesterone on the anesthetic and analgesic requirements for ovariohysterectomy in the dog

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ABSTRACT. The objective of the current study was to investigate the effect of serum progesterone concentration, either endogenous, during the ovarian cycle and pregnancy, or exogenous, when administered during anestrus, and of its active metabolite allopregnanolone, on anesthetic and analgesic requirements, as well as post-operative pain intensity, for the performance of ovariohysterectomy in dogs. One hundred and fifty healthy female dogs, which were admitted to our clinic for elective ovariohysterectomy, were included in the present study. They were allocated to 6 groups according to the stage of the ovarian cycle and the corresponding serum progesterone concentration. The six groups consisted of dogs in anestrus (group A), in anestrus which received intramuscular progesterone injections prior to surgery (group Ap), dogs in diestrus (group D), in diestrus which received subcutaneous aglepristone injections prior to surgery (group Da), in diestrus which received oral trilostane prior to surgery (group Dt) and dogs in pregnancy of duration of 28-42 days (group P). Serum progesterone concentrations were measured in all dogs before and after any hormonal treatment and serum allopregnanolone concentrations were measured in selected dogs from all groups. The required dose of propofol for induction of anesthesia and the required isoflurane concentration for maintenance of anesthesia and the need for intraoperative fentanyl administration and extra postoperative pethidine analgesia were recorded. After statistical analysis, there were no significant differences between groups, regarding their anesthetic or analgesic requirements, that could be attributed to serum progesterone and/or allopregnanolone concentration. However, moderate correlations within certain groups were noted. Serum progesterone or allopregnanolone concentrations do not seem to have an effect on anesthetic and analgesic requirements for ovariohysterectomy in the dog or any potential effect is weak enough to be masked by the action of anesthetic premedication and/or analgesic and/or anaesthetic drugs used.

Keywords: Progesterone, allopregnanolone, dog, anesthesia, ovariohysterectomy.

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Date of initial submission: 21-01-2019
Date of revised submission: 19-08-2019
Date of acceptance: 25-08-2019

ΠΕΡΙΛΗΨΗ. Η παρούσα μελέτη διερεύνησε την επίδραση της συγκέντρωσης της προγεστερόνης, είτε ενδογενούς κατά τον ωοθηκικό κύκλο και την κυοφορία, είτε εξωγενούς, καθώς και του ενεργού μεταβολίτη της αλλοπρεγνανολόνη, στις απαιτήσεις σε αναισθητικά και αναλγητικά φάρμακα και στην ένταση του μετεγχειρητικού πόνου για τη διεξαγωγή ωοθηκυστερεκτομής στον σκύλο. Περιελήφθησαν 150 υγιείς, θηλυκοί σκύλοι, οι οποίο προσκομίστηκαν στην Κλινική Ζώων Συντροφιάς του ΑΠΘ για ωοθηκυστερεκτομή και οι οποίοι κατανεμήθηκαν σε 6 ομάδες με βάση το στάδιο του ωοθηκικού κύκλου και την ενδεχόμενη ορμονική αγωγή που έλαβαν. Συγκεκριμένα, σχηματίστηκαν 6 ομάδες από ζώα στο στάδιο του ανοίστρου (ομάδα Α), ζώα σε άνοιστρο που έλαβαν προγεστερόνη ενδομυϊκώς (Απ), ζώα στο στάδιο του διοίστρου (Δ), ζώα σε δίοιστρο που έλαβαν υποδορίως αγλεπριστόνη (Δα), ζώα σε δίοιστρο που έλαβαν κάψουλες τριλοστάνης (Δτ) και ζώα σε κυοφορία 28-42 ημερών (Ε). Σε κάθε ζώο καταγράφηκαν οι απαιτήσεις σε προποφόλη για εγκατάσταση της γενικής αναισθησίας, οι απαιτήσεις σε ισοφλουράνιο για διατήρηση, οι απαιτήσεις σε επιπλέον αναλγητικά φάρμακα είτε διεγχειρητικά είτε μετεγχειρητικά και έγιναν μετεγχειρητικές εκτιμήσεις πόνου. Επίσης έγιναν αιμοληψίες σε συγκεκριμένες χρονικές στιγμές για ορμονολογικές αναλύσεις. Μετά τη στατιστική επεξεργασία των αποτελεσμάτων δε βρέθηκε στατιστικώς σημαντική διαφορά στις απαιτήσεις σε αναισθητικά ή σε αναλγητικά φάρμακα μεταξύ των ομάδων, ούτε στατιστικώς σημαντική συσχέτιση μεταξύ των απαιτήσεων αυτών και των συγκεντρώσεων στο αίμα της προγεστερόνης ή της αλλοπρεγνανολόνης, εκτός από 2 συσχετίσεις εντός συγκεκριμένων ομάδων. Συμπεραίνεται ότι οι συγκεντρώσεις προγεστερόνης ή αλλοπρεγνανολόνης στο αίμα δεν επηρεάζουν τις απαιτήσεις σε αναισθητικά ή αναλγητικά φάρμακα για τη διεξαγωγή ωοθηκυστερεκτομής στο σκύλο ή ότι ενδεχόμενη τέτοια επιρροή επικαλύπτεται από τη χορήγηση προαναισθητικής αγωγής και αναλγητικών φαρμάκων.

Λέξεις ευρετηρίασης: Προγεστερόνη, αλλοπρεγνανολόνη, σκύλος, αναισθησία, ωοθηκυστερεκτομή.

INTRODUCTION

The anesthetic effect of steroid hormones was first reported by Hans Selye in 1941, who injected progesterone, desoxycorticosterone (DCA) and testosterone in rats (1941a). Selye documented deep sedation immediately after the first two intraperitoneal injections, and about an hour after the testosterone injection, with more pronounced effect on female rats and longer duration of anesthesia in rats that had been partially hepatectomised. The author also noted a synergistic action of progesterone with chloroform, increasing the potency and duration of its action (Selye 1941b). Tanifuji et al. (1986) injected male dogs with progesterone and reported a significant decrease in halothane's minimum alveolar concentration (MAC), the same way Datta et al. reported decreased halothane MAC in rabbits (1989). The most recent relevant study was published by Shimizu et al. (2010) regarding the significant sevoflurane sparing effect of a single subcutaneous injection of progesterone for the loss of righting reflex (LRR) in male mice.

Decreases in MAC of volatile anesthetics attributable to high concentrations of endogenous progesterone were first recorded in animal species during pregnancy, specifically in ewes (Palahniuk et al. 1974) and in rats (Strout and Nahrwold 1981). Chinese researchers studied groups of women receiving anesthesia for termination of early pregnancy, and found decreased MAC for isoflurane, halothane and enflurane compared with non-pregnant women (Chan et al. 1996; Gin and Chan 1994). Consequent studies found decreased thiopentone requirements for hypnosis and anesthesia and decreased propofol requirements for loss of consciousness in the same setting (Gin et al. 1997; Mongardon et al. 2009), and the first study to prove a correlation between the decreased sevoflurane MAC and high progesterone concentration was published in 2006 (Erden et al. 2005). Such correlations were shown again in women receiving anesthesia in 2014. In specific, significant negative correlations were found between progesterone serum concentration and propofol requirements for loss of consciousness in early pregnancy (Fu et al. 2014), as well as sevoflurane requirements in late pregnancy in women receiving anesthesia for caesarian section (Lee et al. 2014).

Atkinson et al. (1965) tested 142 progesterone metabolites on mice and discovered 67 to have anesthetic properties, depending on their chemical structure. Progesterone metabolites site of action was discovered to be the chloride ion channel of the $GABA_A$ receptor (Majewska et al. 1986), and the metabolite responsible for progesterone anesthetic effect was proven to be allopregnanolone (Korneyev and Costa 1996).

The serum concentration of progesterone during the ovarian cycle in the dog is not greatly affected by pregnancy, i.e. even in the case of non-pregnancy, after proestrus and estrus, there is a 2 month period,

called diestrus, during which the progesterone serum concentration is high (Concannon et al. 1975). This makes the dog an ideal candidate for the study of progesterone's effect on anesthetic requirements, excluding any effects of a simultaneous pregnancy, which is known to lower volatile anesthetic requirements through changes in respiratory function (Cugell et al. 1953). In theory, dogs undergoing surgery during a period of increased progesterone serum concentrations (diestrus) should have lower anesthetic requirements than dogs in anestrus, when the concentration of progesterone is baseline (Concannon et al. 1975). The aim of the present study was to investigate the effects of increased progesterone serum concentrations on the anesthetic and analgesic requirements and on the intensity of postoperative pain in dogs undergoing ovariohysterectomy (OHE).

MATERIALS AND METHODS

This double-blind, placebo-controlled, prospective clinical study was conducted at the Companion Animal Clinic, Veterinary School, Aristotle University of Thessaloniki, Greece and was approved by the university's Ethics Committee (number of approval 16/21-2-2012). All owners of animals included in the study were thoroughly informed and signed a consent form. The study included 150 female dogs that were admitted to the clinic for elective OHE. All dogs were classified as ASA status I (American Society of Anesthesiologists physical status I) after clinical examination, complete blood count and examination of the genital system. Exclusion criteria were bodyweight lower than 4kg, age less than 8 months or more than 8 years, abnormal findings in any examination performed, positive results for leishmaniosis, ehrlichiosis or heartworm disease, previous hormonal treatments for the manipulation of the ovarian cycle, blood serum progesterone concentration in the range 2-5 ng/ml, pregnancy of less than 28 days, considered insignificant, or more than 42, considered late pregnancy, as well as overt aggression, which complicates the hospitalization of the dog.

The stage of the ovarian cycle was determined based on the reproductive history of each animal, the results of the examination of the genital system, microscopic examination of vaginal smears and the serum progesterone concentration measurements. During the initial classification of the animals in groups, a commercial chromatographic progesterone kit was used (OVULATION® TEST, VIRBAC), and progesterone concentration was measured in blood serum later on. Animals with a progesterone concentration ≤2 ng/ml were considered to be in anestrus and those with a concentration ≥5 ng/ml in diestrus. Pregnancy was diagnosed by abdominal ultrasonography (micro convex head, 5-10 MHz) and pregnancy duration was calculated using suitable software (EsaoteMyLabOne Vet, Esaote Europe B.V.).

Groups, hormonal treatments, blood sampling and assays

All animals were hospitalized for a minimum of 5 days and OHE was performed on day 3. Dogs in diestrus were assigned to 3 groups according to the hormonal treatment they received prior to surgery, dogs in anestrus were assigned to 2 groups accordingly, and pregnant animals formed 1 group. Assignment of animals in the respective groups was random, based on a random order table. All animals received, on days 1 and 2, two injections plus po capsules per day, either of hormonal treatments or placebos. Placebos were normal saline in the injections and empty capsule shells. In specific, the animals were assigned to 6 groups, as follows:

- group A (anestrus): dogs in anestrus that received only placebos (n=40)
- group Ap (anestrus-progesterone): dogs in anestrus that received progesterone (GESTONE®, NORDIC PHARMA, 5mg/kg im SID) on days 1 and 2 and sc placebo injections and po placebo capsule cells (n=29).
- group D (diestrus): dogs in diestrus that received only placebos (n= 23)
- group Da (diestrus-aglepristone): dogs in diestrus that received aglepristone (ALIZIN®, VIRBAC, 10mg/kg sc SID) on days 1 and 2 and im placebo injections and po placebo capsule cells (n= 17).
- group Dt (diestrus-trilostane): dogs in diestrus that received im and sc placebo injections and trilostane (VETORYL®, ALTAVET, one 60 mg capsule in animals under 20 kg and two 60 mg capsules in animals over 20 kg po BID) from day 1 till the morning of day 3 prior to the surgery (n= 11).
- group P (pregnancy): pregnant dogs that received only placebos (n= 30)

Blood was collected 3 or 4 times for hormonal as-

says. In all dogs, blood sample 1 was collected on day 1, before administration of any hormonal treatment or placebo, sample 2 on day 3 just prior to surgery and after administration of all hormonal treatments or placebo, and sample 3 was collected 5 hours after the end of surgery. Sample 4 was collected only from animals of groups D and Dt on day 5. Progesterone was measured in all animals in samples 1-3, allopregnanolone was measured in sample 2 in randomly selected animals from all groups and cortisol was measured in samples 1-4 in the animals of groups D and Dt.

Progesterone and cortisol concentrations were measured from blood serum using commercial electrochemiluminescence kits (cobas® cortisol II, cobas® progesterone III, Elecsys 2010, Roche Diagnostics) and allopregnanolone was measured using a quantitative sandwich ELISA kit (www.mybiosource.com).

Anesthetic management

All animals received their last meal on the night of day 2 and had free access to water up to 1 hour prior to administration of pre-anesthetic medication. Anesthetic management was supervised in all cases by the same anesthesiologist in charge (CK), who was blinded to the group the animal had been assigned to. After standard pre-anesthetic clinical examination, anesthetic premedication was administered intramuscularly consisting of acepromazine (0.05 mg/kg, CALMIVET®, VETOQUINOL) and pethidine (3 mg/kg, FAMAR), followed immediately by a subcutaneous injection of carprofen (4 mg/kg, RIMADYL®, PFIZER). Thirty minutes later, the cephalic vein was cannulated and anesthesia was induced with propofol (PROPOFOL MCT/LCT/FRESENIUS 1%, FRESENIUS KABI) administered intravenously in consecutive doses of 1 mg/kg, approximately 1 minute apart, until intubation of the trachea was easily feasible. After a cuffed endotracheal tube of appropriate diameter was in place, anesthesia was maintained with isoflurane (Forrane, Baxter Healthcare Ltd) in 100% oxygen administered via a suitable anesthetic circuit. A circle rebreathing circuit was used for animals weighing 8 kg or more and a non-rebreathing circuit for smaller animals. Prophylactic cefuroxime (Zinacef, Glaxo Smith Kline) was then administered intravenously in all animals and an isotonic crystalloid solution (Lactated Ringer's injection, VIOSER) intravenous infusion commenced and was continued until the end of surgery. After clipping and surgical preparation of the abdomen, the dog was transferred to the operating room, where monitoring commenced, consisting of electrocardiography, pulse oximetry, oscillometric blood pressure measurement (PC Scout, SpaceLabs Medical Inc.) and capnography and measurement of inspired and expired isoflurane concentrations (CapnomacUltima, Datex-Engstrom). The monitors and gas analyzer were calibrated according to the manufacturers' instructions prior to the study. Heart rate (HR), respiratory rate (RR) and systolic, diastolic and mean blood pressures (BP), as well as end-tidal carbon dioxide and inspired and expired concentrations of isoflurane were constantly monitored and recorded every 5 minutes until the end of surgery.

The vaporizer dial was initially set to deliver 2.5% isoflurane, but within a few minutes it was adjusted so as to provide the appropriate depth of surgical anesthesia, based on clinical assessment and monitored parameters. The depth of anesthesia was constantly evaluated by the anesthesiologist in charge (CK). In case of a 20% or more increase in HR and/or RR and/or BP associated to noxious stimuli the depth of anesthesia was deemed inadequate and the isoflurane dial-setting was increased by 0.5% and the oxygen flow was temporarily increased to 4 L/min. If this manipulation proved ineffective in normalizing the value of the elevated parameter within 2-3 minutes, fentanyl (Fentanyl, Jannsen Pharmaceutica NV) was administered intravenously (0.002 mg/kg). When surgery was completed, the administration of isoflurane and Lactated Ringer's solution were discontinued and the dogs were allowed to recover. All operations were performed by the same team of surgeons.

Pain and sedation were evaluated 1, 3 and 5 hours after the end of surgery by the anesthesiologist in charge (CK) using a visual analogue scale (0%: no pain, 100%: worst pain imaginable) (0%: no sedation, 100%: very heavily sedated). The pain assessment was based on the animals' posture, movement and response to palpation of the abdomen as described previously by Savvas et al. (2008). Intramuscular pethidine was administered as rescue analgesia in case of a pain score higher than 50% in any assessment and repeated as needed.

Statistical analysis

The required number of animals in each group for an effect size of 0.3 and achieved power at least 0.8 was calculated to be 19 (total number of animals 114).

All data were copied to digital spreadsheets (Mic-

rosoft Excel 2007) and analyzed using the SPSS v.15 software. Propofol requirements were calculated as total mg/kg, fentanyl and rescue analgesia requirements were recorded as "required" or "not required" and isoflurane requirements were calculated using the serial end- tidal concentrations recorded during surgery. Area under the curve (AUC) was calculated from those measurements, as proposed by Matthews et al. (1990), and then divided by the total duration of surgery, which varied between cases, to calculate the time standardized AUC (AUC_std) in each case, as described by Lawrence and De Lange(1997).

The normality of distribution of the data was checked using the Shapiro-Wilk normality test. A generalized linear model was used to evaluate differences between serum cortisol concentrations between groups D and Dt, as well as post operation pain scores between groups. Differences in propofol or isoflurane requirements were evaluated using one way analysis of variance (one way ANOVA), when evaluating 3 or more groups, and Student's T-test when evaluating 2 groups. A chi-squared test was used to evaluate differences between groups regarding fentanyl or rescue analgesia requirements and possible correlations between various factors were checked using Pearson's test. Values of $p \leq 0.05$ were considered statistically significant. Descriptive statistics are presented as mean ± standard deviation.

RESULTS

The distributions of weight, age, duration of anesthesia, duration of surgery, propofol requirements for induction, isoflurane AUC_st, pain scores and sedation scores were found to be normal in all 6 groups ($p = 0.103$-0.983). There were no statistically significant differences between groups regarding age, weight, duration of anesthesia and duration of surgery ($p=0.131$-0.653).

The serum progesterone concentrations in group Ap were significantly higher compared to those of group A after the im administration of progesterone ($p<0.0005$). The serum progesterone concentrations of group Dt were significantly lower than those of group D after the administration of trilostane ($p<0.0005$), but those of group Da were not significantly affected, when compared to group D, after the administration of aglepristone ($p=0.31$).

Propofol requirements ranged from 1 to 10 mg/kg with a mean of 3.88 ± 1.11 mg/kg. There was no significant difference between main groups A, D and P ($p = 0.713$), diestrus groups D, Da and Dt ($p = 0.378$) or between anestrus groups A and Ap ($p = 0.857$). No correlation was found between propofol requirements and progesterone or allopregnanolone concentration, except a moderate inverse correlation between progesterone and propofol in group Da ($r = -0.569$, $p = 0.017$).

Isoflurane requirements (AUC_std) ranged from 0.7 to 2.68% with a mean of 1.65 ± 0.26%. No significant difference was discovered after comparison between groups A, D and P ($p = 0.109$), groups D, Da and Dt ($p = 0.623$) or groups A and Ap ($p = 0.601$). There was no significant correlation between isoflurane requirements and progesterone or allopregnanolone concentration, except a moderate inverse correlation between progesterone and AUC_std in group P ($r = -0.39$, $p = 0.033$).

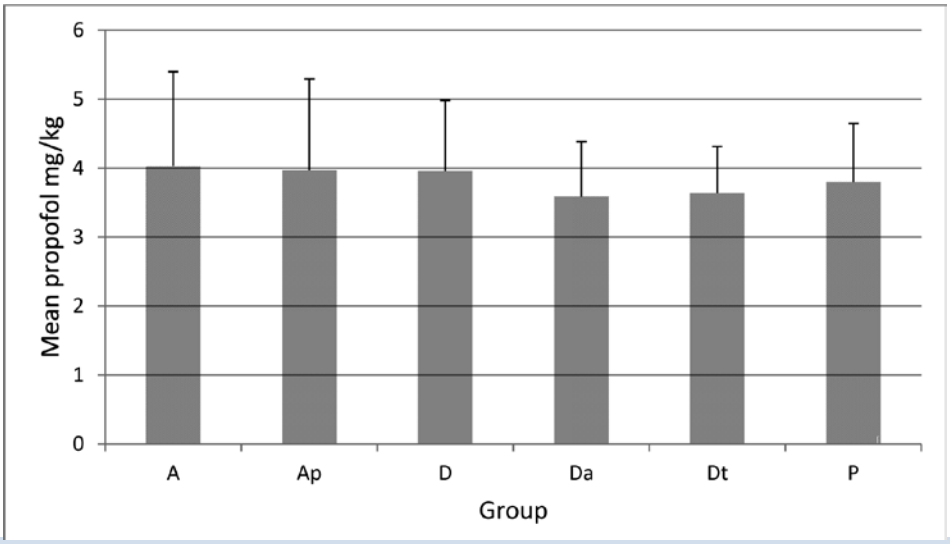


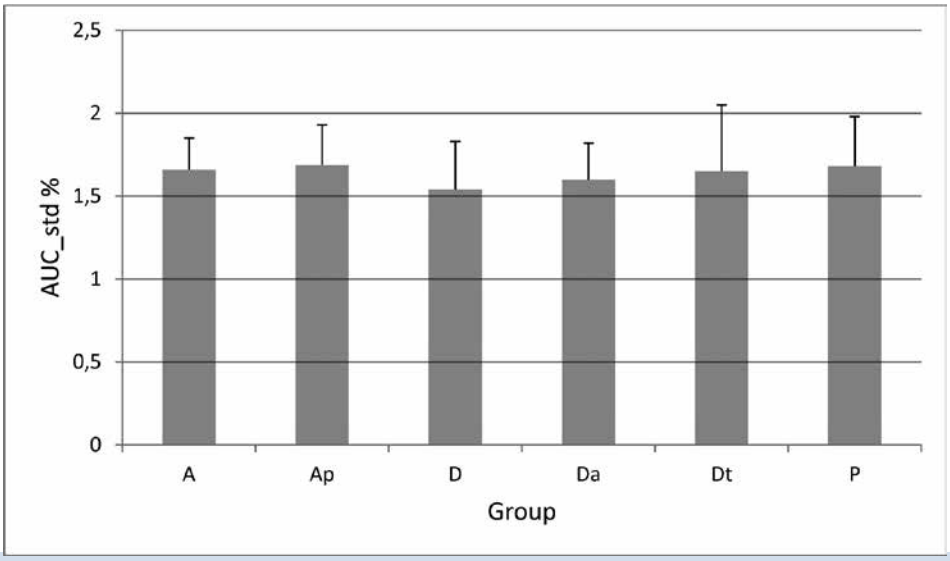
Figure 1.

There were no significant differences between the groups regarding the incidence of fentanyl intra-operative analgesia (p = 0.811) or the incidence of post-operative pethidine rescue analgesia (p = 0.858).

Concerning the post-operative pain scores, there were significant differences within each group over time (p<0.05). There were no significant differences between groups at the specific time points (p>0.05), with the exception of groups D and Dt, where significantly higher scores were found in group Dt at all 3 time points (1h p = 0.033, 3h p = 0.022, and 6h p = 0.01). There was no statistically significant correlation between pain scores (1h) and the progesterone (sample 2) (r=0.068, p=0.405) or allopregnanolone (r=0.064, p=0.565) concentrations.

Sedation scores did not differ significantly between groups at any time point (1h p=0.571, 3h p=0.198, 6h p=0.24).

The serum cortisol concentrations between groups D and Dt were significantly different in blood samples 2 and 3 (p = 0.02 and p = 0.09), the concentrations of group D being higher, as opposed to samples 1 and 4 (p = 0.566 and p = 0.075).



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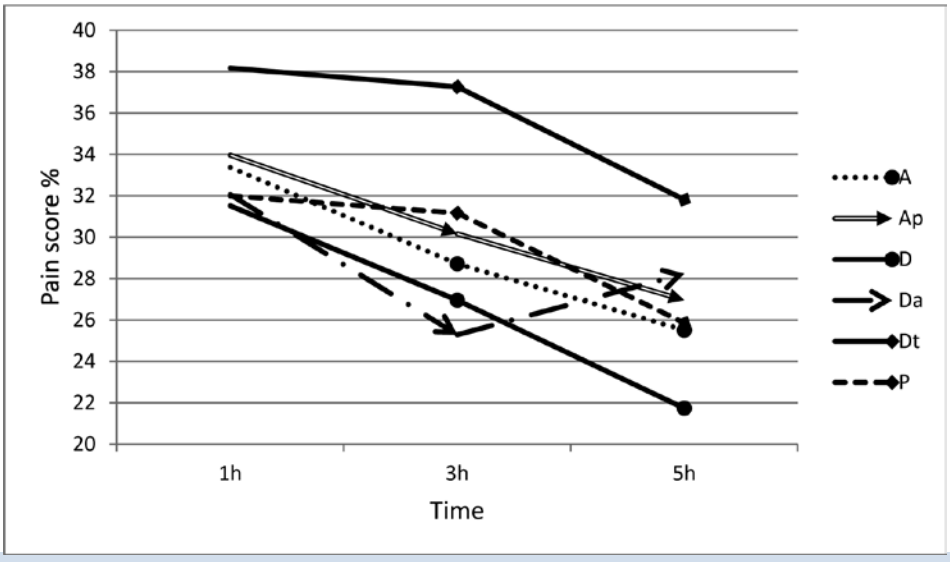

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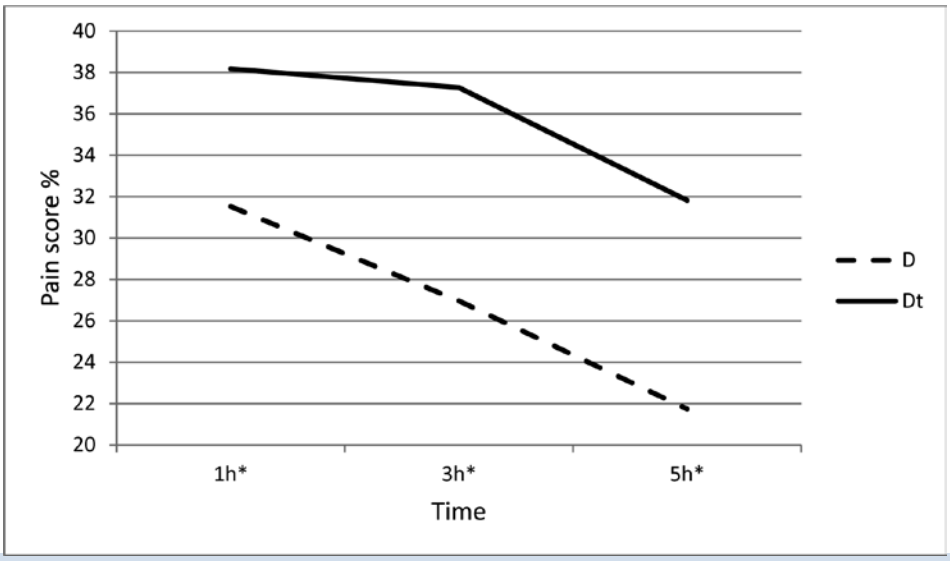

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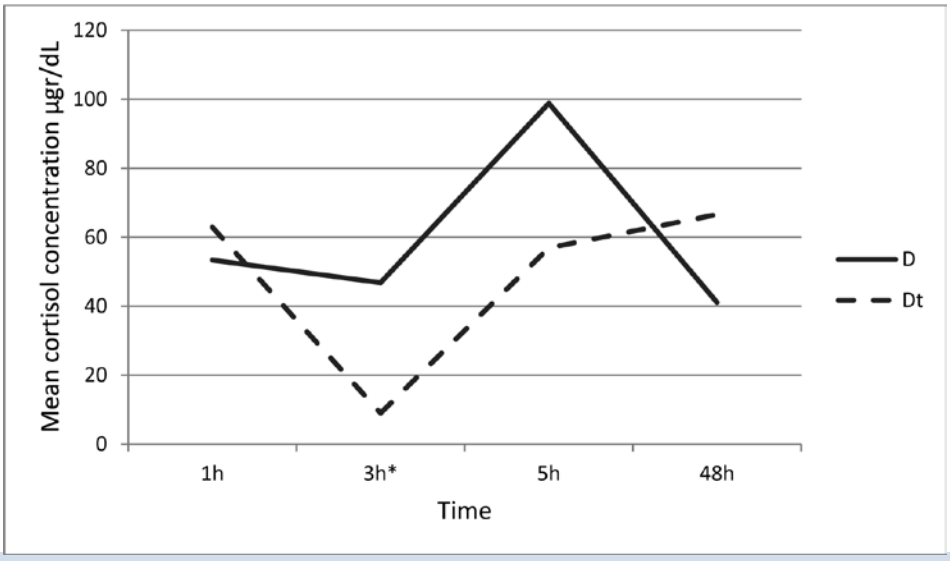

Figure 5.

DISCUSSION

The anesthetic effect of progesterone was discovered in 1941 by Hans Selye (1941a) and its volatile anesthetic sparing effect was documented in dogs, rabbits and mice (Datta et al. 1989; Shimizu et al. 2010; Tanifuji et al. 1986). High levels of endogenous progesterone have also been found to lower requirements for both volatile (Chan et al. 1996; Erden et al. 2005; Gin and Chan 1994; Lee et al. 2014; Palahniuk et al. 1974; Strout and Nahrwold 1981) and injectable anesthetics (Fu et al. 2014; Gin et al. 1997; Mongardon et al. 2009) in various species, including humans. The present study, on the contrary, did not detect any difference in anesthetic or analgesic requirements in dogs, regardless of their baseline or high serum progesterone concentration.

Selye's study was the first report of steroid anesthetic action and inspired all future studies, which eventually lead to the discovery and marketing of steroid anesthetics. However, very large doses of progesterone were used to achieve anesthesia and the blood progesterone concentrations were not reported, so one cannot compare to the endogenous levels normally occurring during the ovarian cycle or pregnancy. Selye injected 100gr rats with 35mg of progesterone, nearly 350 mg/kg. Merryman et al. (1954) tried to reproduce Selye's results in humans, by injecting 500mg of progesterone via infusion over 1 hour, which caused sleep, not anesthesia, similar to Arafat et al. who reported sleep after a 400mg per os

administration (1988). It seems, therefore, that progesterone's anesthetic effect requires large doses.

Progesterone's MAC-reducing effect is reported in many studies (Datta et al. 1989; Erden et al. 2005; Lee et al. 2014; Selye 1941a; Shimizu et al. 2010; Tanifuji et al. 1986). The determination of MAC for volatile anesthetics, although a universal standard for anesthetic potency, is based on a time consuming laboratory procedure and does not correspond to clinical surgical conditions (Eger et al. 1965). It also does not take into consideration preanesthetic or analgesic drugs, which were used in all cases in our study, and could have masked progesterone's anesthetic sparing effect. Other methodologies used in other studies, such as the modified sevoflurane pump used by Lee et al. to calculate the volume of liquid sevoflurane consumed (2014), Mongardon et al.'s automated propofol infusion pump which calculated a predicted effect site concentration of propofol based on a pharmacokinetic algorithm (2009), and even LRR, as used by Shimitzu et al. on mice (2010), are considered laboratory procedures, not common clinical practice, and most are not even applicable to dogs.

The main finding of the present study is that increased progesterone serum concentrations do not reduce the anesthetic and/or analgesic requirements or the intensity of post-operative pain in dogs undergoing OHE. Since no anesthetic-sparing effect of progesterone was shown in the present study, no speculations can be made about the potential role of progesterone receptors (blocked by aglepristone in group Da). Moreover, pregnancy did not influence the anesthetic and/or analgesic requirements or the intensity of post-operative pain. Pregnancy could have influenced via mechanisms unrelated to any potential action of progesterone (e.g. via changes in cardiovascular or respiratory function or via mechanical factors related to the size of the gravid uterus affecting the function of other systems), but no significant effect was shown. Exogenous administration of progesterone in our study did not induce any anesthetic-sparing effect, as one would expect since diestrus and pregnancy did not either induce such an effect.

The most comparable study to the present is the one by Tanifuji et al. (1986), as it was performed on dogs, and serum progesterone concentrations were measured. Tanifuji et al. injected 6 intact male dogs for 1 week with progesterone and calculated halothane MAC using the tail clamp technique. They used 2 dosages of progesterone, 2mg/kg and 5 mg/kg, and in both cases found halothane MAC to be significantly decreased with a strong negative correlation to progesterone concentration. The progesterone concentration increased from control concentration 1.46 ± 0.22 to 4.41 ± 0.7 and 8.91 ± 1.97 ng/ml. In our study, on the other hand, 29 female dogs were injected with 5 mg/kg of progesterone for 2 days and the serum progesterone concentration increased from 1.24 ± 2.97 to 36.44 ± 18.08 ng/ml, and still no statistically significant difference in isoflurane requirements was found when compared to the anestrus group. The difference in the results could be attributed to shorter duration of progesterone administration to female dogs in the present study (2 days) compared to a longer time of administration (1 week) to male dogs in the study by Tanifuji et al. However, it is the authors' opinion that a more likely explanation is that although progesterone shows MAC-reducing effects in a controlled setting, this effect is probably not intense enough to be detected during real-time surgery in the clinical setting.

The current study assessed the isoflurane requirements for performance of surgery in dogs, while in other relevant studies (Datta et al. 1989; Tanifuji et al. 1986) the MAC calculation was used as a simulation of surgical stimulation. It is likely that high serum progesterone levels indeed have an anesthetic effect that can be detected in a laboratory environment, but this effect is probably not so intense as to be shown as anesthetic-sparing effect in the clinical setting during surgery. When trying to answer the question whether the veterinary anesthetist should be prepared for the possibility that lower doses of anesthetics/analgesics might be required for performance of surgery in a dog in diestrus or pregnancy as opposed to a dog in anestrus, the methodology of the current study is more helpful in giving the correct answer than studies based on MAC determination.

The negative correlation that was detected in group P between serum progesterone concentrations and isoflurane AUC_std could be a reflection of this anesthetic effect that high serum progesterone exerts and which is depicted in studies based on MAC determination. Thus, there could be a real progesterone influence, at least in pregnant animals, but which, if present, seems to be too weak to be detected as reduced isoflurane requirements for maintenance of anesthesia, at least with the methodology used in the present study. Concerning the negative correlation detected between serum progesterone concentrations and propofol requirements in group Da, it could be

speculated that unavailability of cellular progesterone receptors occupied by aglepristone could result in more progesterone being available for metabolism to allopregnanolone and thus for action on GABA receptors, leading to central nervous system depression. As in group P for isoflurane, however, it seems that any effect of aglepristone is not intense enough to be detected as a reduction in anesthetic/analgesic requirements during surgery. The two correlations noted in the two groups P and Da taken together could be considered as indications of a potential central nervous system-depressive action of progesterone under specific circumstances that might be required for this action to be highlighted.

Trilostane was administered as it inhibits steroid synthesis from the adrenal cortex, including both cortisol and progesterone (de Gier et al. 2011), since more specific progesterone synthesis inhibitors, like epostane or azastane, were not available even for research purposes. Since trilostane inhibits cortisol synthesis, serum cortisol was monitored in groups D and Dt in blood samples 1 through 4. The serum cortisol concentration was significantly lower in group Dt compared to group D in blood samples 2 and 3 ($p = 0.002$ and $p = 0.009$ respectively) but was not lower in blood sample 4 ($p = 0.075$), indicating that cortisol synthesis returns to pre-administration levels within 48 hours after the last trilostane administration. This is the first report, to our knowledge, of cortisol's synthesis recovery after trilostane administration in healthy dogs, since all relevant publications study cortisol synthesis inhibition in dogs suffering from Cushing's disease.

Post-operative pain scores were significantly higher in group Dt compared to those of control group D, but the incidence of fentanyl intra-operative analgesia or pethidine post-operative analgesia was not higher in group Dt. Detection of higher pain scores in a group of dogs (Dt) that received a drug with a "pro-algesic action" (deprivation of synthesis of an endogenous hormone with anti-inflammatory effects) can be viewed as a confirmation that the pain-assessing tool used in the present study was effective in discriminating animals in more pain.

A limitation of the current study is the small number of animals in group Dt in comparison to the other groups, owing to the fact that trilostane, the only steroid synthesis inhibitor available, was commercially unavailable for a substantial period of the duration of the study. Regarding the methodology used in the current study for the detection of increased isoflurane requirements, instead of MAC calculation, it is the authors' opinion that it is more representative of clinical surgical conditions and would detect differences significant enough to suggest changes in the anesthetic management of such cases. A similar methodology was used by Lawrence and De Lange (1997) in their human study on surgeries of varying duration, and by Columbano et al. (2012) in dogs undergoing OHE.

CONCLUSIONS

In conclusion, progesterone, either endogenous during the ovarian cycle and pregnancy, or administered intramuscularly, does not affect anesthetic or analgesic requirements for the performance of ovariohysterectomy in dogs. In the author's opinion, any anesthetic effect of progesterone is not intense enough and/or is masked by the use of pre-anesthetic medications and analgesic drugs, such as non-steroidal anti-inflammatory drugs and opioids. Dogs receiving trilostane have significantly higher post-operative pain scores than dogs not receiving trilostane, so extra analgesic care is warranted in such animals undergoing surgery.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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