Aus dem Department für Kleintiermedizin

der Veterinärmedizinischen Universität Wien

Institut/Klinik für Anästhesie und Perioperative Intensivmedizin

(LeiterIn: Ulrike Auer)

Comparing the Stress Response of Two Different Locoregional Approaches in Dogs Undergoing Elective Orchiectomy

Diplomarbeit

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vorgelegt von Buchegger Raphael

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Betreuerin: Dr. Christina Braun, Dipl. ACVAA

Interner Betreuer: Christian Lohinger

Begutachter/in:

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1. Introduction

The orchiectomy in dogs is a common procedure performed by small animal practitioners. As in every other surgical procedure, it is important to carry out and reassess the analgesia to improve wellbeing of the dog and avoid negative repercussion of pain. While analgesia can be provided using systemically applied drugs, the use of loco-regional anaesthesia not only avoids systemic side effects, but it is also in principal the only way to provide complete analgesia. In addition loco-regional analgesia has beneficial effects on pulmonary function, gastrointestinal function, reduces thromboembolic and cardiac complications and improves recovery time (Desborough 2000). Indeed, the use of loco-regional anaesthesia has been recommended whenever possible considering its high efficiency and low incidence of negative side effects (Epstein et al. 2015).

The intratesticular block is a common loco-regional method used for orchiectomies. Studies that have investigated this block could show a reduction of mean arterial pressure (MAP) (Huuskonen et al., 2013) and end tidal isoflurane requirement (McMillan et al., 2012), concluding that it adds an analgetic component to the anaesthetic protocol. In another study looking at post-operative analgesia no significant difference was found between patients given the intratesticular block intra-operatively and those with no analgesia (Stevens et al. 2013).

Recently, the intrafunicular block was described as another useful loco-regional technique to apply local anaesthesia/analgesia for orchiectomies (Rodriguez et al. 2016). For this, the local anaesthetic is injected into the funiculus spermaticus instead of the testicle as it is the case for the intratesticular block. To ensure exact application of the local anaesthetic during the intrafunicular block, it is only performed with an ultrasound device to observe the correct position of the needle. In isoflurane-anesthetized donkeys, intrafunicular injection of lidocaine before castration decreased intraoperative nociception and therefore significantly reduced the concentration of the volatile agent necessary to achieve a sufficient surgical anaesthesia (Suriano et al. 2014). This indicates an analgesic effect on the donkeys, however, MAP and respiratory rate (RR) were not different to the control group with only saline solution injected. Haga and Ranheim (2004) reported that applying lidocaine both into the

funiculus spermaticus or into the testes is effective in reducing signs of nociception caused by castration in piglets. Similarly, both blocks reduced the amount of intraoperative analgesics, anaesthetics and nociceptive response in dogs (Rodriguez et al. 2016). In the most recent study dogs given an intra-funicular block prior to orchiectomy had lower pain scores post operatively compared to dogs receiving balanced anaesthesia only (Cicirelli et al. 2021).

None of the studies published so far compared the intratesticular block with the newer method of an intrafunicular block. While studies looked at cardiorespiratory changes, use of inhalant anaesthetics and analgesics, none of them included stress response as an outcome.

The definition of stress response is the hormonal and metabolic change, which follows injury or trauma. It is part of the systemic reaction to injury, which encompasses a wide range of endocrinological, immunological and haematological effects. The stress response can be quantified and therefore the results more easily compared between patients. This makes it a suitable parameter for anaesthetized patients given the same anaesthetic protocol. The stress response to surgery is characterized by increased secretion of pituitary hormones and activation of the sympathetic nervous system. The changes in pituitary secretion have secondary effects on hormone secretion from target organs. For example, release of corticotrophin from the pituitary stimulates cortisol secretion from the adrenal cortex. Cortisol secretion from the adrenal cortex increases rapidly following the start of surgery, due to stimulation by ACTH (Desborough 2000). Because of this, cortisol is used as parameter for stress response and analgesic efficacy in several studies (Church et al. 2006, Garnier et al. 1990, Kona-Boun et al. 2006, Srithunyarat et al. 2016).

The aim of this study was to compare the stress response using plasma cortisol levels in dogs undergoing orchiectomy after either the intrafunicular or intratesticular block. It was hypothesized that male dogs undergoing elective orchiectomy receiving an ultrasound guided intrafunicular block with a local anaesthetic have lower plasma cortisol levels compared to the ones receiving an intratesticular application of such anaesthetic.

2. Material and Methods

This study is part of a doctorate/ larger study.

The study was approved by the Ethics and Animal Welfare Committee of the University of Veterinary Medicine, Vienna in accordance with the University's guidelines for Good Scientific Practice and authorized by the Austrian Federal Ministry of Education, Science and Research (ref BMBWF [2020-0.282.309]) in accordance with current legislation. All owners provided informed consent. This study included 19 privately owned healthy male dogs. Only dogs considered healthy based on clinical exam and bloodwork (CBC/Chem) were included in the study. All dogs came in the morning of surgery for elective orchiectomy to the Gynaecological Clinic of veterinary medicine at the Vetmeduni Vienna.

Pre- surgery

The dogs were fasted overnight for at least 12 hours with free access to water. All dogs got a physical examination prior to premedication. The physical examination included pulse quality and frequency, hydration status, temperature, mucous membrane colour, capillary refill time, internal body temperature, and auscultation of heart and lungs. Blood was taken and examined for abnormalities including the following parameter: leukocytes, erythrocytes, haemoglobin, haematocrit, MCV (mean corpuscular volume), MCH (mean corpuscular haemoglobin), MCHC (mean corpuscular haemoglobin concentration), rod-nucleated granulocytes, segmental granulocytes, lymphocytes, monocytes, eosinophiles, basophiles, large unstained cells, lymphoblasts, juveniles, CHCM (corpuscular haemoglobin concentration mean), MPXI (mean myeloperoxidase activity of neutrophil granulocytes), RDW (red cell distribution width), plasma protein, creatinine, albumin. If the examination showed clinically significant abnormalities, which needed further diagnostics, the patients were excluded from the study.

After physical examination and blood withdrawal the dogs were brought into the preparation room of the anaesthesia ward. There, each got their own cage, where they waited until their appointment. Approximately 45 min before the scheduled start of the surgery dogs were premedicated. Premedication consisted of acepromazine 10-20 µg/kg (10 mg/ml

Vanastress®, VANA GesmbH, Austria) and methadone 0.2 mg/kg (10 mg/ml Methadone Streuli AG, Switzerland) administered intramuscularly in the hind limb, in the musculi semitendinosus or semimembranosus. Afterwards they were returned into their cage and rested for approximately 30 min until they showed signs of sedation. Then a catheter, 18 to 22 gauge depending on the size of the dog (Vasofix® Safety, B. Braun Austria GmbH, Austria), was inserted in one of their cephalic veins. The patients were placed on the preparation table and were preoxygenated. Two to three minutes after the start of preoxygenation, propofol (PROPOFOL 'Fresenius' 1 percent, Fresenius Kabi, Austria) was injected to effect, until orotracheal intubation (RÜSCH Super Safetyclear RÜSCH Austria GmbH, Austria) was tolerated.

General anaesthesia

After endotracheal intubation anaesthesia was maintained with isoflurane (1000 mg/g Vetflurane, Virbac Animal Health Netherlands, Netherlands) in oxygen. All dogs received meloxicam 0,2 mg/kg intravenously (Metacam® 5 mg/ml Injektionsloesung für Hunde und Katzen, Boehringer Ingelheim Vetmedica GmbH, Germany) after induction of anaesthesia. Apparative monitoring was started including pulse oximetry, capnography, endtidal isoflurane, pulse frequency and a respiratory frequency. A blood pressure cuff for non-invasive/ oscillometric bloodpressure was placed but not started until arriving in the surgery room.

Simultaneously a second IV catheter, 20 Gauge, was placed on the contralateral cephalic vein to obtain blood for cortisol measurements. After that the patient got prepared for the surgery by shaving, cleaning and a first disinfection of the scrotum, the prescrotal and the perineal region, the dogs were moved into dorsal recumbency on a movable table to bring them into the surgery room.

Locoregional approaches

Up to that point, all patients were treated the same. After pre-medication, a slip of paper was blindly drawn from a plastic bag to assign them to a group, i.e. either intratesticular or intrafunicular block.

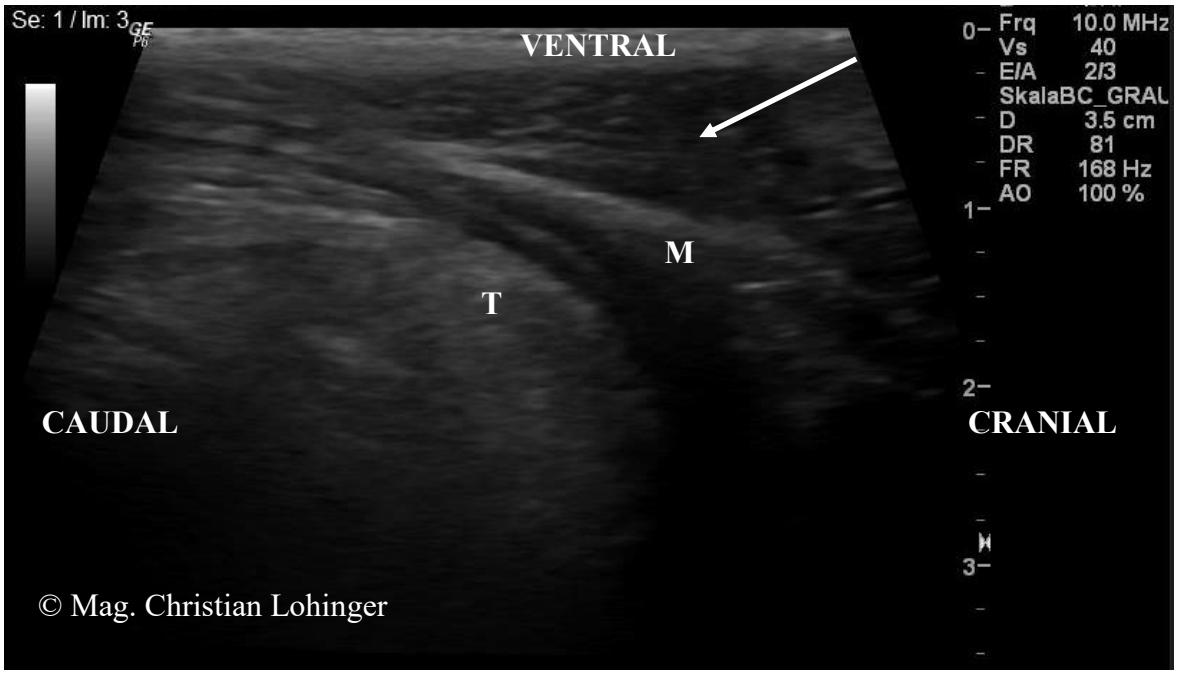
Intratesticular block

Ten dogs received 3mg/kg lidocaine 2% (Xylanaest® purum 2%, Gebro Pharma GmbH, Austria) injected into their testicles, i.e. 1.5 mg/kg or a volume of 0.075 mL/kg per testicle. From the caudal pole, the needle was inserted and lidocaine was injected after aspiration while the needle was slowly pulled back out.

Intrafunicular block

Nine dogs got 3 mg/kg lidocaine (Xylanaest® purum 2%, Gebro Pharma GmbH, Austria) injected into the funiculus spermaticus. The dogs were laying in dorsal recumbency and the skin aseptically prepared. The performing physician (always the same) held the testicle and the ultrasound probe (10-5mHz linear) both in one hand to display the funiculus spermaticus. With the other hand lidocaine was injected with a 23G, 30mm needle while observing the position of the needle on the monitor (SonoSite® Nanomaxx).

Picture 1: funiculus spermaticus in an ultrasonographic image



Sagittal image of the testicle and adjacent part of the funiculus spermaticus.

Testicle (T), Musculus cremaster (M), Injection site (Arrow)

Picture 2: Ultrasound guided intrafunicular block

The image shows how the intrafunicular block is performed. The local anesthetic is injected while the tip of the needle is observed on the screen. (T) testicle

Surgery

After performing the local block, dogs were brought into the surgery room. Before the start of the surgery the scrotum, the prescrotal and perineal region were disinfected a second time. All surgeries were performed by physicians of the Gynaecological Clinic using an approach with one prescrotal incision. The testes were pushed through the incision and bluntly dissected from fat and soft tissue. The spermatic cord was clamped two times and was ligated two times. The first ligation was placed proximal of the proximal clamp and the second distally of the distal clamps. The spermatic cord was cut between the distal clamp and the

distal ligature. The whole process was done for each testis individually. The incision was closed with two sutures, one to close the parietal vaginal tunic and one intradermal. During surgery dogs were monitored continuously and all vital parameters were recorded every five minutes. In case of increases in heart rate, respiratory frequency or non-invasive blood pressure by more than 20% of baseline, dogs would receive a fentanyl bolus of 1 mcg/kg intravenously. When the surgeon was about to finish the isoflurane vaporizer was reduced and eventually turned off to allow for recovery of the dog.

Post- surgery

After the surgery was finished, the dog was placed in lateral recumbency and returned to their cage without apparative monitoring. While in the cage the dog was monitored by a physician and extubated as soon as it regained a gagging reflex.

After 120 min post extubation, dogs received a dose of methadone (0.1 - 0.2 mg/kg IV) and the catheter was removed. Dogs were then taken to the owner. The owner was given a funnel for licking protection and meloxicam 0.2 mg/kg (Metacam® 1,5 mg/ml Suspension zum Eingeben für Hunde und Katzen, Boehringer Ingelheim Vetmedica GmbH, Germany) for the next 2 days.

Study protocol

Time points for blood sampling for cortisol measurements

Catheter placement (T1, baseline), clamping first testicle (T2), clamping second testicle (T3), 30 minutes after extubation (T4) and 120 minutes after extubation (T5). The first blood sample was obtained right after placing the first catheter. Because this one was used for general anaesthesia the other samples were obtained from an additional 20 Gauge IV catheter placed on the contralateral cephalic vein.

Cortisol measurement

Cortisol measurement was performed at the Vetmeduni Vienna, department of Pathobiology. The Immulite 2000 XPi (Siemens Healthcare Diagnostics GmbH ©) was used for testing. The method which was used was a competitive, solid phases-, chemiluminescent-

immunoassay. In this method the sample was placed in a sample tube with a test specific beat and a reagent. Then it was incubated in constant motion for optimized incubation kinetics. Following this the sample was washed four times and chemiluminescent substrate was added. This substrate reacts to an alkaline phosphatase label, which was bound to the bead during the incubation. The machine measured the resulting light reaction multiple times, calculated and displayed the results automatically. In various performance tests the Immulite 2000 Xpi performed good. (Roberts and Roberts 2004) (Tello and Hernandez 2000)

Statistical analysis

The statistical analysis was performed by the department of bioinformatic and biostatistics using the program IBM SPSS v24. A repeated measures analysis of variance was used to test for treatment effects. The Kolmogorov-Smirnoff-Test was used to test for normality in the cortisol measurements by time point for each treatment group. An alpha level of 0.05 was used for all statistical analyses. In addition descripte statistics was performed to better visualize the data. Diagrams were created with Microsoft Office Excel 2008.

3. Results

Of the 19 dogs used in this study, 12 dogs were of mixed breed, 2 were American Staffordshire Terrier and 6 of varied other breeds. One patient of the intrafunicular group was excluded from data analysis because he required rescue analgesia at time point T3. His HR (heart rate) and MAP increased by over 20%.Dogs weighted on average 15 +/- 9.1 kg and 18.3 +/- 11 kg and were 3.2 +/- 2.5 years and 3.4+/- 2.4 old in the intratesticular and intrafunicular group, respectively. For individual details see appendix I.

Cortisol measurements followed a normal distribution (range of Kolmogorov-Smirnoff-Test Z statistic: 0.43 to 0.87). The model testing for the treatment-by-time effect was not statistically significant ($F = 3.498$, $df = 4$)

The test of inner-subject effect between the sampling points, according to Greenhouse-Geisser ($p = 0.012$), was significant. This indicates a significant change in overall cortisol levels during the experiment.

The test of inner-subject effect between the two groups was testet with Greenhouse-Geisser. It was not significant (p = 0.477). While the two groups differed in their absolute cortisol levels they had a similar progression, i.e. relative change over time. See figure 1.

The test of intermediate effect was not significant (p = 0.159). This indicates the significant change over time was the same for both groups, even if the groups differed looking at absolute measurement results. See figure 2.

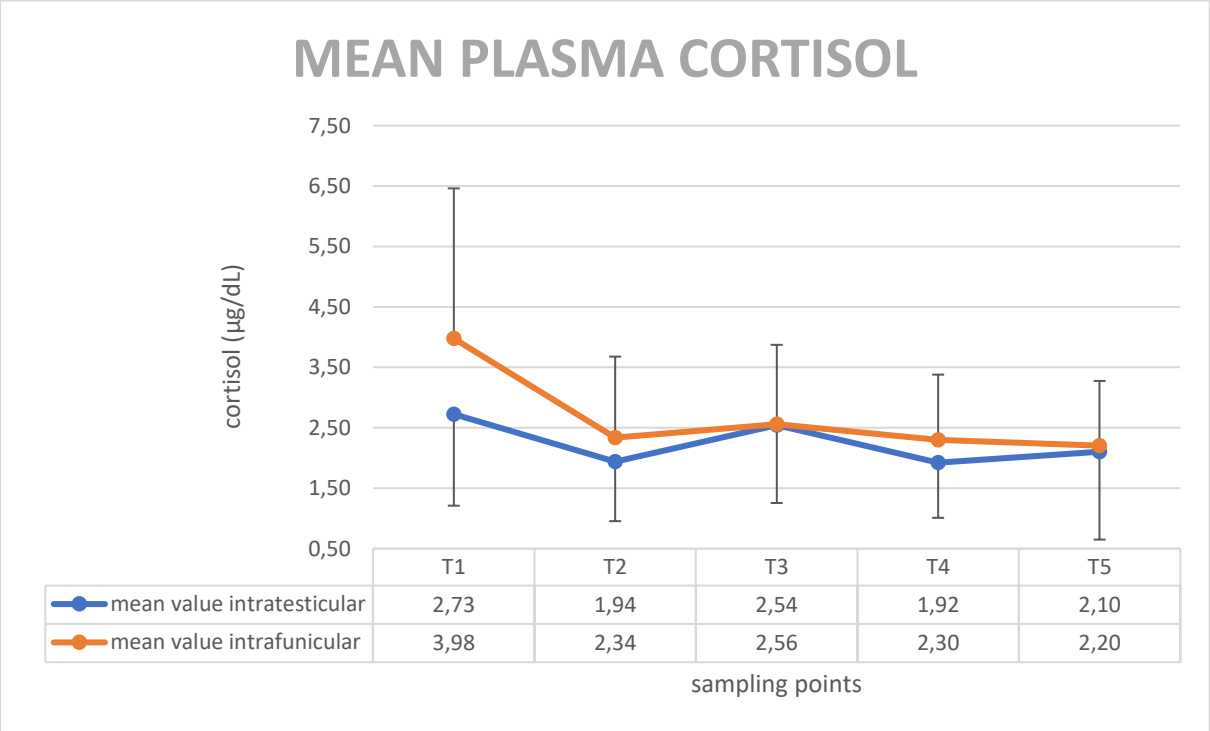


Figure 1: Changes in plasma cortisol in nineteen anaesthetized dogs during orchiectomy after receiving an intratesticular (n =10) or intrafunicular (n = 9) block. Time points: T1: catheter placement, T2: clamping first testicle, T3: clamping second testicle, T4: 30 minutes after extubation, T5: 120 minutes after extubation. The bar represents the upper and lower standard deviation, respectively.

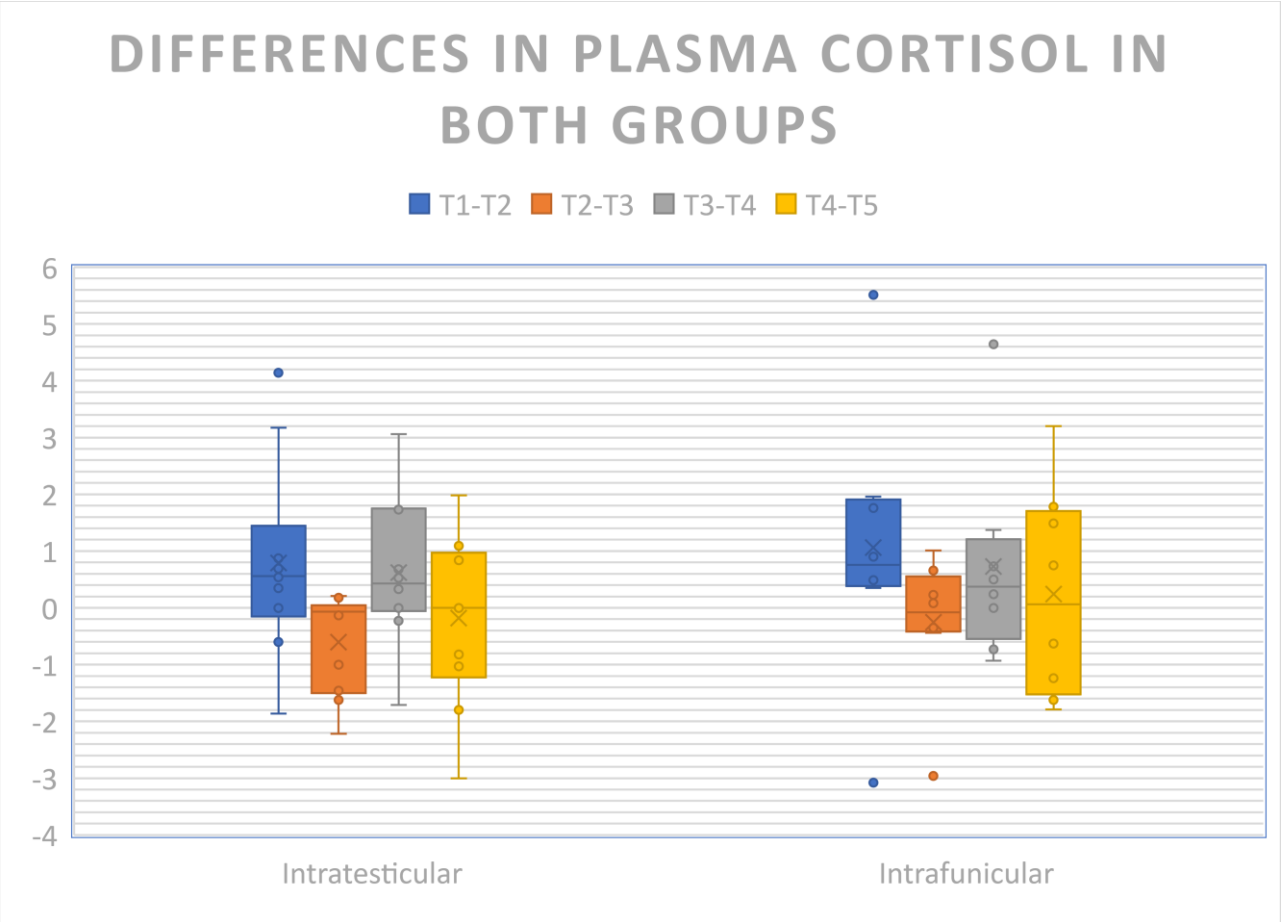


Figure 2. Differences in plasma cortisol between sampling points in nineteen dogs undergoing orchiectomy after receiving an intratesticular (n = 10) or intrafunicular (n = 9) block. Time points: T1: catheter placement, T2: clamping first testicle, T3: clamping second testicle, T4: 30 minutes after extubation, T5: 120 minutes after extubation.

4. Discussion

This study compared plasma cortisol levels as a measure of stress response in dogs that underwent elective orchiectomy and received an intratesticular or intrafunicular block. Contrary to the study hypothesis no significant differences in cortisol levels were found between the two groups.

Baseline cortisol levels were different between the two groups of the present study. In awake, unpremedicated dogs, plasma cortisol is around 150 mmol/L (approximately 5.4µg/dL). It typically increases slightly in the afternoon directly related to activity and drops past 8 pm (Castillo et al. 2009). Dogs, unlike humans, do not have a significant difference in circadian rhythm for cortisol. Premedicated dogs in the current study had lower plasma cortisol levels (2.7-4 µg/dL) compared to the ones by Castillo et al. (2009), but similar ones to dogs in another study that underwent a 24 hour acclimation period prior to sampling (approximately 3+/-2 µg/dL) (Devitt et al. 2005). These results indicate that premedication (acepromazine, methadone) had little to a slight decreasing effect on plasma cortisol but does not explain the difference between groups. While unlikely differences in fasting time could result in differences in baseline cortisol levels between the two groups as 12-24 h fasted dogs in a study by (Reimers et al. 1986) (1986) had lower cortisol than not fasted dogs. All owners were instructed to withhold food throughout the night before surgery. Cortisol secretion in dogs is also dependent on other factors such as individual patterns, breed, living conditions and especially distribution of physical activity, rest and sleep during the day and night (Kemppainen and Sartin 1984) (Kolevska et al. 2003). Blood withdrawal itself has been shown to increase plasma cortisol level (Knol et al. 1992) and it is possible that the individual reaction to this stimulus was different. Another possible cause could be the timing of blood sampling at T1. Engeland et al. (1990) has shown that cortisol changes significantly in dogs 6 minutes after a noise stimulus. Unfortunately, the noise level was not recorded for each dog and thus no inference can be made. In general, the relatively low number of dogs does not allow for further analysis of any of the aforementioned influencing factors.

Studies in dogs undergoing different surgeries report elevated cortisol blood levels during and after the surgery. Devitt et al. (2005) and Church et al. (2006) noted the severity of the

operation apparently does not influence the magnitude of the cortisol response. Similarly different surgeons don't alter the cortisol levels Michelsen et al. (2012). None of those studies used locoregional anaesthesia though. There might be a similar response in the orchiectomy but for ethical reasons in the current study, there was no negative control group without any locoregional anaesthesia.

The effect of anaesthetics on cortisol secretion varies, with the ones used in the present study showing no effect or a decrease in cortisol values. In 2013 a study in dogs compared the serum cortisol in three groups with three different protocols undergoing ovariohysterectomy. In this study one group received IV lidocaine only, one IV meloxicam only and the third group received a combination of the two. There was no difference in serum cortisol between the three groups. (Tsai et al. 2013). A study of Fragen et al. (1987) concluded propofol in induction doses does not suppress the cortisol production. Cortisol was promptly secreted after start of surgical stimulus. Another study has noted that propofol reduces cortisol release at the cellular level. But at a dose necessary for anaesthesia, the reduction is negligible (Kenyon et al. 1985). In a study on humans, which underwent heart surgery, isoflurane reduced cortisol release in a dose-dependent manner. The authors attributed the reduction to the general depth of anaesthesia. (Flezzani et al. 1986)

One of the patients required rescue analgesia. The patient was in the intrafunicular block group. This suggests that the local anaesthetic was not injected in the correct position. Other causes would be a time delay between injection and surgery, which is not obvious based on the anaesthetic record.

Contrary to other studies in the present study blood samples for plasma cortisol measurements were taken immediately after high surgical stimulation, i.e. the orchiectomy, whereby the exact time between orchiectomy and blood sampling was not noted. Engeland et al. (1990) exposed awake dogs to noise and measured cortisol and other hormones in one-minute intervals. Cortisol increased significantly at minute 7 after the noise and stayed elevated until minute 15. This could lead to an additional increase in cortisol if there is not enough time for cortisol to decrease again to baseline prior to the next insult. This could have been in particular problematic between sampling point T2 and T3, the time-points between

clamping testicle one and two. Nevertheless, only a slight, non-significant increase at T3 was visible. On the other hand, if the sample is taken too early after a stimulus, cortisol will most likely be erroneously low. As there was no significant increase, but rather a decrease from baseline, it is unlikely that the timing of sampling created a large distortion in values. The sampling time point in other studies with animals who underwent surgery was earliest 15 minutes after the most painful procedure (Dinnis et al. 1997) or even after extubation (Michelsen et al. 2012) (Fox et al. 1994). In those, cortisol significantly increased at their first sampling point after the start of the surgery. Moreover, the cortisol increased for at least 45 min and stayed high for hours. Sometimes beyond the study period. The fact that cortisol decreased during the experiment in the present study and only one animal needed rescue analgesia suggests that both blocks provided adequate analgesia.

Conclusion

In this small-scale study in dogs undergoing orchiectomy, no significantly different level in plasma cortisol could be detected, following intratesticular or intrafunicular block with lidocaine. In both groups plasma cortisol levels decreased over time indicating sufficient anaesthesia and analgesia using the methods described.

5. Zusammenfassung:

Die Kastration bei Rüden ist eine sehr häufige Operation. Die intratestikuläre Injektion von Lidocain ist eine gängige Methode um ausreichend Analgesie zu gewährleisten. Die Injektion in den Funiculus spermaticus ist eine neue Methode, die zwar aufwendiger ist, aber eine Lokalanästhesie auch bei Verletzungen oder Tumoren des Skrotums erlaubt.

Diese Diplomarbeit findet als Teil einer Doktorarbeit statt (Mag. Christian Lohinger). Die Diplomarbeit behandelt im speziellem das Cortisol im Plasma, als Parameter für die Stressantwort im Kontext einer Rüdenkastration.

Zwanzig erwachsene, ansonsten gesunde Hunde erhielten Acepromazine und Methadon intramuskulär zur Prämedikation, sowie Propofol intravenös(IV) zur Narkoseeinleitung und IV Meloxicam zur Analgesie. Isofluran in Sauerstoff wurde zur Erhaltung der Anästhesie gegeben, sowie eine Infusion mit einer Vollelektrolytlösung zur Kreislaufunterstützung.

Je 10 Tieren erhielten unter Vollnarkose vor OP Beginn entweder eine Regionalanästhesie mit Lidocain 3mg/kg in den Funiculus spermaticus oder dieselbe Menge intratestikulär.

zur Kortisolmessung wurde Blut außer beim Setzen des ersten Katheters (T1) noch beim Abklemmen der zwei Hoden (T2 und T3), 30min nach dem Extubieren (T4) und 120min nach dem Extubieren (T5) genommen.

Die Daten wurden mit SPSS aufbereitet und analysiert. Signifikanz wurde mit $p < 0,05$ festgelegt.

Die beiden Gruppen zeigten einen unterschiedlich hohen Kortisol-Basalwert. Eine Erklärung hierfür konnte im Rahmen dieser Studie nicht gefunden werden. Der weitere Verlauf des Plasmakortisolspiegels verlief hingegen bei beiden Gruppen gleich. Während und nach der Operation fiel dieser ab, mit einem nicht signifikanten, leichten Anstieg beim Abklemmen des zweiten Hodens. Dies ist konträr zu Studien, die einen schnellen und persistierenden Anstieg im Plasmakortisol zeigen.

Beide Lokalanästhesien scheinen eine Stressantwort im Sinne einer erhöhten Kortisolausschüttung während einer Rüdenkastration verhindert zu haben.

6. Summary

Castration in male dogs is a very common operation. Intratesticular injection of lidocaine is a common method to provide sufficient analgesia. Injection into the funiculus spermaticus is a new method, which is more complex but allows local anesthesia even in case of injuries or tumors of the scrotum.

This diploma thesis is part of a doctoral thesis (Mag. Christian Lohinger). The thesis deals specifically with plasma cortisol as a parameter for stress response in the context of male castration.

Twenty adult, otherwise healthy dogs received acepromazine and methadone intramuscularly for premedication, as well as propofol intravenously (IV) for induction of anesthesia and IV meloxicam for analgesia. Isoflurane in oxygen was given to maintain anesthesia, as well as an infusion of whole electrolyte solution for circulatory support.

Each 10 animals received either regional anesthesia with lidocaine 3mg/kg into the funiculus spermaticus or the same amount intratesticularly under general anaesthesia before the start of surgery.

For cortisol measurement, blood was taken apart from when the first catheter was placed (T1), when the two testes were clamped (T2 and T3), 30min after extubation (T4) and 120min after extubation (T5).

Data were processed and analyzed using SPSS. Significance was set at $p < 0.05$.

The two groups showed different levels of basal cortisol. An explanation for this could not be found in this study. In contrast, the further course of plasma cortisol level was the same in both groups. During and after surgery, this dropped, with a nonsignificant, slight increase when the second testis was clamped. This is contrary to studies showing a rapid and persistent rise in plasma cortisol.

Both local anesthetics techniques appear to prevent a stress response in terms of increased cortisol release during male castration.

7. Literature

References

Castillo VA, Cabrera Blatter MF, Gómez NV, Sinatra V, Gallelli MF, Ghersevich MC. 2009. Diurnal ACTH and plasma cortisol variations in healthy dogs and in those with pituitary-dependent Cushing's syndrome before and after treatment with retinoic acid. Research in veterinary science, 86 (2): 223–229. DOI 10.1016/j.rvsc.2008.06.006 (accessed Aug 3, 2021).

Church DB, Nicholson AI, Ilkiw JE, Emslie DR. 2006. Effect of non-adrenal illness, anaesthesia and surgery on plasma cortisol concentrations in dogs. Research in veterinary science, 56 (1): 129–131. DOI 10.1016/0034-5288(94)90211-9 (accessed Aug 3, 2021).

Cicirelli V, Debidda P, Maggio N, Caira M, Lacalandra GM, Aiudi GG. 2021. Ultrasound-Guided Funicular Block: Ropivacaine Injection into the Tissue around the Spermatic Cord to Improve Analgesia during Orchiectomy in Dogs. Animals : an open access journal from MDPI, 11 (5). DOI 10.3390/ani11051275. (accessed Aug 4, 2021).

Desborough JP. 2000. The stress response to trauma and surgery. British journal of anaesthesia, 85 (1): 109–117. DOI 10.1093/bja/85.1.109 (accessed Aug 2, 2021).

Devitt CM, Cox RE, Hailey JJ. 2005. Duration, complications, stress, and pain of open ovariohysterectomy versus a simple method of laparoscopic-assisted ovariohysterectomy in dogs. Journal of the American Veterinary Medical Association, 227 (6): 921–927. DOI 10.2460/javma.2005.227.921 (accessed Aug 3, 2021).

Dinnis A, Stafford KJ, Mellor DJ, Bruce RA, Ward RN. 1997. Acute cortisol responses of lambs castrated and docked using rubber rings with or without a castration clamp. DOI 10.1111/j.1751-0813.1997.tb14380.x (accessed Aug 3, 2021).

Engeland WC, Miller P, Gann DS, Engeland WC, Miller P, Gann DS. 1990. Pituitary-adrenal and adrenomedullary responses to noise in awake dogs. American Physiological Society, 258 (3 Pt 2): R672-7. DOI 10.1152/ajpregu.1990.258.3.R672 (accessed Aug 3, 2021).

Epstein M, Rodan I, Griffenhagen G, Kadrlik J, Petty M, Robertson S, Simpson W. 2015. 2015 AAHA/AAFP Pain Management Guidelines for Dogs and Cats. Journal of the

American Animal Hospital Association, 51 (2): 67–84. DOI 10.5326/JAAHA-MS-7331 (accessed Aug 3, 2021).

Flezzani P, Croughwell ND, McIntyre WR, Reves JG. 1986. Isoflurane Decrases the Cortisol to Cardiopulmonary Bypass. https://europepmc.org/article/med/3767009 (accessed Aug 4, 2021).

Fox SM, Mellor DJ, Firth EC, Hodge H, Lawoko CRO. 1994. Changes in plasma cortisol concentrations before, during and after analgesia, anaesthesia and anaesthesia plus ovariohysterectomy in bitches. DOI 10.1053/rvsc.2000.0375 (accessed Aug 3, 2021).

Fragen RF, Weiss HW, Molteni A. 1987. The Effekt of Propofol on Adrenocortical Steroidgenesis: A Comparative Study with Etomidate and Thiopental. DOI 10.1097/00000542-198706000-00026 (accessed Aug 3, 2021).

Garnier F, Benoit E, Virat V, Ochoa R, Delatour P. 1990. Adrenal cortical response in clinically normal dogs before and after adaptation to a housing environment. DOI 10.1258/002367790780890356 (accessed Aug 3, 2021).

Haga HA, Ranheim B. 2004. Castration of piglets: the analgesic effects of intratesticular and intrafunicular lidocaine injection. DOI 10.1111/j.1467-2995.2004.00225.x (accessed Aug 3, 2021).

Huuskonen V, Hughes JML, Estaca Bañon E, West E. 2013. Intratesticular lidocaine reduces the response to surgical castration in dogs. Veterinary anaesthesia and analgesia, 40 (1): 74–82. DOI 10.1111/j.1467-2995.2012.00775.x (accessed Aug 3, 2021).

Kemppainen RJ, Sartin JL. 1984. Evidence for episodic but not circadian activity in plasma concentrations of adrenocorticotrophin, cortisol and thyroxine in dogs. DOI 10.1677/joe.0.1030219 (accessed Aug 3, 2021).

Kenyon CJ, McNeil LM, Fraser R. 1985. Comparison of the effects of etomidate, thiopentone and propofol on cortisol synthesis. DOI 10.1093/bja/57.5.509 (accessed Aug 4, 2021).

Knol BW, Dieleman SJ, Bevers MM, van den Brom WE, Molt JA. 1992. Effects of methods used for blood collection on plasma concentrations of luteinising hormone,

testosterone, and cortisol in male dogs. The veterinary quarterly, 14 (4): 126–129. DOI 10.1080/01652176.1992.9694348 (accessed Aug 4, 2021).

Kolevska J, Brunclik V, Svoboda J. 2003. Circadian Rhythm of Cortisol Secretion in Dogs of Different Daily Activities. DOI 10.2754/avb200372040599 (accessed Aug 4, 2021).

Kona-Boun J-J, Cuvelliez S, Troncy E. 2006. Evaluation of epidural administration of morphine or morphine and bupivacaine for postoperative analgesia after premedication with an opioid analgesic and orthopedic surgery in dogs. JAVMA, (229). DOI 10.2460/javma.229.7.1103 (accessed Aug 4, 2021).

McMillan MW, Seymour CJ, Brearley JC. 2012. Effect of intratesticular lidocaine on isoflurane requirements in dogs undergoing routine castration. The Journal of small animal practice, 53 (7): 393–397. DOI 10.1111/j.1748-5827.2012.01233.x (accessed Aug 3, 2021).

Michelsen J, Heller J, Wills F, Noble GK. 2012. Effect of surgeon experience on postoperative plasma cortisol and C-reactive protein concentrations after ovariohysterectomy in the dog: a randomised trial. Australian veterinary journal, 90 (12): 474–478. DOI 10.1111/j.1751-0813.2012.01013.x (accessed Aug 3, 2021).

Reimers TJ, McGarrity MS, Strickland D. 1986. Effect of fasting on thyroxine, 3,5,3'-triiodothyronine, and cortisol concentrations in serum of dogs. American Journal of Veterinary Research,, (47(12)): 2485–2490. https://europepmc.org/article/med/3800108 (accessed Aug 4, 2021).

Roberts RF, Roberts WL. 2004. Performance characteristics of five automated serum cortisol immunoassays. Clinical biochemistry, 37 (6): 489–493. DOI 10.1016/j.clinbiochem.2004.01.009 (accessed Aug 3, 2021).

Rodriguez Á, Raul A, Buriticá Gaviria EF, Echeverry Bonilla DF. 2016. Redalyc.Evaluación del efecto analgésico de la lidocaína vía intratesticular o intrafunicular en perros sometidos a orquiectomia electiva bajo un protocolo de anestesia disociativa. https://www.redalyc.org/pdf/636/63647456009.pdf (accessed Aug 4, 2021).

Srithunyarat T, Höglund OV, Hagman R, Olsson U, Stridsberg M, Lagerstedt A-S, Pettersson A. 2016. Catestatin, vasostatin, cortisol, temperature, heart rate, respiratory rate,

scores of the short form of the Glasgow composite measure pain scale and visual analog scale for stress and pain behavior in dogs before and after ovariohysterectomy. BMC research notes, 9: 381. DOI 10.1186/s13104-016-2193-1 (accessed Aug 3, 2021).

Stevens BJ, Posner LP, Jones CA, Lascelles BD. 2013. Comparison of the effect of intratesticular lidocaine/bupivacaine vs. saline placebo on pain scores and incision site reactions in dogs undergoing routine castration. Veterinary journal (London, England : 1997), 196 (3): 499–503. DOI 10.1016/j.tvjl.2012.11.019 (accessed Aug 3, 2021).

Suriano R, Varasano V, Robbe D, Carluccio A, Straticò P, Contri A, Petrizzi L. 2014. Intraoperative Analgesic Effect of Intrafunicular Lidocaine Injection During Orchiectomy in Isoflurane-Anesthetized Martina Franca Donkeys. Journal of Equine Veterinary Science, 34 (6): 793–798. DOI 10.1016/j.jevs.2014.02.002 (accessed Aug 3, 2021).

Tello FL, Hernandez DM. 2000. Performance Evaluation of Nine Hormone Assays on the Immulite 2000®Immunoassay System. DOI 10.1515/CCLM.2000.154 (accessed Aug 4, 2021).

Tsai T-Y, Chang S-K, Chou P-Y, Yeh L-S. 2013. Comparison of postoperative effects between lidocaine infusion, meloxicam, and their combination in dogs undergoing ovariohysterectomy. Veterinary anaesthesia and analgesia, 40 (6): 615–622. DOI 10.1111/vaa.12064 (accessed Aug 4, 2021).

8. Appendix

Intratesticular group			Cortisol (µg/dL)				
	Weight (kg)	Age (Years)	T1	T2	T3	T4	T5
	8	2.54	2.04	3.9	4.9	1.84	1
	5.9	6.52	2.99	3.59	3.6	1.87	3.67
	6.8	1.98	2.27	1.58	1.71	3.42	1.44
	16	0.76	1	1	1	1	1
	25	1.24	1.58	1	2.46	1.93	1
	16.5	1.79	1.35	1	1	1	1.82
	25.2	2.64	5.4	2.23	2.05	1.37	4.37
	3.25	8.89	2.4	1.86	3.48	3.71	4.74
	31.5	3.26	2.55	1.68	3.9	2.09	1
	11.3	3.23	5.68	1.54	1.33	1	1
mean	14.95	3.28	2.73	1.94	2.54	1.92	2.10
SD	9.11	2.39	1.51	0.98	1.29	0.91	1.45
Intrafunicular group			Cortisol (µg/dL)				
	Weight (kg)	Age (Years)	T1	T2	T3	T4	T5
	6.8	1.01	1.98	1.08	1	1	1.63
	30	1.47	2.4	1.79	4.75	4.25	1.05
	19	4.70	7.12	5.16	4.5	3.13	4.37
	34	1.06	2.76	1	1.24	1	2.62
	24.5	0.94	1.47	1.12	1.56	2.49	1
	9.4	4.11	4.03	7.11	7.46	2.82	2.07
	5.3	5.65	8.93	3.42	2.41	3.14	1.36
	9	3.89	3.13	2.64	2.41	1.68	3.47
	27.2	7.74	7.71	2.48	2.61	1.2	2.27
mean	18.36	3.35	4.39	2.87	3.10	2.30	2.20
SD	10.37	2.26	2.62	1.96	1.98	1.08	1.07

Table 1: Basic data for the statistical evaluation, with mean and SD (standard distribution) Time points: T1: Catheter placement, T2: clamping first testicle, T3: clamping second testicle, T4: 30 minutes after extubation, T5: 120 minutes after extubation.

9. Figures:

10. Picture