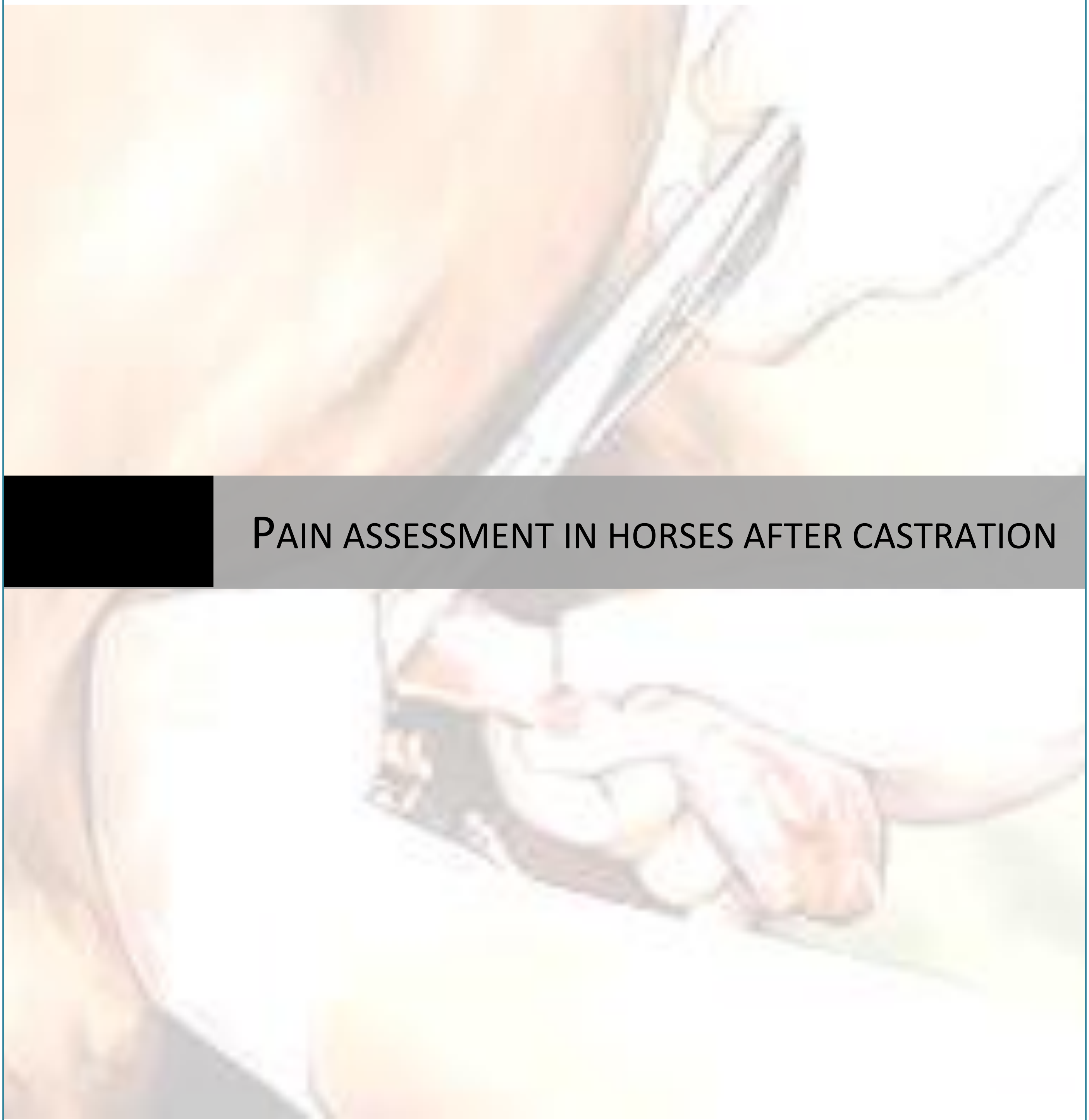
PAIN ASSESSMENT IN HORSES AFTER CASTRATION



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Summary

The assessment of pain is important to estimate the wellbeing of horses, especially induced by common veterinary procedures such as castration. Various existing pain assessment methods (e.g. the Post Abdominal Surgery Pain Assessment Scale (PASPAS), the Composite Pain Scale (CPS) and the Numerical Rating Scale (NRS)) have their limitations and therefore it makes them less useful in practice. Pain assessment methods based on facial expression however, seem to have some beneficial properties that surpass the limitation of other types of pain assessment. The purpose of this study is to assess the usefulness of the EQUUS-FAP in the assessment of pain following the castration of horses and to compare it to the Horse Grimace Scale.

Seven stallions of different breeds (1-6 years), admitted for castration to the Equine Health Department of the Faculty of Veterinary Medicine in Utrecht between 01/03/2015 and 01/05/2015, were used for this study. The control groups consisted of different breeds (4-14 years) admitted to the Equine Health Department of the Faculty of Veterinary Medicine in Utrecht for either a special farrier treatment (7 geldings), a CT scan or an MRI (1 gelding and 2 mares).

Two pain scales were used to assess the amount of pain: Equine Utrecht University Scale for Facial Assessment of Pain (EQUUS-FAP) and the Horse Grimace Scale (HGS). The scoring of EQUUS-FAP and HGS were performed independently at the same time by two observers, who were not blinded. The scoring of the EQUUS-FAP and HGS was performed at several predetermined time points: a baseline score was performed at the day of arrival, and at the morning of the operation. The next assessments were 4 and 8 hours after surgery and followed for two more days with assessments in the morning and the late afternoon. This whole procedure was performed to monitor the pain and to register the changes in pain over time. The farrier-control group of horses, were assessed only once for both the EQUUS-FAP and HGS, following the same protocol as the castration patients. The control group of CT and MRI patients, used to investigate the impact of general anaesthesia on the EQUUS-FAP and HGS, was assessed for the EQUUS-FAP and HGS on the day of arrival and/or just before the performing the CT or MRI, so before starting of premedication and anaesthesia. Afterwards the pain assessments were conducted 2, 4, 5, and 6 hours after anaesthesia.

Overall the pain assessment from both the EQUUS-FAP as well as the HGS, gave the comparable low scores after castration. Most likely this can be explained by a good anaesthetic and analgesic protocol as is discussed extensively. It was encouraging that both scales clearly picked up the two animals with post operative painful complications. Considering this, the EQUUS-FAP seems to be useful in the assessment of pain following castration in horses. However, the low number of patients and controls; the caused a impossibility to calculate statistic support. Future extension of the research is advised.

Contents

Introduction

Pain

'Animal pain is an aversive sensory and emotional experience representing awareness by the animal of damage or threat to the integrity of its tissues; it changes the animal's physiology and behaviour to reduce or avoid damage, to reduce the likelihood of recurrence and to promote recovery' [Molony 1997]. Another source defined pain as 'an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage'. Both definitions enclose those neurophysiologic processes that warn and protect the horse from actual or potential tissue damage and help prevent further injury and promote healing [Reed 2010].

Nociception and perception are the multifaceted components from which pain results. Both facets are assimilated to provide the immediate recognition and, if possible, elimination of the painful stimulus. Nociception is the detection of a noxious (mechanical, thermal, chemical) stimulus. This stimulus by itself is not pain, because the transformation of noxious stimuli to electric impulses (transduction), their transfer to the spinal cord (transmission) and their eventual projection on the brain, will not ensure that the electric impulses will be recognized. After this there is the phenomena that can be called perception. Perception is the recognition of that which nociception has developed or is continuing to take place and triggers responses that protect the horse from further hurt and helps to maintain homeostasis [Reed 2010]. Pain relates to the way the unpleasant or aversive sensation is experienced by the animal [Hellebrekers 2000], so it is the emotional and subjective processing of the nociceptive information.

The difference between acute and chronic pain is the time development: acute pain does not outlive the healing, where chronic pain remains beyond the presumed healing time for an injury. Usually acute pain is guided by autonomic changes and responds to analgesic treatment. Cancer pain, which has a describable cause and consists of repeating attacks of acute pain, is an example of prolonged pain, or in other words recurrent pain. Chronic pain may have no apparent cause or temporal onset [American Pain Society 1987, Molony 1997]. Another division is that of somatic and visceral pain. This classification is based on the different parts of the body where the pain originates. Somatic pain is generally well localized like orthopedic pain, whereas visceral pain is poorly localized and may be referring to outlying parts of the body, as for example colic pain [Molony 1997].

The response to pain can be multifarious. At first it is possible to have an increase activity in the afferent nerves, the sympathetic nervous system and of the hypothalamo-pituitary-adrenal axis. Also changes in posture, locomotor activity and evoked behaviour can appear [Molony 1997]. These parameters can help to make an assessment of the amount of pain that an animal experiences. But the value of every response needs to be brought into perspective. For example, if the focus is on the changes in activity of the sympathetic nervous system, monitoring of the heart rate is an option. But changes in heart rate can be caused by many interfering variables, such as: eating, exercise and extraneous noises. This also holds for measuring changes in plasma cortisol concentrations. Individual variation, diurnal changes and wide variety of stressors that activate the hypothalamic-pituitary-adrenal system, can limit the use of such measurements for the assessment of pain [Molony 1997].

Pain scales

In recent years, there has been an increasing interest in pain recognition and management in veterinary medicine, because animal welfare is contemporarily receiving increased public interest [Van Loon 2010]. The welfare of animals involves both physical and mental aspects, and supports the notion that animals should be free from pain, injury or disease [Prunier 1992]. Also, in the world of horses, the quality of life of a horse is receiving a growing interest. That makes the ability to objectively assess the amount of pain of a horse of considerable value. But pain recognition and interpretation is considered to be difficult, because animals are not capable of expressing themselves with words. That is why the use of the Visual Analogue Scale, used a lot in human medicine to interpret the amount of pain of a patient by asking, is not working in animals. Furthermore, horses have the natural ability to suppress the exhibition of obvious signs of pain in the presence of possible predators (like humans) as is similar to other prey species [Dalla Costa 2014].

It is clear that pain evaluation of a horse is a difficult task. It would be of great value if there were a reliable and objective method to estimate the degree of pain a horse experiences. It would be helpful when deciding how to adapt treatment to a patient and it would make certain decisions, as for example euthanasia, easier [Bussières 2008].

The pain assessment of horses is a field of scientific research wherein several performers are active. In this field different methods have been used to assess pain in horses objectively with the help of behavioural expressions and, most of the time, together with physiological parameters. However, the physiological variables show least specificity for pain, such that our attention has turned more towards investigating the behavioural changes induced by pain (Price 2003, Ashley 2005). Some existing scales are: simple descriptive scales, numerical rating scales, visual analogue scales and composite pain scales [Love 2013, Pritchett 2003, Bussières 2008].

In the study of Love et al. (2013) they used, among others, a simple descriptive scale modified from a feline study. This scale consists of a score from 0 till 3, where the 0 stands for a state of 'normal behaviour', a score of 1 for 'a little subdued', 2 for a 'subdued' state and a score of 3 for a 'no interaction at all' state. So there are no evaluated subdivided categories of behaviour in this pain scale [Love 2013]. In the study of Pritchett et al. (2003) they introduced a numerical rating scale. In this pain scale they assigned a numeric score to each behaviour category that was weighted based on a descriptive definition of the behaviour. The numerical rating scale yields a total behaviour score for each time point that is the sum of the scores assigned for each behaviour category. This pain scale consists of 9 behavioural categories; each of them can be scored 0-4 [Prittchet 2003]. The Visual Analogue Scale is a line with two end points: the left end equalizes no pain (=0) and the right end unbearable pain (=10). Observers of an animal place a mark on the line corresponding to their interpretation of the patients' pain intensity. VAS scales consist not of any descriptive definitions [Hielm-Bjorkman 2011]. The Composite Pain Scale is a composite numerical rating scale combining physiological data with behaviour expressions (like responses to stimuli and interactive behaviour). All measurements are rated from 0 (normal, no pain) to 3 (physiologic or behaviour changes due to presence of pain). The sum of all these scores leads to a total score between 0 and a certain maximum [Van Loon 2010].

A specific approach of the pain scales based on behavioural changes, is the assessment of facial expression of pain. In human medicine facial expressions are commonly used to assess pain in patients, especially in those who are unable to communicate understandably with their clinicians (e.g. neonates and patients with cognitive impairment). Recently a comparable assessment tool for facial expression for rodents is accomplished. This method describes, as it does in humans, the changes to the surface appearance of the face resulting from muscle actions, in ‘action units’. These action units are incorporated into species-specific ‘’grimace scales’’ [Sotocinal 2012, Langford 2010].

For example, the mouse grimace scale and the rat grimace scale are formations of 'no-pain' (baseline) and 'pain' photographic coding systems, as shown in figure 1. The scales are used for pain assessment in the laboratory mice and rats for veterinary care and drug development. Orbital tightening, nose and cheek bulge, ear position and whisker changes are the ‘pain face’ action units tallied in the mouse grimace scale and rat grimace scale. It is a standardized behavioural coding system with high accuracy, reliability and validity [Sotocinal 2012, Langford 2010].

Love et al. (2011) used a kinematic analysis to discover that certain facial expressions of horses changed during the administering of an injection [Love 2011]. In the study of Dalla Costa et al. (2014) the facial expression in horses was also the principal part of the study. They developed and validated a pain scale based on facial expressions in horses, the ‘Horse Grimace Scale’ (HGS). Using routine castration they tried to assess whether the HGS could be effectively implemented with minimal training. So the aim was to develop a practical on-farm pain assessment tool [Dalla Costa 2014]. In this study researchers observed different facial actions to identify pain. Stiffly backward ears, orbital tightening, tension above the eye, strained mouth and chewing muscles, pronounced chin, strained nostrils and flattening of the profile are all elements of this scoring system, as seen partly in figure 2. This total of six Facial Action Units of the (HGS) are shown in the appendix [Dalla Costa 2014].

__Figure 1:__ ‘pain face’ action units scored in the Mouse Grimace Scale (Langford 2010).

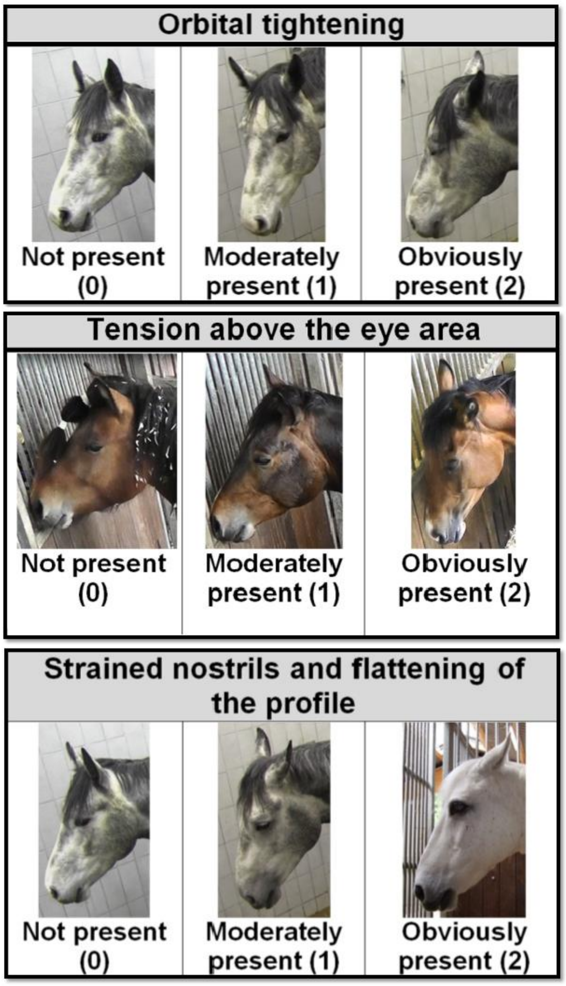


__Figure 2:__ Three out of six Facial Action Units of the Horse Grimace Scale (Dalla Costa 2014).

This research group has also created an Android application to bring the Horse Grimace Scale in practice. For appropriate pain relief to be provided it is crucial that veterinarians, farmers, horse owners and riders are able to recognize pain in a reliable way. With this application users are taught to recognize and then assess pain in horses using facial expressions [Website 'Animal Welfare Science hub'].

The use of facial expressions to assess the pain has come with a sum of advantages in comparison to the regularly used pain scales in animals. With facial expressions the assessment can be done quickly, furthermore it is easily learned by observers and could improve the safety of the observer, because for the assessment there is no need to approach the subject and palpate the painful area [Dalla Costa 2014]. Also, many of the existing pain scales had been developed for situations wherein there are patients with severe pain conditions (e.g. colic and laminitis) [Graubner 2011, Bussières 2008]. The grimace scales seems to be suitable to assess the postoperative castration pain in horses. Another substantial profit of the HGS is the high inter-observer reliability and a reasonably high degree of accuracy (73.3%) [Dalla Costa 2014].

So facial expressions of pain may be a valuable addition to the existing pain evaluation tools. But it is important to differentiate changes in facial expression due to pain from changes in facial expression due to stress, analgesics, anaesthetics and other interfering factors such as the influence of humans. In the study of Gleerup et al. (2014) they investigated the facial expressions in horses during induced acute pain and they described these facial signals in detail. In this study the researchers avoided treatment with analgesics or anaesthetics and tried to avoid stress as much as possible. Two noxious stimuli were used to induce this acute pain: a tourniquet on the antebrachium and topical application of capsaicin. Alterations in facial expressions were observed in all horses during all noxious stimulations. Through explanatory illustrations the researchers have tried to show the facials expressions during control and pain trials. Some of the conclusions of this study are that an equine pain face comprising: 'low' and/or 'asymmetrical' ears, an angled appearance of the eyes, a withdrawn and/or tense stare, mediolaterally dilated nostrils and tension of the lips, chin and certain facial muscles. These expressions can be recognized during induced acute pain [Gleerup 2014].

Van Loon and Van Dierendonck (2015) have created a scale based on facial expressions: the Equine Utrecht University Scale for Facial Assessment of Pain (EQUUS-FAP). This scale is a bit comparable to the Horse Grimace Scale of Dalla Costa et al. (2014). The EQUUS-FAP is comprised of a multifactorial numerical rating scale based 10 parameters with a total range from 0 to 20. In an earlier study the pain scale consisted of 9 parameters, but the patients in this earlier study, acute colic patients, made the parameter 'startle' not (yet) necessary. The EQUUS-FAP describes several parts of the facial expressions of pain in horses (appendix 1). They have also developed the Equine Utrecht University Scale for COMposite Pain ASSessment (EQUUS-COMPASS). This pain scale includes physiological and behavioural data, in other words: a composite pain scale. In their study they were monitoring acute equine visceral pain in colic patients with the EQUUS-COMPASS and the EQUUS-FAP. The results of this work showed good inter-observer reliability and high sensitivity and specificity for the EQUUS-COMPASS and EQUUS-FAP. This establishes validity and clinical applicability of the EQUUS-COMPASS and the EQUUS-FAP in horses with acute colic [van Loon and van Dierendonck 2015].

Adjacent to this study, in another later study, the aim was to create reliable objective scoring systems to monitor facial pain in horses. Horses with facial pain that were presented to the Equine Health Department of the Faculty of Veterinary Medicine in Utrecht during autumn 2013, were used for the assessment of pain with the EQUUS-COMPASS and the EQUUS-FAP. In this study 'facial pain' is defined as pain caused by eye-problems (e.g. uveitis, cornea-ulcers, injuries), ear pain, trauma (e.g. jaw-fractures, wounds), tooth problems (with secondary sinusitis) or surgery on the head. The conclusion of this study was that the EQUUS-FAP was a reliable and useful pain scoring system for assessing facial pain in horses [van Loon & van Dierendonck, 2015].

Castration

Castration is one of the most common surgical procedures performed in equine practice. Potential reasons for performing this procedure include: a need to reduce or prevent masculine or aggressive behaviour in animals unsuitable for breeding, testicular trauma or neoplasia, or inguinal herniation [Schoemaker 2004]. On a yearly basis it is estimated that 240,000 horses are castrated in Europe [Dalla Costa 2014].

As mentioned earlier, progress has been made in our understanding of the physiology and treatment of pain in animals. But the assessment of pain in horses during common management procedures, such as castration, remains insufficient [Price 2004, Love 2009, Sanz 2009]. The requirement for administration of analgesia to horses following castration has been debated within the literature. There are studies that suggested that the procedure is not painful and that administration of analgesia is not required [Green 2001]. However, other studies in horses have shown that castration is associated with some degree of pain that can go on for several days and, because of that, requires adequate analgesic therapy [Love 2009, Sanz 2009]. Recent statistics of the status of analgesic treatment with the castration of horses in the Netherlands are not available. But a survey of 282 equine veterinarians in the United Kingdom in 2005, showed that 45.4% of veterinarians did not administer supplemental analgesics after castration, 17.7% administered analgesics only occasionally, and 36.9% administered analgesics routinely [Price 2005]. A clarification for this, as said before, could be the fact that it is difficult to quantify pain in horses [Bussières 2008, Dalla Costa 2014, Sanz 2009, Love 2009].

Aim of the study

A suitable manner for the assessment of pain after castration will improve equine well-being, because the estimation of the need for analgesic treatment will be easier, which subsequently leads to a faster recovery of the horse.

The Horse Grimace Scale (HGS) potentially offers an effective and reliable method of assessing pain following routine castration in horses [Dalla Costa 2014]. An earlier study made clear that the EQUUS-FAP is useful for the assessment of pain in horses with acute colic and facial pain. But what is the applicability of the EQUUS-FAP in other types of pain?

Because the EQUUS-FAP is, like the HGS, a pain scale based on the facial expressions of horses, the question arises whether or not this pain scale is also applicable for the assessment of pain after a common procedure like castration.

This leads to the following aim of this study:

'The aim of this study is to assess the validity and applicability of the EQUUS-FAP in horses following castration.'

To investigate this aim both the HGS and EQUUS-FAP were assessed before and after castration. The hypothesis was that the EQUUS-FAP and the HGS are suitable objective scoring systems to monitor post-operative pain after castration.

Materials and Methods

Animals and husbandry

Seven stallions of different breeds, coat colour and aged between 1 and 6 years, admitted to the Equine Health Department of the Faculty of Veterinary Medicine in Utrecht, between 01/03/2015 and 01/05/2015, for castration, were used for this study (see table 1 for details). All horses were recruited from the faculty's clinical cases. The control group consisted of ten horses admitted to the Equine Health Department of the Faculty of Veterinary Medicine in Utrecht for a special farrier treatment, a CT scan or a MRI. This control group included 8 geldings and 2 mares of different breeds, coat colour and aged between 4 and 14 years old (see table 2 for details). The owners of all the animals that were used for this study were asked for a written permission to enrol their horse in this study and to use photo and film material for this research and education. In order to be included in this study, all the subjects had to be assessed as healthy and only pain-free horses were included in the control group. All the castration patients and CT/MRI subjects were kept in the same housing and management conditions: they were housed in standard single horse boxes (5 x 5 m) on wood shavings, in some boxes with visual contact with other horses. This because of the fact that different boxes were used in this study, some of them without horses beside and/or at the opposite of it.

Horse	Age (year)	Breed	Type of castration	Analgesics after surgery?
Cas01	6	unknown	Dorsal recumbency, both sided inguinal approach with primary wound closure	Yes: 3 days after surgery Meloxicam (1dd p.o.) for 5 days because of a wound infection
Cas02	2	KWPN	Dorsal recumbency, both sided inguinal approach with primary wound closure	No
Cas03	3	Andalusian	Dorsal recumbency, both sided inguinal approach with primary wound closure	Yes: 5 days Meloxicam (1dd p.o.) because of the removal of a chip from the left tarsus.
Cas04	6	Tinker	Standing, both sided laparoscopic procedure because of cryptorchid testicles	Yes: 1 day Meloxicam (1dd p.o.)
Cas05	3	KWPN	Dorsal recumbency, both sided inguinal approach with primary wound closure	No
Cas06	3	Oldenburger	For the right: standing, laparoscopic procedure because of cryptorchid testicles, for the left: dorsal recumbency, inguinal approach with primary wound closure	No
Cas07	1	unknown	For the right: dorsal recumbency, inguinal approach with primary wound closure, for the left: standing, laparoscopic procedure because of cryptorchid testicles	No

Table 1: *Castration cases in this study.*

The patients were fed twice a day with silage grass or hay (approximately 2-3 kg/100 kg body weight) and basic concentrates to the owner's wishes. Water was provided ad libitum by automatic drinkers. Food was withheld from all horses for at least 8 hours before and 4 hours after anaesthesia. The exception on this were the stallions who where castrated laparoscopically. These animals needed to fast for a longer period (36 hours) since their abdomen needed to be empty enough for the laparoscopic procedure.

Horse	Sexe	Age (year)	Breed	Type control
Con01	gelding	11	KWPN	Farrier
Con02	gelding	8	Fries	Farrier
Con03	gelding	7	Onbekend	Farrier
Con04	gelding	4	Arabier	Farrier
Con05	gelding	14	KWPN	Farrier
Con06	gelding	7	NRPS	Farrier
Con07	gelding	10	KWPN	Farrier
Con08	mare	12	KWPN	CT (general anaesth.)
Con09	mare	6	KWPN	MRI (general anaesth.)
Con10	gelding	10	KWPN	Uveitis patient (general anaesth.)

Table 2: *Control cases in this study.*

Analgesic treatment and surgery

Horses that underwent castration received analgesia (local anaesthesia with 10 cc lidocain for each testicle and a single perioperative systemical injection of Flunixin 5%) together with (general) anaesthesia. The general anaesthesia protocol at the Equine Health Department of the Faculty of Veterinary Medicine in Utrecht consisted of a pre-medication with detomidin (Domosedan: 0.01-0.02 mg/Kg) and butorphanol (Dolorex: 0.01-0.02 mg/Kg), induction with diazepam (Midazolam: 0.06 mg/Kg) and ketamine (Ketamin: 2 mg/Kg) intravenously via a jugular catheter, maintenance with isoflurane and a detomidin CRI.

The surgeries were all carried out by one of two experienced veterinary surgeons. Different techniques of castration were performed, due to the nature of the cases. Four out of the seven castrations were achieved in dorsal recumbence under general anaesthesia; with primary wound closure using an inguinal approach. The vaginal process was closed, afterwards crushing and ligation was performed. The other three castration patients were cryptorchids. One of these three animals was both sided chryptorchid and total laparoscopic castration in a standing position was accomplished. The other two stallions were one-sided cryptorchid, so the procedure was first in a standing position and with laparoscopic guidance this cryptorchid side was castrated. The other testicle was castrated in a dorsal recumbence as well, under general anaesthesia, with primary wound closure, using an inguinal approach (see table 1 for details). Surgery lasted 30 – 75 minutes, afterwards the horses were moved to a recovery box. Later, as soon as they were able to walk (around 30-60 minutes after anaesthesia) they returned to their own box. All of the horses recovered from anaesthesia with assistance.

One castrated animal received Meloxicam (Metacam p.o., 1 dd) post-surgery, for one day. Another castrated horse received 5 days Meloxicam post-surgery because of another surgical operation together with the castration (they also removed a chip from his left tarsus). One of the castrated horses had a complication of a wound infection after castration and received, 3 days after surgery, Meloxicam for 5 days.

To assess the impact of general anaesthesia on the EQUUS-FAP and HGS, a control group of horses was recruited. These horses underwent the same general anaesthesia protocol as the horses of the castration group except for the fact that this control group did not receive a perioperative injection of Flunixin or any postoperative analgesics because no surgical procedure was accomplished, only a MRI, CT-scan or a minimal invasive surgical procedure.

Pain assessment

Two pain scales were used in this study: Equine Utrecht University Scale for Facial Assessment of Pain (EQUUS-FAP) and the Horse Grimace Scale (HGS), the details of which are presented in the appendix. The HGS used in this study is the pain scale described by Dalla Costa et al. [Dalla Costa 2014]. The EQUUS-FAP used in this study was created at Equine Department of the University and has been used in previous research [van Loon and van Dierendonck 2015]. Both pain scales are based on the facial expression of the horse, they have some similarities, but also some differences. The HGS consists of 6 elements: 'stiffly backwards 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 element can be scored by a 0 (= not present), 1 (= moderately present) and 2 (= obviously present). This numerical scale results in a total maximum score of 12. The original EQUUS-FAP used in a earlier study for the colic patients however contained 9 elements: 'head', 'eyelids', 'focus', 'nostrils', 'corners mouth/lips', 'muscle tone head', 'flehmen reaction/yawn/ smacking', 'teeth grinding/moaning' and 'ears'. For this study a tenth element 'headshaking/startle' has been added to the EQUUS-FAP. Also in this pain scale each element can receive a score of 0, 1 or 2, which results in a total maximum score of 20.

For every pain assessment moment, a two-minute video was recording using a smartphone. In each minute the left side, front side and right side were recorded for 20 seconds each. The pain assessment for this study was performed by direct observation during the first minute. The film material will be used for training, blinded scoring, and additional future extra control assessments. Scoring of EQUUS-FAP and HGS were accomplished independently at the same time by two observers, not blinded. Thus, the first minute of filming was used for the assessment of the EQUUS-FAP as well as for the HGS. The possible need for post-operative treatment of the castrated horses was adjusted to the normal clinical process and was independent on the outcome of the pain score measured for this study.

The scoring of the EQUUS-FAP and HGS was performed at eight separate moments. The first moment of scoring ($T=0^1$) was performed on the day of arrival of the stallion, the second moment was completed on the morning before surgery ($T=0^2$). The third moment was achieved after 4 hours and the fourth 8 hours after surgery (originally also observations at 6 hours post surgery were planned, but not performed due to miscommunication). After this, the horse was followed for two more days and pain assessment was completed in the morning and in the late afternoon (figure 3). This whole procedure was performed to monitor the pain and to take note of the changes in pain over time.

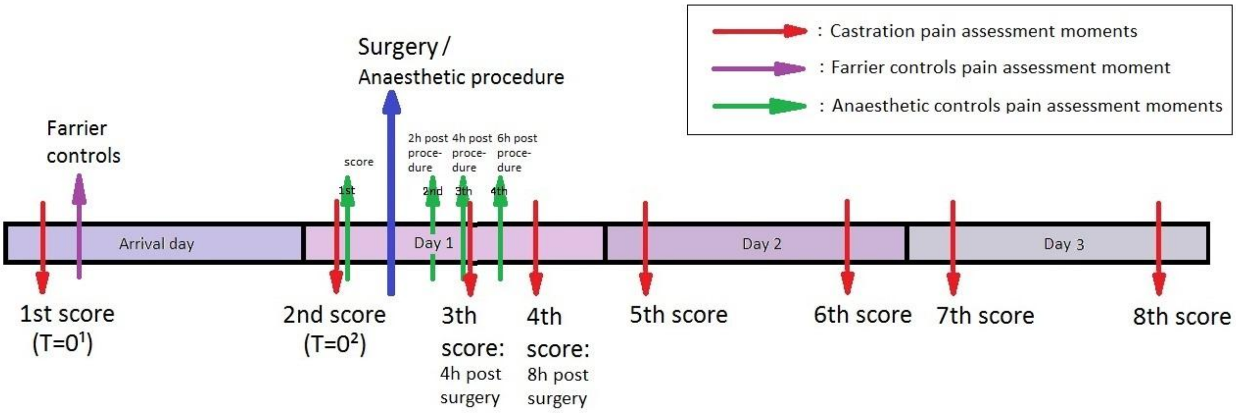


Figure 3: *Timeline of all assessment moments of the castrated animals (red arrows), farrier controls (purple arrow) and anaesthetic controls (green arrows).*

The control group of horses, that were admitted for special farrier treatments, were scored for the EQUUS-FAP and HGS just once, following the same protocol at the same location. The control group of CT and MRI patients, used to investigate the impact of general anaesthesia on the EQUUS-FAP and HGS, was assessed for the EQUUS-FAP and HGS on comparable moments as the patients: The first assessment was achieved on the day of arrival and/or just before the performing of the CT or MRI. Afterwards the pain assessment moments were planned on 2, 4, 5, and 6 hours after anaesthesia. It is important to consider that the castrated horses enter the university clinic in a healthy state. So the T0 measurements can be used as own control for the stallions that underwent castration as well.

During the scoring of pain, the castrated horses stayed in their own stables to minimize any environmental influences on the results. The same applies to the control group of CT and MRI animals. Unfortunately, the control group of 'farrier horses' usually did not have their own stable in the clinic. Sometimes they were already presented to the farrier by their owner before checking with the researchers and went home after treatment immediately. It was decided to perform the assessments in the room where the farrier had treated them, since a temporary stable would possibly have more impact on the behaviour of the horses than the surrounding of the farrier would. At the farriers place they already had settled down a little.

Methods

To evaluate whether the EQUUS-FAP and HGS were useful for the assessment of pain following the castration in horses, the repeatability/reproducibility of the pain scoring systems was examined. The inter-observer-reliability of observer 1 with observer 2 was analysed using Spearman's rho correlation analysis. This determined the degree of reproducibility of measurements of the two observers under the same circumstances.

With the help of a paired two-sample t-test the significance between the mean results of the HGS pre-procedure and post-procedure has been made for the castration pool and the control pool. For the EQUUS-FAP the same comparison has been made for only the castration pool.

Data and statistical analysis is performed using IBM SPSS statistics 20 and Microsoft Excel 2010.

Results

Comparison of mean scores

In the figure 4 the comparison between the mean scores of the EQUUS-FAP and the anaesthetic control group is made (one MRI patient, one uveitis patient and one CT patient). The mean HGS scores and the control group are represented in figure 5 similarly. The measurement point T=0[1] in figure 4 gives for the control group a lower mean pain assessment outcome in comparison to the castration group at T=0[1]. This is not the case for the HGS results. The measurement point T = 4h post surgery shows in figure 4 for the EQUUS-FAP a higher score for the control group in comparison to the castration group.

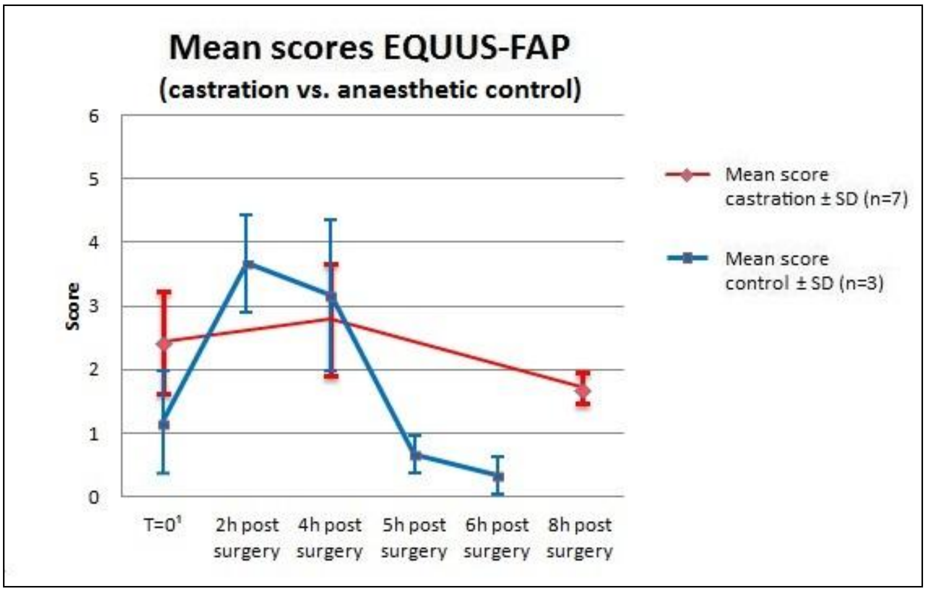


Figure 4: *Mean scores EQUUS-FAP castration against anaesthetic control group. The anaesthetic control group (n=3) consisted of a MRI, CT and an uveitis patient.*

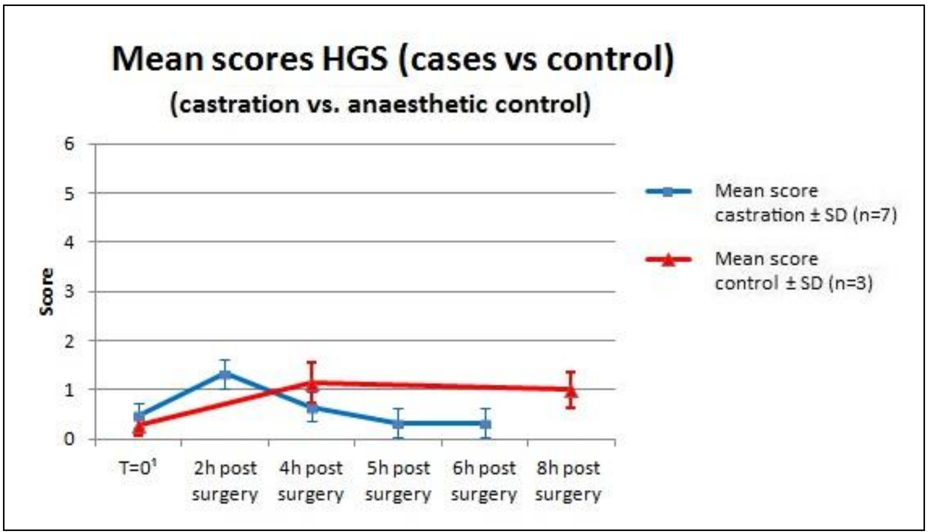


Figure 5: *Mean scores HGS castration against anaesthetic control group. The anaesthetic control group (n=3) consisted of a MRI, CT and an uveitis patient.*

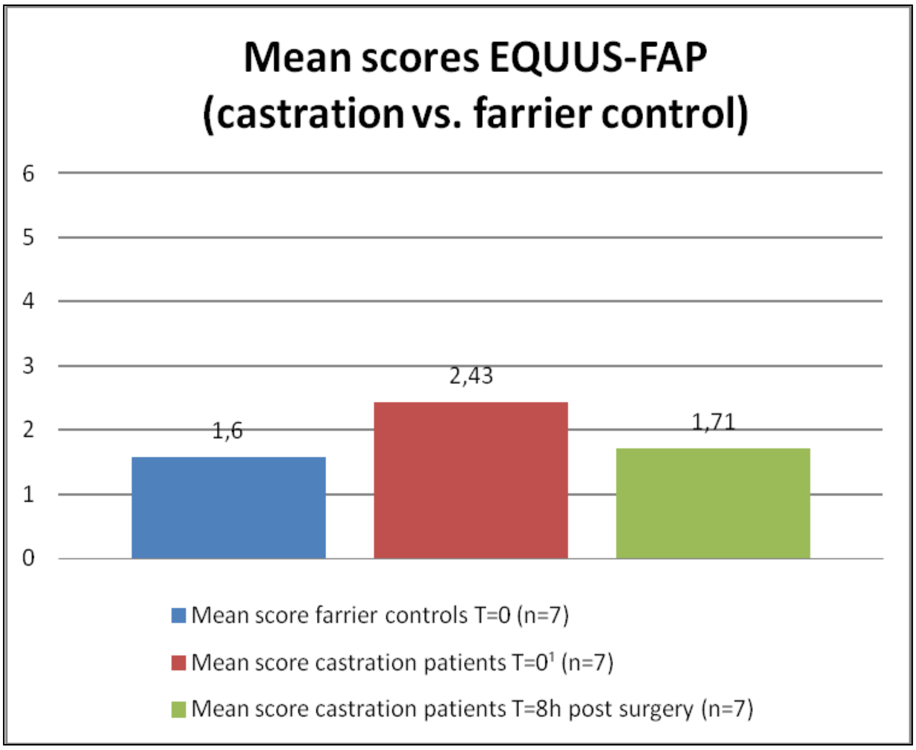


Figure 6: *Mean scores EQUUS-FAP castration against farrier control group.*

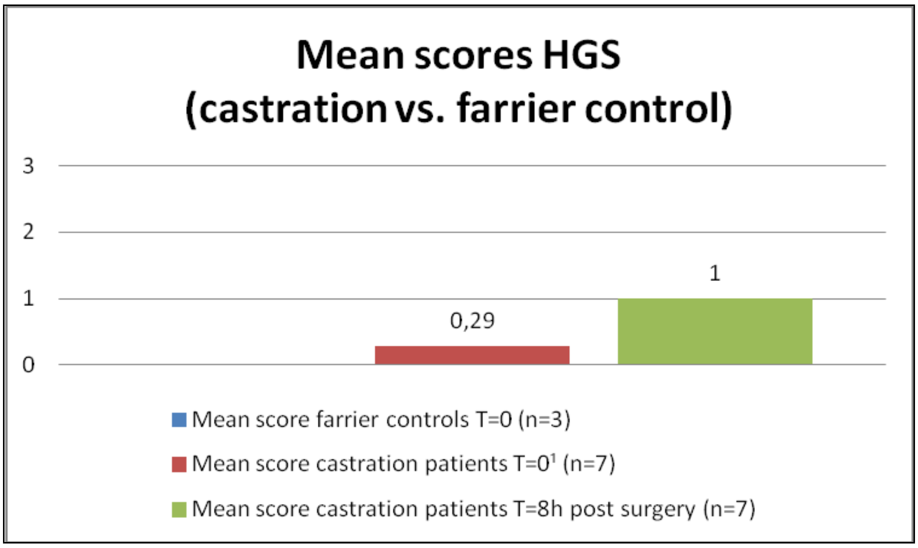


Figure 7: *Mean scores HGS castration against farrier control group.*

Mean scores

In figure 8 the development of the mean scores of the EQUUS-FAP as well as the HGS, during the observation period, are shown.

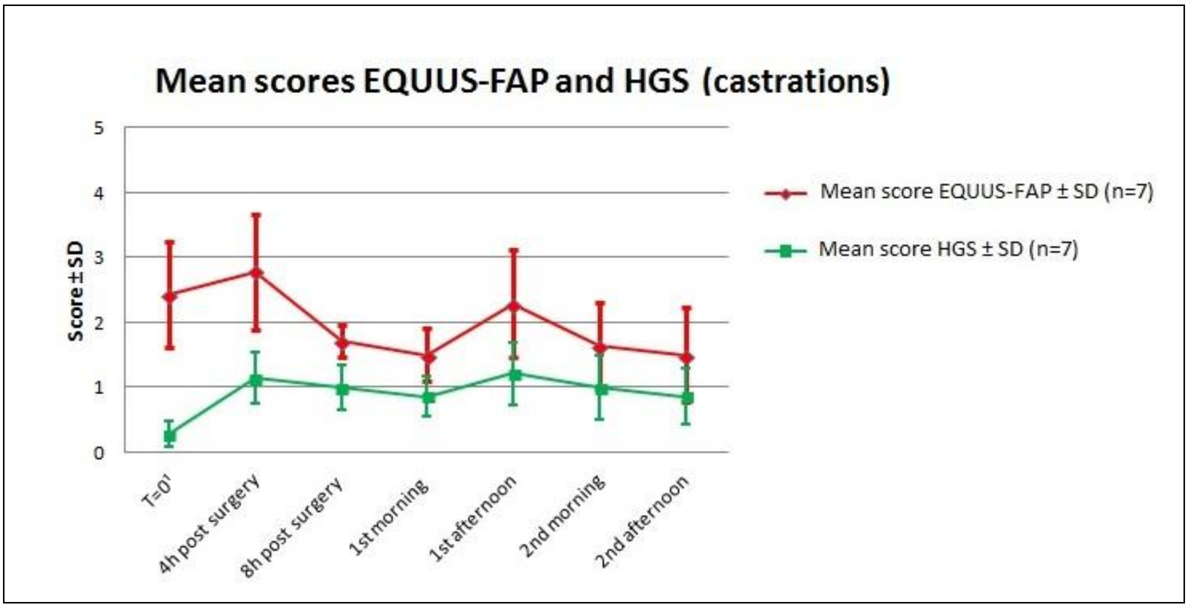


Figure 8: *Mean scores EQUUS-FAP and HGS castration group.*

Deviating results

Two out of the patient's group of seven castrated horses show some deviating development of pain assessment results. The first of these animals, Cas01, had elevated pain scores after the first morning post surgery for the EQUUS-FAP as can be seen in figure 9. This gelding had a wound infection after the surgery. The pain score development of this horse turned out to be quite a bit higher than the mean score development of the five geldings with a 'normal' pain assessment for the same time period. For the HGS this gelding also had overall a higher pain score in comparison to the mean scores (figure 10).

The development of pain scores of Cas04 demonstrated an unstable development of pain score results for both the EQUUS-FAP, as well as for the HGS, (figure 11 and 12). This horse was castrated laparoscopic both sided, because of a complete cryptorchid situation. Maybe this totally laparoscopic approach has lead to a more painful post surgery period.

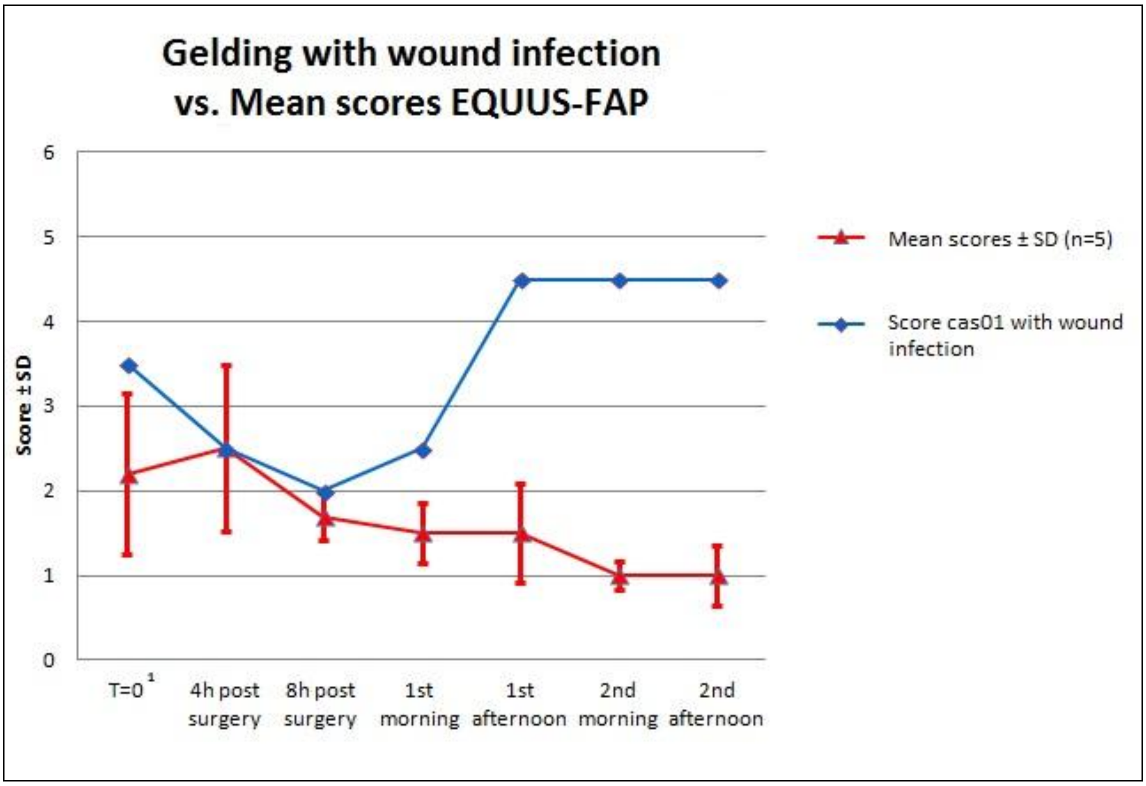


Figure 9: *Mean scores castration group against scores cas01, with wound infection, for the EQUUS-FAP. N=5 because the total amount of castrated horses that show a 'normal' pain assessment development, were used.*

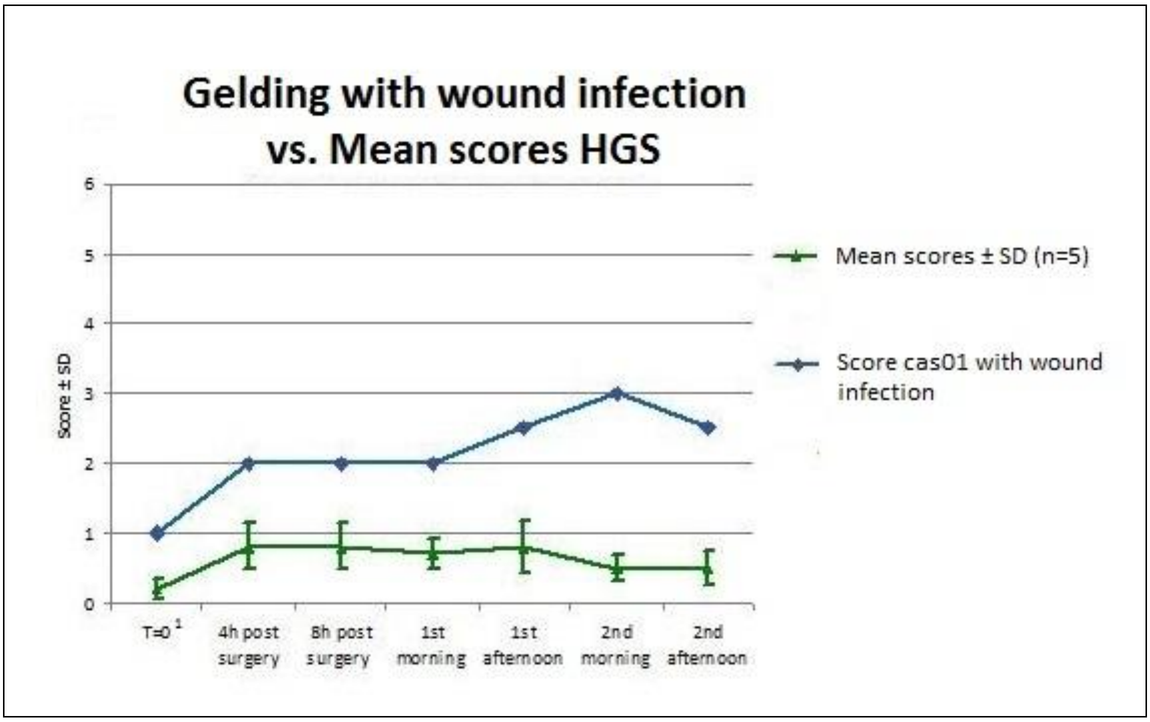


Figure 10: *Mean scores castration group against scores cas01, with wound infection, for the HGS. N=5 because the total amount of castrated horses that show a 'normal' pain assessment development, were used.*

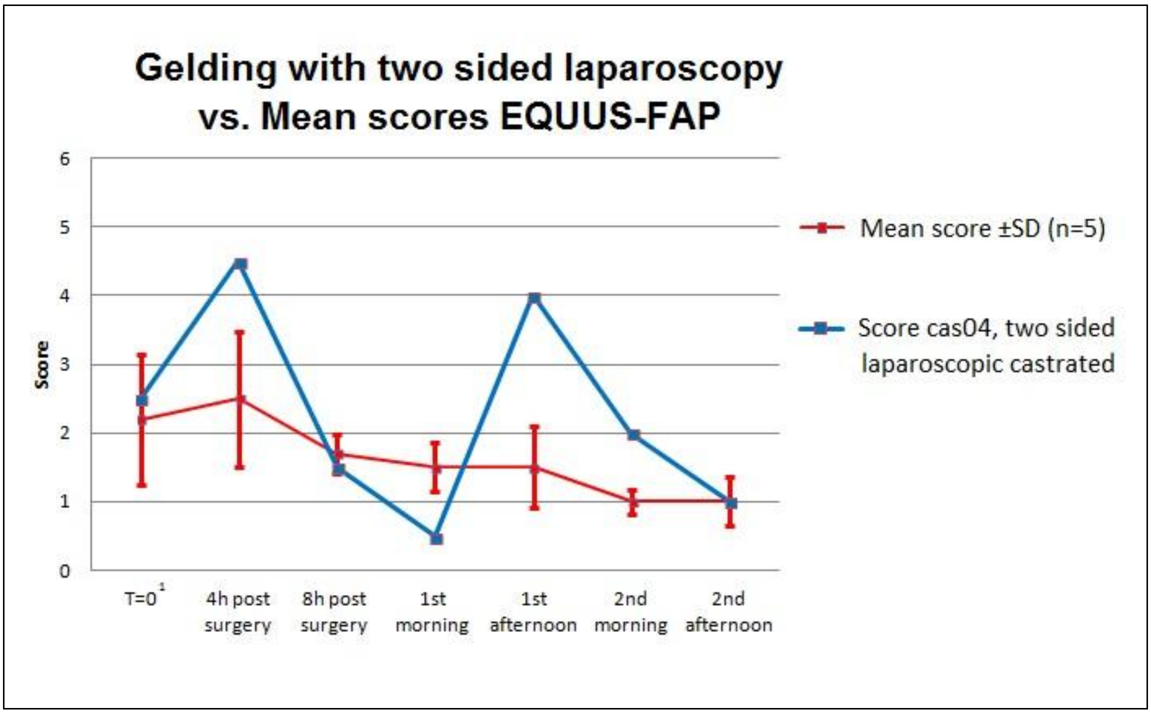


Figure 11: *Mean scores castration group against scores cas04, two sided laparoscopic castrated, for the EQUUS-FAP. N=5 because the total amount of castrated horses that show a 'normal' pain assessment development, were used.*

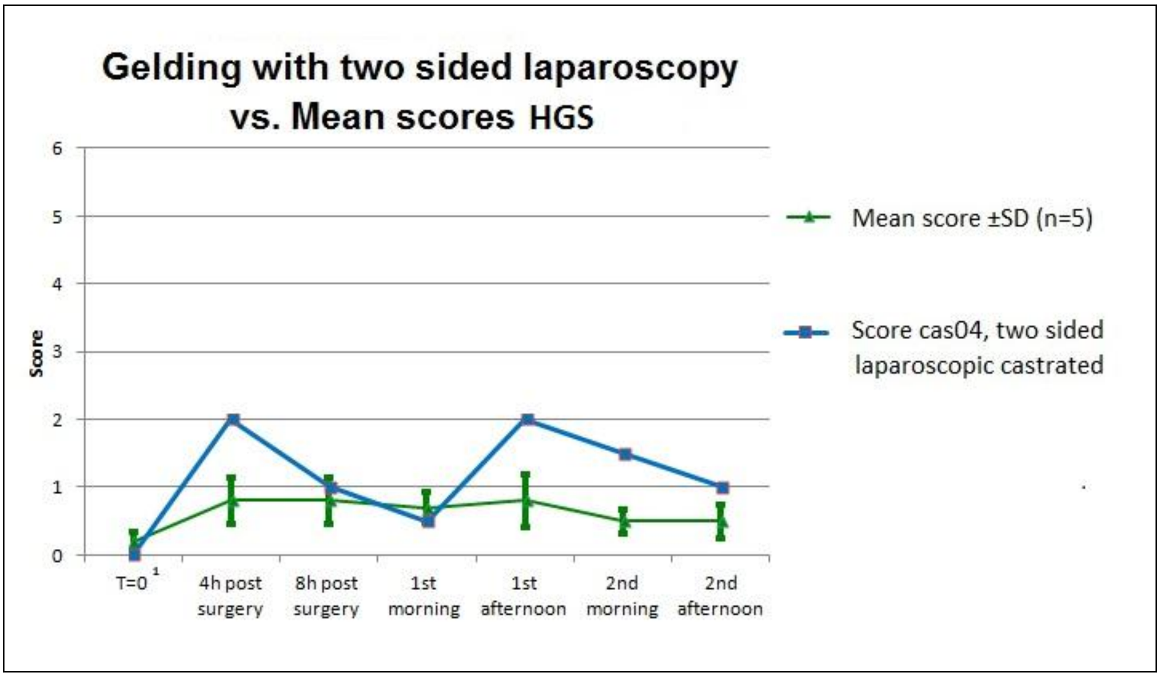


Figure 12: *Mean scores castration group against scores cas04, two sided laparoscopic castrated, for the HGS. N=5 because the total amount of castrated horses that show a 'normal' pain assessment development, were used.*

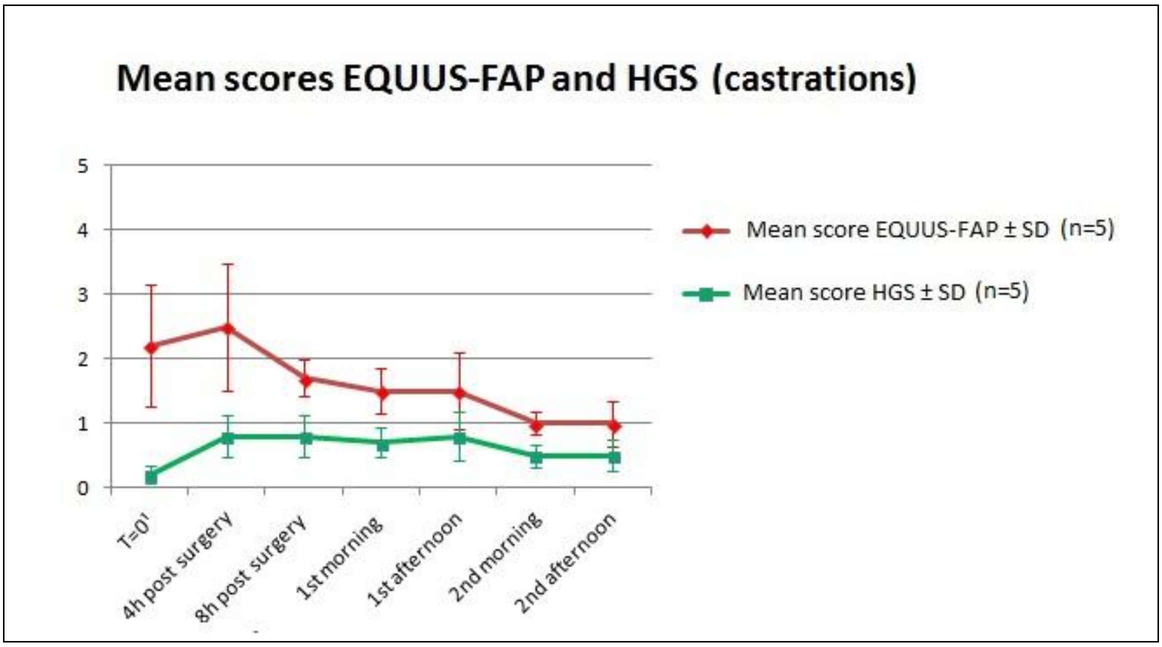


Figure 13: *Mean scores EQUUS-FAP and HGS castration group. N=5 because the total amount of castrated horses that show a 'normal' pain assessment development, were used.*

Comparison with Dalla Costa et al. (2014)

In the following figures 14 and 15 the comparison of the results in this study and the results of the research of Dalla Costa et al. (2014) is made.

Mean Horse Grimace Scale (HGS) scores pre and 8-hours post-procedure (Dalla Costa)

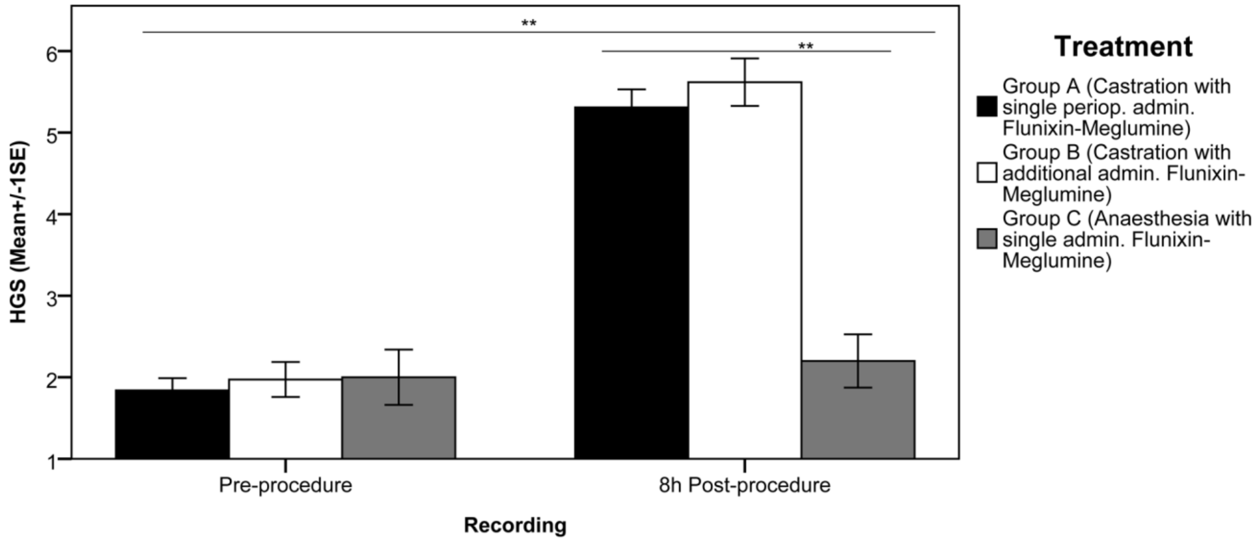


Figure 14: *Results of the study of Dalla Costa et al.* [*Dalla costa 2014, page 7*].

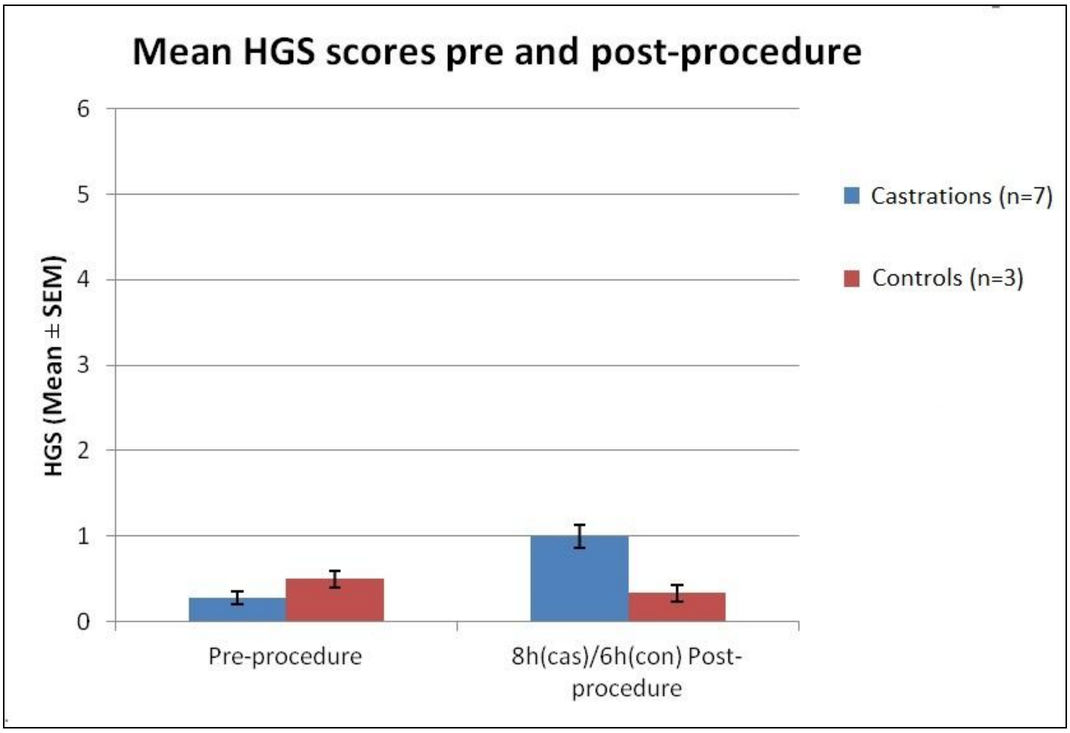


Figure 15: *Results pre- and post procedure of the HGS for the castration and the anaesthetic control group.*

With the help of a paired two-sample t-test the significance between the mean results of the HGS pre-procedure and post-procedure has been made for the castration pool and the control pool. In table 3 the results of this statistical analysis are shown.

Group	Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference		t	df	Significance (2-tailed)
				lower	upper			
Castration (n=7) HGS pre- vs. post-procedure	-0.714	0.636	0.240	-1.303	-0.126	-2.970	6	**0.025**
Control (n=3) HGS pre- vs. post-procedure	0.16667	1.04083	0.60093	-2.41891	2.75224	0.277	2	0.808

Table 3: *Paired two-sample t-test for the HGS mean results pre-procedure versus post-procedure, divided in the castration and control animals .*

Since the p-value of the castrated animals is 0.025, i.e. smaller is than 0.05, it can be concluded that there was a significant difference between the means. This result for the control group is a partly predictable outcome, because it is clear that between the pre-procedure en post-procedure for the control group no differences exists. The castration group experienced probably pain, so it is more obvious that the difference between the pain assessment scores pre- and post-surgery is significant.

The patient group was not homogeneous, because different types of castration were used. To make a more homogeneous patient group for additional statistical comparison, the (partly) laparoscopic surgeries and the castrated animals with complications, were removed. This separate group consisted of three animals: cas02, cas03 and cas05. With the help of a paired two-sample t-test a comparison between the mean results of the EQUUS-FAP and HGS pre-procedure and post-procedure has been made for this two groups of animals. NB the mean results post-procedure were in this comparison 8 hours post surgery. In table 4 the results of this statistical analysis is shown. There was no significant difference between any of these comparisons.

Group	Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference		t	df	Significance (2-tailed)
				Lower	upper			
Castration (n=3) HGS pre- vs. post-procedure	-1.0	0.5	0.2887	-2.2421	0.2421	-3.464	2	0.074
Castration (n=3) EQUUS-FAP pre- vs. post-procedure	0.1667	2.0207	1.1667	-4.8531	5.1864	0.143	2	0.899

Table 4: *Paired two-sample t-test for the HGS and EQUUS-FAP mean results pre-procedure versus 8 hour post-procedure for a more homogeneous group (n=3).*

Reproducibility of the pain scoring systems

In table 5 and figures 16 and 17 the strength of the linear association between the two independent observers is shown. The black line in the figures represents a perfect linear association, which would have occurred if both observers had given the same pain score to a horse. The purple line is the regression line of the results. In figure 16 and 17, all the pain assessment observations are incorporated, for the castration animals as well as for the farrier and anaesthetic control animals.

Pain scale	Correlation coefficient (r)	R^2	p-value	N	Figure
EQUUS-FAP	0.571**	0.3885	0.000	59	16
HGS	0.515**	0.3141	0.000	59	17

Table 5: *Inter-observer association of the EQUUS-FAP and the HGS. All the observation are incorporated. ** correlation is significant at 0.01 level.*

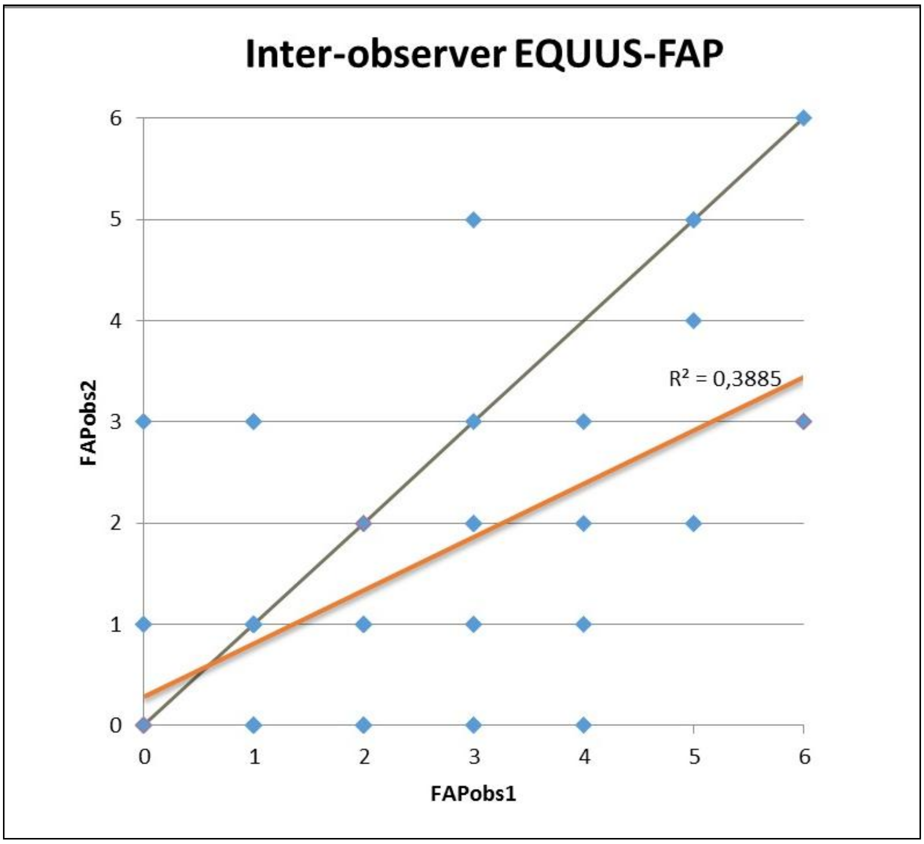


Figure 16: *Inter-observer association EQUUS-FAP. FAPobs= score observer 1 or 2, n=59.*

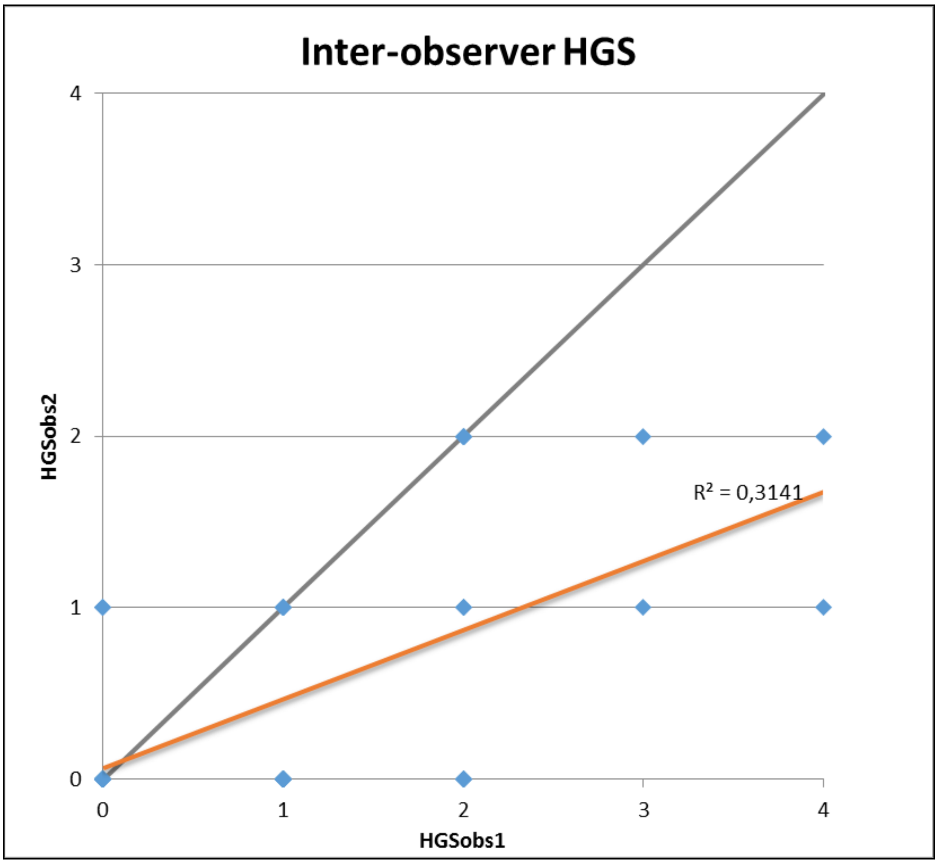


Figure 17: *Inter-observer association HGS. HGSobs= score observer 1 or 2, n=59.*

If only the castrated horses with 'deviating' pain assessment scores are taken along in the reproducibility calculation, another correlation coefficient is noticeable. In table 6 is the outcome of this deviating calculation visible.

Pain scale	Correlation coefficient (r)	p-value	N
EQUUS-FAP	0.722**	0.002	15
HGS	0.468**	0.078	15

Table 6: *Inter-observer association of the EQUUS-FAP and the HGS. Only but all the observations of Cas01 and Cas04 are incorporated. ** correlation is significant at 0.01 level.*

Discussion

Considerations of two horses with post castration complications

The outlying observations of the patients cas01 and cas04 could be explained by the next observations: Cas01 had a complications due to a wound infection after the surgery. The swollen and warm wound area (swollen preputium and scrotum) and fever made the stallion more uncomfortable. So this provides higher scores for this animal in comparison to the mean scores after surgery. The scores corresponded good with the clinical appearance over time of this patient.

The development of pain scores over time of patient Case04 demonstrated an unstable development of pain score for both scales. A reason for this could possibly be the fact that this horse was castrated laparoscopic both sided, because of a complete cryptorchid situation. For laparoscopic surgery carbon dioxide is used to inflate the abdomen. The advantage of this gas is that carbon dioxide is widely available, affordable, unlikely to cause gas emboli, is non-combustible and only mildly irritating to the peritoneal and serosal surfaces. Despite these generally good experiences with carbon dioxide, some mild peritoneal inflammation is associated with carbon dioxide insufflation and has been documented in standing horses [Smith 2005]. One of the complications of a laparoscopic cryptorchidectomy is that some horses will experience minor colic signs, because of the lack of complete extravasation of abdominal CO_2 [Joyce 2008]. Since this horse had a double dose of CO_2 and over a longer time this could result in a more painful abdomen during the post-surgery period. Resulting in fluctuating scores in both scales. The question could arise why the other laparoscopic castrated horses in this study did not show the same pattern. Perhaps the amount of carbon dioxide used and the duration of surgery was playing a part in this. The other laparoscopic surgeries were only one-sided cryptorchid, so the whole surgery was performed quicker and less CO_2 was used. There is no scientific literature that confirms that a longer exposure to CO_2 and the use of more $CO_{2,}$ could lead to a bigger change of developing colic signs in horses after surgery. Even though no studied evidence did support the possibility, it seems an biologically valid suggestion.

When looking at the group of castrated horses that did not undergo a (partly) laparoscopic cryptorchidectomy or got complications afterwards, a small group of horses (n=3) results were most homogeneous. The comparison between the mean pre-surgery (T=0) pain assessment and the mean 8 hours post-surgery pain scores, gave for the EQUUS-FAP as well as for the HGS no significant difference (table 4). As a result of this analysis it is questionable if the castrated horses of these homogeneous group experience serious pain after castration.

The good news is that the used EQUUS-FAP and HGS noticed the outlying observations in this study. So these pain scales seems both functional in taking out the patients who needs more care.

Inter-observer reliability

The linear association between the two independent observers did not turn out to be that strong. A correlation coefficient of respectively 0.571 and 0,515 for the EQUUS-FAP and HGS is not that high. This is probably attributable to the fact that overall the scores for the EQUUS-FAP as well as for the HGS turned out to be low in this research. The possible maximum score for the EQUUS-FAP is 20, in this study the maximum measured score was 6. For the HGS these numbers were respectively 12 and 4. The low pain score results illustrate that the difference between the two observers in this study has a relative bigger impact. For example, when observer 1 gave a castrated animal, at some measurement moment, in total a score of 1 point and observer 2 in this same situation gave only a total pain score of 2 points, than the difference is only 1 point, but the score has doubled between the two observers. With higher total pain scores, like for example a total score of 5 against a total of 7 points, then the absolute difference is bigger, but the relative difference in percentage of change is smaller. So in short, proportionally, the differences between the results of the two observers, is always unfavourable for the lower scores. This also could mean that the analgesics after castration is good. Therefore, the question could arise if the used pain scales are applicable to assess subtle pain in horses. In other studies it became clear that the EQUUS-FAP was clinically applicable for horses with acute facial pain, like trauma, sinusitis and postoperative surgical pain to the head (n=30; max FAP scores = 14; mean 5.1 ± 2.1) as well as horses with acute colic (n=50; max FAP score = 11; mean 5.3 ± 1.3). The results of these higher pain scores, showed a much better correlation (R^2 = 0.84) in the association between the two independent observers for the horses with acute colic [Van Loon & Van Dierendonck, 2015]. To study this assumption in the current castration study a calculation for the Inter-observer association was calculated for the two geldings with complications after surgery. These geldings experienced more pain than the "normal" five animals, probably because of more pain, and gave a better correlation in the linear association between the two independent observers (table 6: correlation coefficient (r) of 0,722) .

Pain scales

The HGS is a more stationary assessment in comparison to the EQUUS-FAP. The EQUUS-FAP focuses on the changes of expression of the head for example: the evaluation of the movement of the head, the focus of the horse on its environment or the sounds that the horse produces during the pain assessment. The HGS has a greater focus on the profile of the head during a single moment. These differences are probably responsible for the differences in pain score outcomes in some way. Although the development of the mean scores of both pain scales (figure 8) is comparable.

In the past a couple of studies tried to assess the pain after castration in horses. In the study of Sanz et al. (2009) there was a comparison between the analgesic effect of butorphanol, phenylbutazone, or both drugs in combination, in young stallions undergoing castration. Physical and physiological variables, plasma cortisol concentration, body weight, and water consumption were assessed before and at intervals after surgery. Observers assessed signs of pain by use of a visual analogue scale (VAS) and a numerical rating scale (NRS). Significant changes in gastrointestinal sounds, fecal output, and plasma cortisol concentrations were evident in each treatment group over time, compared with preoperative values. At any time point, assessed variables of the NRS and signs of pain did not differ significantly between groups. Two types of behaviours were measured by use of the NRS: undisturbed behaviour that was visible to the observer without disturbing the horse and socialization behaviours observed in response to interactions with the observer. Each behaviour (9 elements) got a score. A total score was calculated as the sum of scores for all behavioural observations at each time point. A mean 24-hour behaviour NRS scores was calculated for each horse for the time prior to surgery (day 1), the day of surgery (day 2), and the 2 days following surgery (days 3 and 4). The results made clear that the VAS scores varied over time in all groups. The highest VAS scores were evident at 4 and 8 hours after castration. The mean 24-hour NRS behaviour scores did not change significantly over time in any group. The development of a perfect pain scale was not the aim of this study [Sanz 2009]. The used pain scales in this study were not ideal, since the VAS is highly subjective [Bussières 2008, van Loon and van Dierendonck 2015]. The disadvantage of the NRS seems that this pain scale did not observed any difference in the scores prior to surgery in comparing with scores after surgery. So maybe this pain scale is not sensitive enough to notice post castration pain in horses?

In the study of Love et al. (2009) the analgesic effects of butorphanol administration following castration of ponies was examined. The post castration pain was assessed by one person with the use of a dynamic interactive visual analogue scale (DIVAS) of 100 mm in length (0 = no pain, 100 mm the maximum possible pain for that procedure). This DIVAS is comparable to the earlier discussed VAS. Pain was assessed by observing the behaviour of ponies for 10 min from a distance each 1, 2, 3, 4, 6 and 24 hr after time 0 (the assessment moment before castration). In particular, the animals' posture and presence or absence of activities, such as grooming, foraging, eating, bruxism, vocalization or kicking at their abdomen were assessed. The observer then entered the stable and approached the pony while noting the willingness to interact. Once caught, the ponies' response to touch on their neck and flank was assessed as well as their willingness and ability to walk when led round the stable. Pain was judged to be unacceptable if a score ≥50 mm was awarded using DIVAS. Higher pain scores (>50 mm) were awarded to ponies that reacted to gentle pressure on their flank and abdomen, were grinding their teeth, lifting a hindleg, weight shifting, reluctant to walk and had a stiff hindlimb gait. These ponies were often also quiet and not engaging in activities such as grooming, foraging or eating. The conclusion of this study was that castration was noticeable painful and administration of a single preoperative dose of butorphanol did not provide adequate post operative analgesia [Love 2009]. The pain scale used in this study seemed to be useful because changes in pain assessment pre- and post castration were recorded, but the question arises if the scale was sufficient valid and reliable as well as easy to use? Observers needed a lot of training before they could use this pain scale and it seems a time-consuming method. A third disadvantage of this pain assessment method was that it is not that safe in use because observers needed to approach and palpate the horses.

With facial expressions the assessment can be done quickly, are easily learned by observers and could improve the safety of the observer, because for the assessment there is no need to approach the subject and palpate the painful area [Dalla Costa 2014].

Also in other animal species an attempt was made to assess the pain after castration. In the study of Wagner et al. (2008) they evaluate among other aspects, the degree of postoperative pain in dogs undergoing elective castration or ovariohysterectomy. The baseline pain assessment scores (pre-procedure) of the dogs in this study, were used for the comparison post surgery. Thus the control group were the animals by themselves. To assess this pain they used a pain evaluation form modified from the University of Melbourne Pain Scale. This scale consists of 9 elements: 'posture', 'comfort level', 'pupils', 'salivation', 'vomiting', 'vocalization', 'mental status', 'movement' and 'response to palpation'. Each element can be scored with 0-4 points. Overall pain score was calculated by summing the scores for individual pain-related behaviours. Overall pain scores ranged from 0 (least painful) to 20 (most painful). In the treatment groups, overall pain scores were significantly higher after surgery, compared with baseline scores. The aim of this study was not to fine-tune the used pain scale but to determine whether an association exists between surgeon experience, incision length, or surgery duration and degree of postoperative pain and to determine whether analgesic treatment decreases expression of postoperative pain [Wagner 2008]. The used pain scale seemed to be appropriate to evaluate the degree of post castration pain in dogs, because it seems to be not necessary that observers needed a lot of training before they could use it. Beside this it seems to be a quick and safe method. Facial expressions seems not (yet) common in the pain assessment of dogs.

Comparison with the study Dalla Costa et al. (2014)

The results for both the EQUUS-FAP and HGS show quite low pain score results post surgery compared with the results of Dalla Costa (2014) (see figures 14 and 15). For this observation, a couple of possible explanations could be hypothesized: maybe the horses are not in that much pain after their surgery due to sufficient analgesics. Or maybe the pain scoring systems used were not sensitive enough to discover the post castration pain? In short, the question arose whether or not the EQUUS-FAP and the HGS were able to identify the pain after castration. This last question can at least for the HGS immediately answered: in the study of Dalla Costa et al. (2014) the HGS assessed pain after castration in horses. In this study the HGS has been used in the same manner.

The next step is to compare the study design of this study with the design of Dalla Costa et al. (2014). The patient's group of Dalla Costa consisted of different breeds and ages were between 1 and 5 years (mean age 2.3 years). The animals in this study aged between 1 and 6 years and different breeds were used as well. So the patient group was not different. Likewise the surgical procedure could not influence the difference between the results. In the study of Dalla Costa et al. (2014) the horses underwent routine castration with closed technique through a scrotal approach without primary closure of the wound. In our study the surgical procedures were variable due to the nature of the animals (normal routine castrations of young animals are usually not admitted to the Equine Health Department of the Faculty of Veterinary Medicine in Utrecht, also resulting in a relative low number of animals anyway). In this study group for removal of the normal testes also a closed technique was used; the vaginal process stays closed and crushing and ligation was also performed. The difference between the surgical approach in this study and in that of Dalla Costa et al.(2014) was that most of the time in this study an inguinal approach and primary closing of the wound was performed. The critical point of the entire intervention is the crushing and ligation of the ductus deferens. This is probably the most painful part of the castration and this action was present in both castration procedures. So the surgical procedure would probably not be the cause of the dissimilarity between the results of this study and that of Dalla Costa et al. (2014).

Finally, the difference in anaesthesia and analgesic treatment design could be the reason for the discrepancy between the results. The general anaesthesia protocol in the study of Dalla Costa et al.(2014) consisted of a pre-medication with Romifidine, induction with Diazepam and Ketamine and when necessary the general anaesthesia was maintained by another injection of Ketamine. All of the horses received a single injection of Flunixin perioperatively. The anaesthesia and analgesic protocol of the Equine Health Department of the Faculty of Veterinary Medicine in Utrecht differed in the fact that an opiate (butorphanol) is used in the pre-medication and the horses received a local anesthetic block with 10 cc lidocaine in each testicle. Both these analgesic differences could possibly be an explanation for the differences in pain scores postoperatively.

The administration of analgesia to horses following castration has been debated within the literature. Also in practice this topic seems to be debatable. In the survey under 282 equine veterinarians only 36.9% administer analgesics routinely [Price 2005]. The questioned veterinarians > 40 years of age were almost 3 times as likely to provide no analgesia after castration compared to the respondents < 30 years old [Price 2005]. Of course these are no Dutch figures, but perhaps the circumstances are comparable and are also the relatively older Dutch equine veterinarians sceptical about the use of analgesics after castration? There are studies in horses that have shown that castration is associated with some degree of pain that can go on for several days and, because of that, requires adequate analgesic therapy [Love 2009, Sanz 2009]. Our study in comparing with the study of Dalla Costa (2014) supports this known fact also.

The difference between our study and Dalla Costa (2014) supports to use an opiate in the pre-medication and a local anaesthetic block in each testicle before the castration is performed.

Castration versus control group

The control group consisted of a CT patient, an MRI patient and one horse that was anaesthetized for implantation of a cyclosporine implant in the eye. The control group was used to investigate the impact of general anaesthesia over time on the EQUUS-FAP and HGS. The castrated group at T=0[1] were healthy animals. As was the same situation for the CT/MRI/eye patient; they did not suffer from acute pain. The CT control patients was suspected of a primary sinusitis. For the MRI patient the diagnosis was probably a digital deep digital flexor tendinitis, together with collateral desmitis of the coffin joint. Of course, this horse had some serious lameness problems, but it was chronic pain specifically present during the locomotion of the horse. The horse that was anesthetized for implantation of a cyclosporine implant in the eye suffered from Equine Recurrent Uveitis, but at the moment of surgery the eye with the problems resided in a steady state. Thus there was not an uveitis attack present at the moment of pain scoring and the eye surgery itself is a relatively mild surgical procedure. Thus, at the T=0, the castrated group, as well as the control group, are relatively (acute) pain free.

Unfortunately, the study scores showed higher pain scores for the castrated horses in comparison to the anaesthetic control group with the EQUUS-FAP at T=0[1]. This was not the case for the HGS at T=0[1]. Explanation for this could be the fact that young stallions were more nervous in a new environment compared to the older (and most likely more experienced) horses in the control group, so they showed possibly more facial expressions, which in turn could be linked to a higher pain score. Like, for example, nostrils or eyelids opened more, and this is shown more obvious with the EQUUS-FAP. These facial expressions have nothing to do with pain, so this will give a misleading pain assessment result, which was picked up better by the HGS. This means that an attempt must be made to separate stress from pain and/or assess the different individual elements in relation to stress versus pain. This observation could also be the support for the fact that at T0[1] there is quite a big difference between the results of the EQUUS-FAP and HGS (figure 8).

At measurement point T=4 hour post procedure, there was something happening as well. For the EQUUS-FAP, the scores of the control group at this measure point were higher in comparison to the T=0[1] values (figure 4). Also at T=2 hours post procedure, the mean score of the control group was higher in comparing to the baseline (T=0[1]). At the measurement point T= 5 hour post procedure, the mean pain score was decreased below the baseline values. This suggests that probably four hours post surgery, the anaesthetics and or analgesics still has some impact on the pain score results. Perhaps it is too early to do the first pain assessment four hours post surgery. Of course, this conclusion also needs to be considered with some restraint, because only three animals in the control group remains a very limited number.

Mean scores

When comparing the T=0[1] values with the complete development of the mean scores of the castrated horses, then the T=0[1] values are quite high. As said before, the fact that in this research the T=0 pain assessment is assessed in young stallions in a new environment, the results will be distorted. The increased sympathetic tone in young inexperienced stallions allows the difference between pre and post surgery to be less evident. Within the design of this study, this incident had been predicted and some consideration was taken. The plan was to do a pain measurement at the moment of arrival of the stallions, so the day before surgery, and at the day of surgery in the morning, just before the castration. The expectation was that the second measurement would give more reliable results, because the stallions would be more confident in their new environment. But the opposite happened. During the study the second pain measurement, in the morning, turned out to give higher results than the first one. The stallions were more nervous because of the fact that they were fasted before surgery, so all the animals in the clinic got their food in the morning, but the stallions were deprived of it. Also, they need to wear a, for them, strange basket around their nose, to prevent them from eating shavings. This object makes them shake their head in some cases and makes them walking through the stable more. Eventually, during the study the decision was made to do the T=0 measuring not on arrival of the stallions or in the morning before surgery, but later on in the evening before surgery. The stallions could in this case get acclimatized more to their new environment and it does not let them suffer from the fasting stress in the morning. Even with this solution, the values at T=0 were quite high for the EQUUS-FAP, for the HGS the T=0 values stays comparably low. The reason for these quite high T=0 results for the EQUUS-FAP could be the same as before; the fact that the stallions stayed quite nervous and that this distorted the results, or maybe, after the surgery, the animals were not in any real pain, so that the contrast between pre- and post-surgery is not evident at all. The T=0 pain assessment score just after arrival of only one stallion needed to be used in this study. Of the remainder castration patients we used a T=0 pain score from an assessment in the evening.

The seven castrated animals that were used, were generally at ease with people around them. Despite this, the observers had the idea that (castrated) stallions were distracted by their environment more during the pain assessment than the mares and geldings normally do. This could be also a support for the comparably high T=0 results of the castration patients.

Future studies

To give our conclusions more value, the number of patients and control animals needs to be larger. Especially more MRI/CT patients are necessary to see if the observations with the anaesthetic control group can be confirmed. In this study the pain assessment moments were not always consistent in use. As for example we did not perform a measurement point at 6 hours post procedure for the castrated horses. Also no measurement point 8 hours post procedure for the anaesthetic control group was carried out. These for example made the results incomplete, so in future study everything needs to be tested at comparable measurement moments. Thus every patients needs to be tested pre-procedure and at least (2), 4, 5, 6 and 8 hours post procedure. It would be of great value to test horses that undergo routine castration procedures in the field practices and to try to explore the complication patients out of them.

Conclusion

The aim of this study was to investigate the possible usefulness of the EQUUS-FAP and HGS in the assessment of pain following castration in horses.

The hypothesis was that the EQUUS-FAP and the HGS are suitable (objective) scoring systems to monitor post-operative pain in horses after castration.

The results do show that the two horses which had (painful) complications were picked up clearly! So the outliers can be indicated with both pain scales. The conclusion can be made that the EQUUS-FAP and HGS are useful to follow up the castrated horses, so that in case of complications it is picked up by both scales. Also the fact that the castrated horses showed not that much pain after surgery, would suggest that the used anaesthetic and analgesic protocol at the Equine Health Department of the Faculty of Veterinary Medicine in Utrecht is of good quality.

But the number of patients and controls were limited (due to the nature of the clinic) so follow-up with more patients in the castration and control groups will be necessary. The result of this study leads to the suggestion that the EQUUS-FAP is more fitting for the assessment of pain of horses with more obvious pain. Subtle or mild pain is possibly a bit more difficult to register with the EQUUS-FAP. The differences between the outcome of this study and that of Dalla Costa et al. (2014) could possibly be attributed to the deviation in anaesthetic and analgesic protocol. It would be interesting in the future to do more research on the impact of both protocols on the outcomes of the pain assessment.

Appendix

Score form castration patient

Date:
Time:
Observer:

Patients label

Time frame:	T0 =	T1 =	T2 =
	T3 =	T4 =	T5 =
	T6 =	T 7=	T8 =

EQUUS-FAP (new)

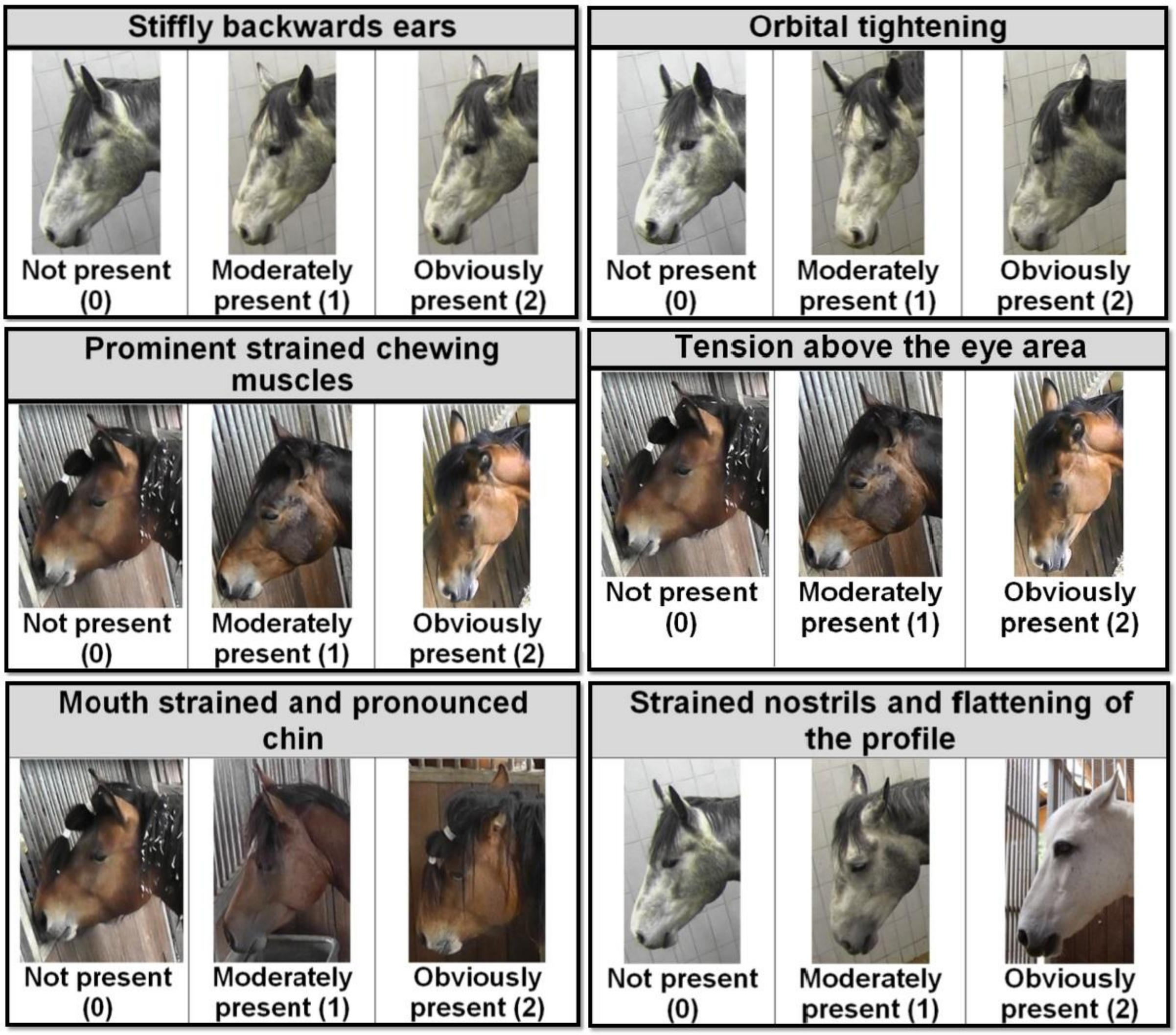
Data	Categories	Score
Head	Normal head movement/ Interested in environment Less movement No Movement or excessive movement	0 1 2
Eyelids	Opened, sclera can be seen in case of eye/head movement More opened eyes or tightening of eyelids. An edge of the sclera can be seen for 50% of the time Obviously more opened eyes or obvious tightening of eyelids. Sclera can be seen more than 50% of the time	0 1 2
Focus	Focussed on environment Less focussed on environment Not focussed on environment	0 1 2
Nostrils	Relaxed A bit more opened Obviously more opened, nostril flaring and possibly audible breathing	0 1 2
Corners mouth/ Lips	Relaxed Lifted a bit Obvious lifted	0 1 2
Muscle tone head	No fasciculation's Mild fasciculation's Obvious fasciculation's	0 1 2
Flehming and/or Yawn	Not seen Seen	0 2

Teeth grinding and / or moaning	Not been heard Heard	0 2
Ears	Position: Orientation towards sound/ clear response with both ears or ear closest to source Delayed / reduced response to sounds Position: backwards / no response to sounds	0 1 2
Startle/headshakin g	No startle/no headshaking At least one startle (a sudden abrupt movement with the head as if suddenly aware of danger) or headshaking	0 2
Total		.../20

Horse Grimace Scale (HGS)

Data	Categories	Score
Stiffly backwards ears	Not present Moderately present Obviously present	0 1 2
Orbital tightening	Not present Moderately present Obviously present	0 1 2
Tension above the eye area	Not present Moderately present Obviously present	0 1 2
Prominent strained chewing muscles	Not present Moderately present Obviously present	0 1 2
Mouth strained and pronounced chin	Not present Moderately present Obviously present	0 1 2
Strained nostrils and flattening of the profile	Not present Moderately present Obviously present	0 1 2
Total		.../12

The Facial Action Units (FACs) of the Horse Grimace Scale (Dalla Costa 2014):



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