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Local mepivacaine before castration of horses under medetomidine isoflurane balanced anaesthesia is effective to reduce perioperative nociception and cytokine release

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Summary

Background: In horses castration with primary intention healing is usually performed under balanced inhalation anaesthesia. To optimise analgesia, the use of local anaesthesia was tested.
Objectives: To investigate the effect of local mepivacaine before castration with first intention healing under balanced medetomidine-isoflurane anaesthesia and flunixin meglumine, morphine analgesia on perioperative cytokine levels and pain in horses.
Study design: Prospective blinded clinical study.
Methods: Twenty stallions were randomly assigned to control or mepivacaine groups. Flunixin meglumine was administered before sedation with medetomidine and followed by ketamine/diazepam intravenously (i.v.). Anaesthesia was maintained with isoflurane and 3.5 μg/kg per hour medetomidine. Mepivacaine horses were given mepivacaine 2% (3.5 mL SC, 1 mL/100 kg intrafunicularly, 2 mL/100 kg intratesticularly) on each side. For recovery, horses were given 2 μg/kg medetomidine i.v. and 0.1 mg/kg morphine i.m. and oral phenylbutazone (0.02 mg/kg q12h) for post-operative analgesia. One hour before premedication and 4, 8 and 24 h post-incision, pain was scored with three different pain scales (Equine Utrecht University Scale for Facial Assessment of Pain, Horse Grimace Scale, Equine Utrecht University Scale for Composite Pain Assessment) and plasma cytokines (interleukin-6 and tumour necrosis factor alpha) were measured. Data were analysed using repeated measures ANOVA, linear regression and unpaired *t*-test, significance level $P \leq 0.05$.
Results: Horses in both groups showed a significant increase in pain scores and cytokines compared to baseline. Post-operatively the mepivacaine group exhibited significantly lower pain scores and cytokine levels. Mean heart rate during anaesthesia was significantly lower in the mepivacaine group compared to control group (28.8 ± 1 and 33.2 ± 1.7 respectively). Otherwise there were no differences between the groups.
Main limitations: The decision to provide additional analgesia was based on the attending surgeon's assessment rather than a standardised rescue analgesia plan based on pain scores. The study was only conducted for 24 h post-castration and complications were not recorded.
Conclusion: Local mepivacaine before castration with primary wound closure improved anaesthesia quality, attenuated post-operative increases in cytokines and reduced post-operative pain despite balanced anaesthesia with multimodal analgesia in control horses.

Keywords: horse; analgesia; IL-6; TNF-α

Introduction

Castration of stallions is a very common surgical procedure in equine practice [1]. Castration with primary intention healing has low complication rates and short post-operative recovery periods [2]. The duration of surgery necessitates the use of balanced inhalation anaesthesia [3]. Recently pain recognition and management in animals has improved and animal welfare is receiving increased public interest. Nevertheless, following common management procedures, such as castration, assessment of pain still remains insufficient [4]. Possibly as a consequence, the requirement for analgesia in horses undergoing castration has been debated within the literature. Some authors have suggested that analgesia is not required following uncomplicated castrations [5–7]. However, many specialists agree that the procedure can be associated with a significant degree of pain, and that analgesia is an ethical requirement [8–10]. As clinical pain has a negative impact on patient well-being, causes suffering by definition, suppresses the immune system and delays wound healing [11], it should be controlled. Balanced anaesthetic protocols help to prevent nociception during surgery and have become a common concept in equine anaesthesia [12]. Locoregional anaesthetic techniques may provide a useful contribution to such protocols. Local analgesia is a simple and cheap way to relieve pain, patient comfort is improved and hospitalisation time shortened [13–15]. In horses castrated under total intravenous anaesthesia, local lidocaine reduced the need for incremental intravenously (i.v.) anaesthetic [16] and under inhalation anaesthesia reactions to surgical stimulation were reduced [17]. However, although investigated in other species [18,19], the influence of presurgical local anaesthesia on perioperative pain has not been documented in horses yet.

A wide array of soluble mediators plays a key role in the early wound repair cascade. Activated immune cells release cytokines in response to trauma, inflammation or infection. Interleukin-6 (IL-6) and tumour necrosis factor-α (TNF-α) are two important pro-inflammatory cytokines [20]. IL-6 reflects severity of injury and is considered the most reliable prognostic indicator of outcome and TNF-α is a primary mediator of the inflammatory response [21]. The consistent overlap between neuroendocrine and immune function with wound healing and pain might explain why local anaesthesia techniques have an impact far beyond their local anaesthetic effects [22].

The current study was designed to document the perioperative effects of local anaesthesia with mepivacaine 2% added to modern balanced anaesthesia for castration of horses and to investigate its effects on IL-6 and TNF-α, two important proinflammatory cytokines [20]. Our hypothesis was that with local anaesthesia for castration during anaesthesia, less reaction to surgical stimulation would be detectable and that post-operative pain and cytokine levels would be reduced.

Materials and methods

Animals and study design

The study was performed as a randomised blinded prospective clinical trial. Twenty stallions (ASA category I) scheduled for castration via inguinal approach with primary wound closure [3] under general anaesthesia were included in the study. Eight to twelve hours before surgery, food was withheld but free access to water was provided. Castration was performed by one of three board certified surgeons. The same anaesthetist (R.B.) who was unaware of the treatment group assignment, performed all anaesthetic procedures. The same veterinarian (M.A.) who was also unaware of treatment group assignment, performed all perioperative pain scorings.

Included horses were randomly allocated into two different groups, mepivacaine treatment group (n = 10) and control group (n = 10), using an envelope system. Following aseptical preparation of the area above both testicles the mepivacaine group was infiltrated subcutaneously, two minutes before surgical incision with 0.5 mL/cm of mepivacaine 2% (Mepivacaine Sintetica 2%)[a]. When the vaginal process was opened and the testes and spermatic cord were exposed, the testes (2 mL/100 kg) and the spermatic cord, just distal to the proposed ligation site (1 mL/100 kg), were infiltrated with mepivacaine 2%. Again, two minutes elapsed until castration was performed. The control group did not receive a sham injection as this could result in local irritation and is not routinely performed; however, two minutes were allowed to elapse prior to beginning surgery as for the mepivacaine group. Drapes were used to separate the surgical site from the head region so that the anaesthetist and veterinarian performing the perioperative assessment remained unaware of the treatment group assignment.

Anaesthesia premedication and induction

Prior to surgery, a 14-gauge, 16 mm catheter (SecalonT)[b] was introduced into the jugular vein. Thirty minutes before anaesthesia induction, all horses received 30,000 IU/kg of penicillin Na^+ (Penicilline Natrium Streuli)[c], 9 mg/kg of gentamicin (Vetagent)[d] and 1 mg/kg of flunixine meglumine (Fluniximin)[e] i.v. and 0.03 mg/kg of acepromazine (Prequillan)[f] intramuscularly (i.m.).

The horses were sedated with 7 μg/kg of medetomidine (Domitor)[g] and left undisturbed. Horses that still lifted the head when approached 5 min after administration of medetomidine were administered another 2 μg/kg medetomidine and anaesthesia was induced within 5 min. Anaesthesia induction was performed with an i.v. bolus of diazepam 0.02 mg/kg (Valium)[h] and ketamine 2.2 mg/kg (Ketasol-100)[e].

Anaesthesia maintenance and monitoring

Immediately after anaesthesia induction and endotracheal intubation, the tube was connected to a large animal anaesthesia machine (Mallard 2800)[i]. Anaesthesia was maintained with isoflurane (AttaneTM Isoflurane)[j] in oxygen and air with an initial inspiratory fraction of oxygen (FIO_2) of 0.5. Horses were mechanically ventilated with a positive inspiratory pressure of 15–20 cmH_2O, a tidal volume of 10–12 mL/kg and a respiratory frequency which aimed at maintaining end-tidal expired carbon dioxide pressures ($P_E'CO_2$) of 40–50 mmHg (5.3–6.7 kPa). As soon as the horses were connected to the anaesthetic circuit, they received a constant rate infusion (CRI) of medetomidine[g] (3.5 μg/kg per hour) by means of an infusion pump (Phoenix 700)[k]. Isoflurane[j] was delivered to the minimal expiratory fraction of isoflurane ($F_E'ISO$) required to keep the patient immobile, to prevent muscle contraction, nystagmus, spontaneous blinking and to allow for evocation of sluggish palpebral reflexes. If the horses developed nystagmus or breathing against the ventilator, ketamine[e] (0.1 mg/kg) was administered i.v. to deepen anaesthesia and endtidal isoflurane concentration was increased by 0.1%. If sudden movement occurred, 0.5 mg/kg thiopental (Pentothal)[l] was administered i.v. and endtidal isoflurane concentration increased by 0.2%. When signs of superficial anaesthesia occurred, the surgeons were asked at what stage of surgery they were and this was noted. Endtidal isoflurane concentration was lowered again in steps of 0.1% every 10 min, once testis was removed and no nystagmus had occurred. Lactated Ringer's solution (Ringer-Lactat-Lösung)[m] was infused throughout anaesthesia (8–10 mL/kg per hour).

Dobutamine (Dobutrex)[n] was administered to maintain mean arterial blood pressure (MAP), starting with 0.63 μg/kg per min. The rate of dobutamine was adjusted every 5 min according to a prepared rate scale based on the MAP. If MAP remained <65 mmHg after maximal dose of 1.25 μg/kg per min dobutamine had been infused for 5 min, a bolus of Lactated Ringer's solution[m] (10 mL/kg/10 min) was infused twice. If MAP still remained <65 mmHg a 500 mL i.v. bolus of hetastarch (Voluven 6%)[o] was infused and repeated every 5 min, until MAP was >65 mmHg. The total amount of dobutamine administered over the whole anaesthetic period was summed up and the average dose of dobutamine given per kilogram per minute calculated.

A urinary catheter was placed before surgery in all horses.

A 22-gauge catheter (Surflo)[p] was placed in a transverse facial artery for MAP measurement and to collect blood for arterial blood gas analysis. HR, MAP, respiratory rate (RR), FIO_2, $F_E'ISO$ and endtidal expired carbon dioxide pressure ($P_E'CO_2$) were continuously displayed, using a multiparameter anaesthesia monitor (Datex-Ohmeda Cardiocap/5)[q] and manually recorded every 5 min.

Arterial blood samples were anaerobically collected at 15 and 30 min after anaesthesia induction and, thereafter, at 30-min intervals. The arterial blood pH, partial pressures of oxygen (P_aO_2) and carbon dioxide (P_aCO_2) and lactate concentration were determined without delay, using RAPIDPoint® 500 System[r].

Anaesthesia recovery and post-operative analgesia

Thirty minutes before the end of anaesthesia, 0.1 mg/kg of morphine (Morphin HCl Sintetica)[s] was administered i.m. At the end of surgery, the administration of isoflurane[j] and infusions were discontinued, and horses placed into a padded recovery box. Ventilation was assisted using a demand valve until the horses started to breathe spontaneously. Subsequently medetomidine (2 μg/kg) i.v. was administered to both groups for post-anaesthetic sedation. If horses showed excessive nystagmus within five minutes of the end of isoflurane administration, another bolus of medetomidine[g] (2 μg/kg) i.v. was administered. Phenylephrine (Phenylephrini hydrochloridum)[t] was administered intranasally and supplemental oxygen was delivered (15 L/min). At the first sign of awakening (swallowing, nystagmus, movement of ears, head or legs), the endotracheal tube was removed. The entire recovery phase was timed and scored. The quality of the anaesthesia recovery was evaluated using a previously reported recovery score system for horses [23] (1 = very good, horse standing at the first attempt, 2 = good, two attempts until standing, 3 = more than two attempts, horse remains calm, minimal ataxia when standing, 4 = bad recovery, several attempts to get up, horse becomes excited or in panic, risk of injury, 5 = very bad, recovery resulting in injury of the horse).

Post-operatively, the horses received 2 mg/kg phenylbutazone (Butadion)[u] orally twice per day (PO, q12h). The first dose was administered 4 h following surgery.

Pain scoring and additional analgesia

Scoring of pain was performed at the following time points: at baseline = before the horses had received any drugs (in the morning 1 h before premedication) and at T4, T8 and T24 h post-surgical incision. Following recovery until first scoring, the horses were supervised by caretakers. If restlessness or colic signs were reported during this phase, horses were examined by a veterinarian and if considered appropriate by the examining veterinarian, dipyrone (first-line treatment) followed by flunixin meglumine (within 15 min, if pain persisted) was administered. Also during the 24 h post-surgery, the decision to provide additional analgesia was based on the surgeon's assessment and not according to a standardised pain-score-based rescue analgesia plan.

Three different pain scales were used for pain scoring. The first one was the Equine Utrecht University Scale for Facial Assessment of Pain (FEPS) [24]. Briefly, nine items are scored, seven of which differentiate three scores (0, 1, 2) including head position, opening of eyelids, focus, nostrils, corners mouth/lips, muscle tone head, ears. The remaining two items are only differentiated by two scores (0, 2) flehmen and/or yawing, teeth grinding and or moaning. For each item 0 means no, 1 means indicative of slight pain and 2 severe pain. The sum of all these score results in a maximum pain score of 18.

The second pain scale used was the Horse Grimace Scale (HGS) [9] that judges six items: stiffly backward ears, orbital tightening, tension above the eye area, prominent strained chewing muscles, mouth strained and pronounced chin, strained nostrils and flattening of the profile. Each judgement point contains 3 grades from 0 = no pain, 1 = mild pain and 2 = severe pain. The maximum total score is 12.

The third scale the Equine Utrecht University Scale for composite Pain Assessment (CPS) [24]. Briefly the scale has two divisions: the first one is a physiological division that contains 4 items (heart rate, respiratory rate, body temperature, digestive sounds) and the second one is a behavioural division that contains 10 items of spontaneous behaviour (posture, laying down, sweating, tail flicking, kicking of abdomen, pawing at floor, head movements, pain sounds) or response to stimuli (response to observer or reaction to palpation of painful area). Each item has four scores (0 = no pain/normal behaviour, 1 = mild pain, 2 = moderate pain, 3 = severe pain). The maximum total score is 42.

Cytokine plasma concentrations

For determination of cytokines, venous blood samples were collected from the venous catheter just before anaesthesia premedication (baseline) and at T4, T8 and T24 h post-incision. Each time, 5 mL of blood were withdrawn and discarded before sampling. The blood was collected in 2 heparinized tubes (Vacutainer®)[v]. The sample was centrifuged immediately (280 rpm) for one minute following collection. Plasma was harvested and stored at −80°C. TNF-α and IL-6 were measured by enzyme-linked immuno-absorbent assay (ELISA) using a specific equine kit according to the manufacturer's instructions (IL-6 and TNF- α kits)[w].

All undiluted samples (plasma, detection antibodies, standards, conjugate) were incubated at room temperature (RT) for 1 h and then were centrifuged one minute at 6000× ***g***. All plasma samples and standards were duplicated and directly transferred to plates pre-coated with the specific primary antibody. Then they were incubated for 90 min in the dark at room temperature (RT), plates were washed 3× with the buffer solution and antibodies were added and incubated for 60 min in the dark at RT. After 3× washing conjugates were added and incubated for another 20 min in the dark at RT, followed by 3× washing. The substrate was added and after 20 min incubation at RT the reaction was terminated by addition of a stop solution. The signal was detected using a microplate reader set at 450 and 540 nm and using ELISA reader machine (Tean Genios)[x].

The level of quantification of the IL-6 and TNF-α ELISA kits assay was 0.8 pg/mL and the intra-assay coefficient of variance (CV) was 6% and the inter-assay CV was 8%.

Data analysis

All statistical analyses were conducted using R libraries (R version 3.4.0)[y] Data were tested for normality using Kolmogorov–Smirnov test. Composite clinical scores that were shown to be normally distributed were analysed by *t*-test, nonparametric data with Mann–Whitney and repeated measures outcomes were analysed by repeated measures ANOVA. Repeated measures general linear regression model was used to analyse composite pain score data and plasma analysis data with the different time points as the within-subjects factor and the treatment group as the between-subjects factor. Differences were considered significant when $P \leq 0.05$.

Results

There were no significant preoperative differences among the groups in age, body weight, body temperature, HR, RR, cytokine levels or pain scores (Figs 1 and 2), for details see supplemental information.

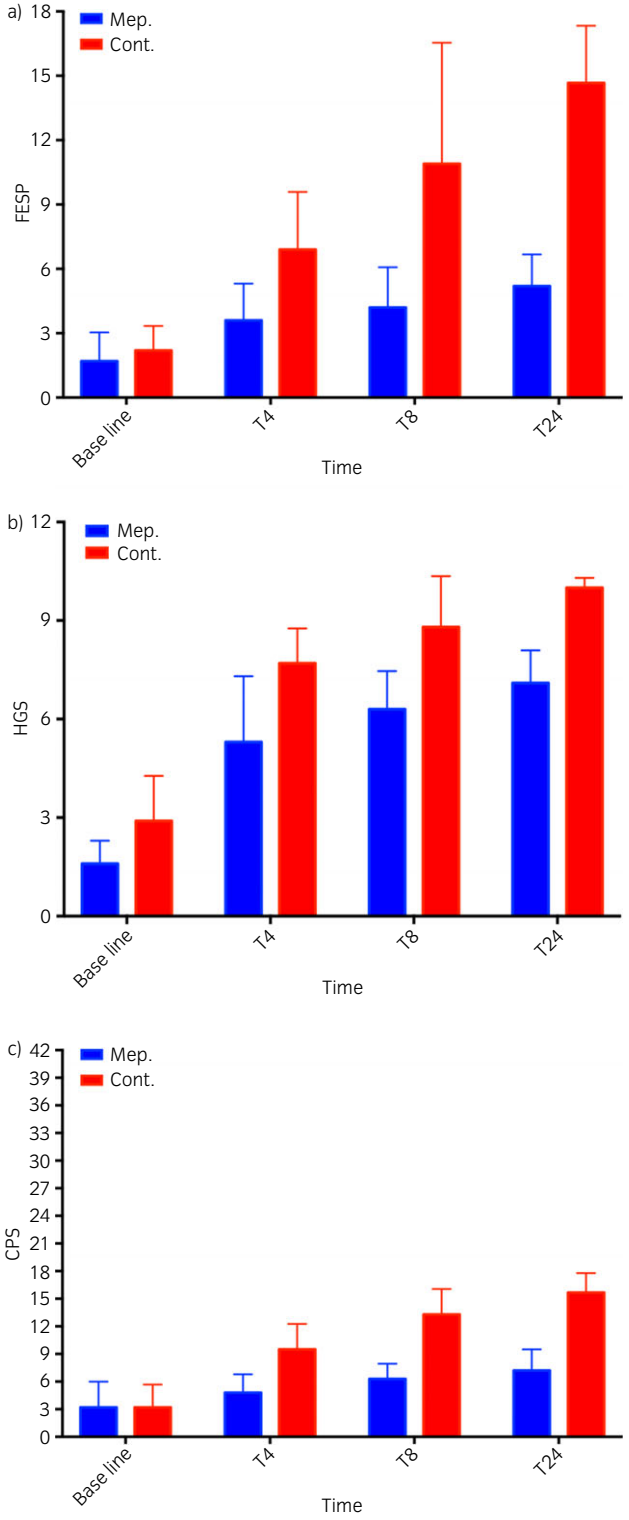

Fig 1: Pain Scores a) Equine Utrecht University Scale for Facial Assessment of Pain (FEPS) at baseline, T4, T8 and T24 h post-surgical incision in mepivacaine and control groups (median ± interquartile range [IQR]). Pain Scores b) Horse Grimace Scale (HGS) at baseline, T4, T8 and T24 h post-surgical incision in mepivacaine and control groups (median ± IQR). Pain Scores c) Equine Utrecht University Scale for composite Pain Assessment (CPS) at baseline, T4, T8 and T24 h post-surgical incision in mepivacaine and control groups (median ± IQR).

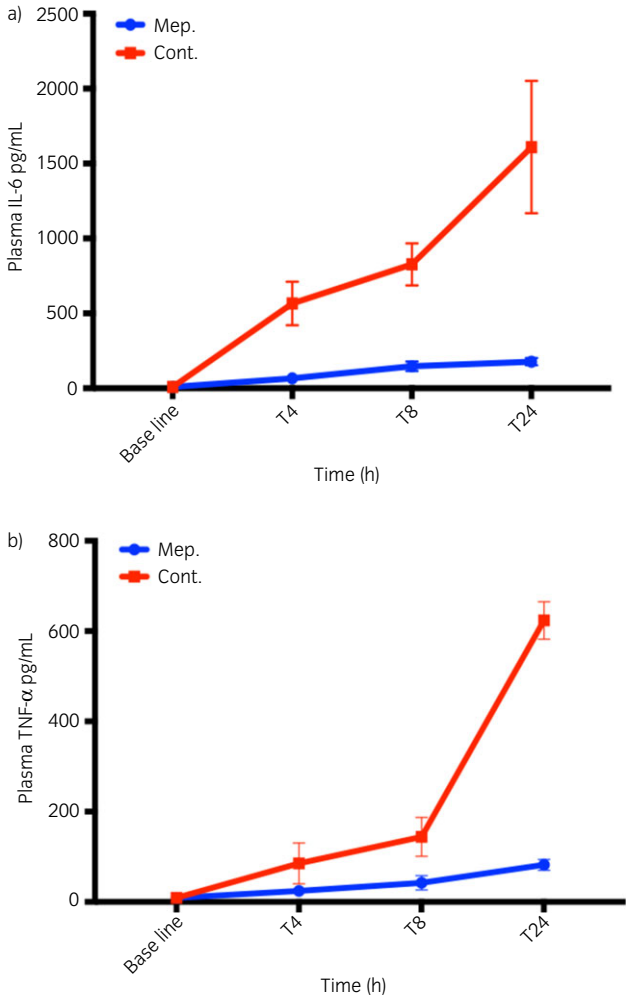

Fig 2: a) Interleukin-6 (IL-6, pg/mL) plasmalevels at baseline, T4, T8 and T24 h post-surgical incision in mepivacaine and control groups (mean ± standard deviation [s.d.]). B) Tumour necrosis factor-α (TNF-α, pg/mL) plasmalevels at baseline, T4, T8 and T24 h post-surgical incision in mepivacaine and control groups (mean ± s.d.).

Breeds investigated were the following: Island pony (5 mepivacaine, 4 control), Spanish Warmblood (1 mepivacaine, 2 control), Achal-Tekiner (1 control), Freiberger (1 mepivacaine), German Warmblood (1 mepivacaine, 2 control), Swiss warmblood (1 mepivacaine), Quarter Horse (1 control), Thoroughbred (1 mepivacaine).

There was no statistically significant difference between the groups in duration of anaesthesia, F_E'Iso, FIO_2, $P_E'CO_2$, MAP, pH, P_aCO_2, P_aO_2, and lactate concentration. The mean HR in mepivacaine horses was significantly lower than in the control horses (mepivacaine: 28.8 ± 1, control: 33.2 ± 1.7 bpm; P = 0.03). In the mepivacaine group, the HR ranged from 25 to 29 bpm and in the control group rose from 28 up to 43 bpm during surgery.

Two horses from the control group had MAPs <70 mmHg (lowest MAP of 61 mmHg) that did not respond to boluses of Lactated Ringer's solution and dobutamine 1.25 μg/kg per min. They were given hetastarch (2.17 and 1.77 mL/kg respectively). There was no difference between the groups in dobutamine dose infused or the amount of Lactated Ringer's solution administered.

During anaesthesia, the use of ketamine when nystagmus or breathing against ventilator occurred and thiopental when movement of horses occurred was needed significantly more often in control horses than in mepivacaine ones, resulting in higher mean dose rates of ketamine per horse (mepivacaine: 0.07 ± 0.1, control: 0.3 ± 0.4 mg/kg; P = 0.04) and thiopental per horse (mepivacaine: 0, control: 0.4 ± 0.4 mg/kg; P = 0.01) in the control horses.

Despite administration of 2 μg/kg medetomidine during recovery, two control horses showed severe nystagmus right after disconnection from isoflurane and they were given another 2 μg/kg medetomidine. The recovery quality was good and not different between groups. Recovery scores of 1 were recorded in all but 3 horses which had scores of 3 (2 mepivacaine horses and 1 control). The horses in the mepivacaine group remained in sternal recumbency for a significantly shorter time (14.9 ± 2.7 min) than control horses (20.9 ± 5.6 min; P = 0.03). The time taken to stand in mepivacaine horses was shorter (61.2 ± 4.5 min) than control horses (72 ± 7 min; P = 0.01).

At baseline, there was no difference in pain scores between the groups and cytokine levels were below levels of detection. In comparison to baseline, at T4, T8 and T24 h post-surgical incision there was a significant increase in pain scores and cytokine levels within the same group (P<0.001, Figs 1 and 2). In addition, the pain scores and plasma cytokine levels were significantly lower in mepivacaine horses than control horses at T4, T8 and T24 h post-surgical incision (P<0.001).

No serious post-operative complications were noted within the first 24 h.

Discussion

In the current study, local anaesthesia for castration resulted in less intraoperative measurable reactions to surgical stimulation and more stable anaesthesia, lower post-operative pain scores and lower cytokine levels for the 24-h post-operative observation period. As we used multimodal analgesia in both groups and mepivacaine action is much shorter than 24 h, these results were not expected and hopefully will provide an impetus for improving post-castration analgesia regimens. It is likely that the action of local anaesthesia on the release of local inflammatory mediators like interleukin-6 and tumour necrosis factor-α are mainly responsible for the reduced pain in the local anaesthesia treatment group [20–22].

Intraoperative cardiopulmonary function was well maintained in both study groups and we could not detect relevant differences between the groups. Ideally to assess the influence of local anaesthesia, recording these parameters after each stage of the surgical procedure and not just every five minutes may have been a better approach. Recovery quality was good in all horses and only clinically irrelevant differences between groups regarding recovery duration were identified. The incidence of poor recoveries following medetomidine-isoflurane balanced anaesthesia is low [25–27] and the number of horses included in this study was too low to detect a significant difference. Because the measured beneficial effects of local anaesthesia on cytokine levels and pain were so pronounced, including additional horses to detect a significant difference in anaesthesia recovery was considered unethical.

We used a combination of subcutaneous, intratesticular and intrafunicular injection of mepivacaine to achieve an optimal local block for castration. Intratesticular lidocaine injection leads to diffuse distribution into the spermatic cord and only poor distribution into the cremaster muscle [17]. Size of testicles differs with age and, therefore, some authors prefer to dose according to presumed weight of the testicle [16]. We considered the approach of mL/cm of cut length and mL/100 kg of horse's bodyweight a more practical approach. To inject 10 mL per testicle, as commonly performed in equine practice [28], would have resulted in volumes that seemed too high in younger, smaller individuals with the potential for local stimulation during injection. We chose to compare local anaesthesia before surgery to the standard surgery without sham injection of saline. Sham injection would have carried the risk of local haematoma formation or local mechanical nociceptive stimulation that was considered unethical, as discussed in a recent review of the use of sham injection for local anaesthesia studies [29].

Pain scoring in horses is not easy. Behaviour can be difficult to interpret in stallions in an unknown environment [30]. Specific pain scales have been established and validated in order to optimise judgement of horses under clinical circumstances [9,24,31]. Previously it has been shown in studies

investigating reliability of different scoring systems, that trained veterinarians yield more reliable results than students [13] and better results are achieved if scores are performed by the same person [13]. Therefore, all horses were scored by the same veterinarian who was unaware of the treatment group assignment and trained to perform pain scoring of horses. Three pain scales that have been validated either for acute visceral pain or for post-castration pain were used in the present study. All scales showed the same trends and differences between groups. The magnitude of the total scores assigned, however, was different. This highlights the need for more studies in horses to establish valid scales for everyday practice incorporating guidelines for additional analgesia.

In the current study, the predetermined post-operative analgesia protocol was comparable to other recent studies [2,9]. Administration of additional analgesia was based on the surgeon's judgement rather than a clear scoring-based cut-off number, as reported for dog pain management [32].

The numerical scores recorded in the mepivacaine group were comparable to other castration studies in horses [9,33] but the control group scores were of magnitude that would have necessitated additional analgesia. It is not clear why our control horses showed higher scores than in other studies despite multimodal analgesia. Possible factors are differences in surgical technique and resulting tissue trauma or relatively high age and testicular size of our castration population. Clear guidelines for perioperative pain-scoring-directed analgesia should be established. Judgement of patient pain is difficult and in horses the need for perioperative administration of potent analgesics included opioids remains a subject of debate.

Horses that had received local anaesthesia prior to castration were significantly less painful in the 24 h observation period, a duration far beyond mepivacaine's local anaesthetic action [34]. Cytokine levels were positively correlated to the pain scores, a finding which has also been noted in studies in humans [35]. In humans, levels of IL-6 and TNF-α correlate with severity of trauma [36,37], and have modulating effects on inflammation, nociception and pain [20,38]. It is likely that in the current study, the significant effect of local anaesthesia on cytokines is mainly responsible for the relevant perioperative pain reduction.

Limitations of the current study are that there was no rescue analgesia plan, that pain-scoring and cytokine level determination ceased 24 h post-castration and we did not score castration wounds or swelling or record complications.

In conclusion, the use of the local anaesthetic mepivacaine during castration with primary intention healing castration results in more stable anaesthesia and reduces post-operative pain and inflammatory cytokine levels for at least 24 h post-operatively.

Authors' declaration of interests

No competing interests have been declared.

Ethical animal research

The study was reviewed by the internal University Ethical commission and was approved by the animal experimentation commission of the canton of Zuerich, Switzerland (ZH 239/15) Owner consent was obtained.

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Authorship

M. Abass contributed to study design, study execution, data analysis and interpretation, and preparation of the manuscript. S. Picek contributed to study execution, and data analysis and interpretation. J. Glaus Garzón contributed to study execution, and data analysis and interpretation. C. Kühnle contributed to study execution. A. Zaghlou contributed to study design. R. Bettschart-Wolfensberger contributed to study design, data analysis and interpretation, preparation of the manuscript, and gave final approval of the manuscript.

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's website:

Supplementary Item 1: Pre- and intra-anaesthetic parameters with no significant differences between groups.