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PROTOCOL FOR SEDATION AND ANTAGONISM (DEXMEDETOMIDINE-ATIPAMEZOLE) TO PERFORM DIAGNOSTIC ANALGESIA IN FRACTIOUS HORSES: PRELIMINARY STUDY

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INTRODUCTION

Lameness investigation in the horse consists of different parts. The first part is anamnesis followed by static and dynamic examination that sometimes includes ridden assessment.

In certain conditions, characteristic gait abnormalities allow immediate and straightforward recognition and localization of the problem. Sweeny, fibrotic myopathy, upward fixation of the patella, stringhalt, shivers, and radial nerve paresis may be some examples. However, similar gait deficits are due to a variety of lameness problems, complicating recognition and localization. A fundamental concept in lameness diagnosis is the application of diagnostic painful techniques to localize the source of pain causing lameness. The sequence of properly determining the lame leg (recognition) and then abolishing the clinical sign of lameness by use of diagnostic analgesia (localization), only to have lameness return when the local anaesthetic effects disappear, is essential for accurate diagnosis. In essence, diagnostic analgesia establishes clinical relevance, a most important concept to the lameness diagnostician. With experience and under certain circumstances, this step in lameness diagnosis can be omitted. The degree of lameness, certain gait characteristics, and palpation findings allow the clinician to strongly suspect a diagnosis. The next step may be diagnostic imaging examination (Ross 2011). However, because distinctive or peculiar signs are rare, science and proficiency in diagnostic analgesic techniques is mandatory for the lameness diagnostician.

However, in some cases, it is not possible to safely perform diagnostic analgesia due to the horse demeanor (Buchner 1999). Therefore in these cases pharmacological restrain of the horse is the only option available in order to safely and precisely perform the procedure (Barr 1997). Moreover all the different drugs used to provide an adequate degree of sedation may also providing a dose-dependent degree of ataxia (Alitalo 1986; Ricketts 1986), which can subsequently influence the final lameness assessment (Barr 1997).

Different authors have suggested a solution to overtake this problem. In a previous study Dyson and Kidd (1993) suggested that the use of xylazine should be considered in order to safely perform navicular bursa block. In the same study it was proven that there was no difference between horses that received xylazine prior performing the block and those who did not. Another study looking for the influence of a bolus of detomidine ($10\mu g/kg$) administered to horses (17) undergoing lameness investigation, showed that there was no change in the lameness scores. However, a 6% of increase in the amplitude of the stride was noticed as well as a 3% increase of the time of the stance

phase. Even though the degree of lameness was not changed these changes can affect the naked eye of the clinician and lead to a wrong clinical judgment particularly in cases of mild alteration of the gait.

The alpha-2 agonists are the family of drugs more frequently used in equine practice in order to provide an adequate degree of sedation. These molecules possess many of the qualities required for an ideal preanesthetic drug, including predictability, anxiolysis, marked sedation, stupor, and indifference to mildly painful procedures (England *et al* 1992; Daunt & Steffey 2002). They vary considerably in potency, depending on their alpha2-adrenoceptor selectivity (Virtanen *et al* 1985). Xylazine serves as the prototype and was the first alpha-2 adrenoceptor agonist approved for use in horses (Clarke & Hall 1969). The alpha 2-adrenoceptor agonists produce sedation and analgesia and potentiate the effects of other sedative-hypnotic and anaesthetic drugs by activating receptors in the locus coeruleus and spinal cord (Doze *et al* 1989) by two different mechanisms (Guo *et al* 1996). Stimulation of alpha 2-adrenoceptors in the locus coeruleus (A6 area) hyperpolarizes neurons and inhibits norepinephrine and dopamine storage and release. These effects decrease the discharge rate of locus coeruleus neurons, resulting in sedation, analgesia, and muscle relaxation. Conversely, dampening of locus coeruleus activity disinhibits activity in the adjacent cell bodies of A5 and A6 areas, resulting in increased release or norepinephrine from their terminal in the dorsal horn, which in turn produces peripheral analgesia (Gaynor Muir 2009). Alpha2-Adrenoceptor agonists decrease central nervous system (CNS) sympathetic output and peripheral sympathetic tone (Schmitt *et al* 1970). Parasympathetic (vagal) tone is initially increased as a result of transient increases in arterial blood pressure and increases in baroreceptor sensitivity (Lemke 2007). Most alpha 2-adrenoceptors agonists, especially xylazine, also produce some degree of alpha1-adrenoceptor activation, although the more selective alpha 2-adrenoceptor agonists (detomidine, medetomidine, dexmedetomidine) are relatively devoid of this effect. Interestingly, large (pharmacological) doses of alpha 2-adrenoceptor agonists, including dexmedetomidine, produce minimum sedation initially, followed by a much longer period of pronounced sedation. This response is believed to be caused by activation of CNS alpha 1-adrenoceptors, which are known to functionally antagonize the hypnotic effects of alpha 2-adrenoceptor agonists (Guo *et al* 1991). Other neuromodulators, including endogenous opioids, purines, and cannabinoids, are speculated to be important contributors to the CNS effects of alpha 2-adrenoceptor agonists and are believed to be responsible for synergistic and additive interactions when alpha 2-adrenoceptor agonists are coadministered with opioid analgesics (Tham *et al* 2005). The alpha 2-adrenoceptor agonists can be antagonized by a variety of alpha 2-adrenoceptor antagonists, including atipamezole (ATZ), tolazoline, and yohimbine (Yashamita *et al* 1996). This propriety makes alpha2-agonists the best choice for sedation and antagonism during

lameness investigation. In the past the use of ATZ has been reported after sedation with medetomidine (Virtanen 1989) and xylazine (Luna *et al* 1992). In another study 100 µg/kg of ATZ and 160 µg/kg were used in order to antagonize respectively the effect of 10 µg/kg and 20µg/kg of detomidine administered intravenously (Ramseyer *et al* 1998). In these studies was noticed a complete antagonism of the sedative effects on blood pressure, heart rate (HR) and behaviour, even though a residual sedative effect was still present. Furthermore 30 minutes after the ATZ had been administered some signs of sedation were reappearing. Due to the different results obtained, a new study was planned using previously proven scales of sedation in order to extrapolate objective data. The more selective alpha-2 agonist currently available for use in equine medicine is dexmedetomidine, the active isomer of the detomidine (racemic mixture of equal parts of two optical enantiomers, dextro and levomedetomidine). Complete antagonism after dexmedetomidine administration was obtained in people (Karrhuvaara 1991), in cats (Granholm *et al* 2006) and in dogs (Granholm *et al* 2007). These encouraging results obtained in different species made us postulate that a complete antagonism could have been possible in the horse.

As general matter, this study will be introduced by a short description of the orthopaedic examination of the horse, including history, static (conformation and palpation) and dynamic assessment (manipulation, diagnostic analgesia) and by a review of the pharmacological and clinical effects of the two drugs used (dexmedetomidine-atipamezole).

ORTHOPAEDIC EXAMINATION OF THE HORSE

Orthopaedic examination of the horse begins with collection of the **history** (*anamnesis*) from the owner and/or trainer. The importance of a detailed clinical history, cannot be overemphasized. Information is divided into two categories: basic facts necessary for every horse, and additional information from questions tailored to the specific horse. The veterinarian must understand the breed, use, and level of competition of each horse, because prognosis varies greatly among different types of sports horses. First hand experience of the particular type of sports horse being examined is useful but is not essential. Clinicians must understand the language associated with the particular sporting event, and this may be a challenge. For some sporting events, understanding the clinical history and having the ability to ask the right questions are like speaking a different language. The veterinarian must seek out as much information as possible, particularly if the problem is complex or not readily apparent. Videotapes are useful, particularly if the gait deficit, behavioural problem, or any other circumstances necessary to elicit the suspected lameness cannot be duplicated during the examination. Paraprofessionals working with the horse provide useful information, but not every- one may agree about the source of the problem, and in some instances diplomacy is key to negotiating among concerned individuals.

The second part of the lameness investigation is the **static assessment** that involves assessment of *conformation* and *palpation*.

The thought that the way a horse is *conformed* determines the way it moves is well accepted. The relationship of conformation, especially of the distal extremities, and lameness also is well recognized. "Conformation determines the shape, wear, flight of the foot, and distribution of weight"(Adams 1957). Veterinarians often are asked to comment on conformation during lameness and prepurchase examinations, especially with regard to the suitability of the horse to perform the intended task. In some instances, as in the case of presale yearling evaluations, the veterinarian's opinion is paramount, and purchase is contingent on judgment of the yearling's potential to perform as a racehorse, given its conformation, or in some instances its conformational faults. "It is by a study of conformation that we assign to a horse the particular place and purpose to which he is best adapted as a living machine and estimate his capacity for work, and the highest success in this connection will be best attained by the judicious blending of practice with science" (Belloy 1996). Evaluation of conformation and its influence on lameness is based largely on observation, experience, and pattern recognition. Recognizing desirable conformational traits in horses suited for

a particular sporting activity and learning when to overlook a minor fault that has little clinical relevance are important.

Palpation is an important part of a lameness examination. In some sports horses, it becomes more important because, for example, suspensory desmitis often is not associated with overt lameness but may compromise performance.

The veterinarian must develop a system to evaluate comprehensively all parts of the musculoskeletal system. Each forelimb should be palpated followed by neck, back, pelvic regions, and then the hindlimbs. Each limb should be assessed when bearing weight and then again with the limb elevated from the ground. Deep palpation is used to describe direct, digital palpation, with the limb in an elevated position.

If time permits, palpation should be completed before the horse is moved, because if the lame limb is identified first, the other limbs may be overlooked and compensatory problems may be missed. Comprehensive palpation may allow the clinician to make predictions about lameness, to "read" the horse. Palpation before exercise also facilitates identification of localized heat or swelling, because limb temperature increases with exercise, and swelling often decreases.

The third part of the lameness examination is constituted of assessment of ***dynamic assessment***, ***manipulation*** (when appropriate) and ***diagnostic analgesia***.

Although all parts of the lameness examination are important, the key is the determination of the limb or limbs involved. This is best performed with the horse *moving* at the trot on straight line, lunge and on different surface. Different grading systems have been proposed in order to objectively assess the degree of lameness (Tab.1 and 2).

Flexion or other *manipulative tests* often are used to induce or exacerbate lameness during lameness or prepurchase examinations.

Despite the many technological advances in equine sports medicine over the past three decades, *diagnostic analgesia* arguably remains the most valuable tool in the equine clinician's arsenal to localize the source of pain causing lameness. Although the technique requires a thorough understanding of anatomy, basic technical skill, and clinical experience, the equipment and expense are minimal. In addition, diagnostic analgesia can be performed on site, with the outcome immediately obvious.

Degree	Lameness
0	Lameness not perceptible under any circumstances.
1	Lameness is difficult to observe and is not consistently apparent, regardless of circumstances (e.g. under saddle, circling, inclines, hard surface, etc.).
2	Lameness is difficult to observe at a walk or when trotting in a straight line but consistently apparent under certain circumstances (e.g. weight-carrying, circling, inclines, hard surface, etc.).
3	Lameness is consistently observable at a trot under all circumstances.
4	Lameness is obvious at a walk.
5	Lameness produces minimal weight bearing in motion and/or at rest or a complete inability to move.

Tab. 1 – AAEP lameness assessment scale.

Degree	Lameness
0	No lameness observed
1	Lameness is difficult to observe. The lameness is inconsistent, and might only be observed while the horse is turned
2	Lameness difficult to detect at the walk or at the trot on a straight line. Constant lameness when the horse is turned or under similar 'stress'
3	Lameness difficult to detect at the walk but consistent at the trot regardless of the circumstances
4	Lameness consistent at the walk and trot regardless of the circumstances
5	Obvious lameness with marked head-nod at the trot
6	Marked head-nod at the walk, and has problems with trotting
7	Lameness is marked with minimal weight bearing. The horse is reluctant to move (the horse 'carries the leg')

Tab. 2 – Lameness grading system proposed by Lindegaard *et al* in 2010.

SEDATION OF THE HORSE

ASSESSMENT OF THE SEDATION

During the past years different ways of assessing the degree of sedation have been proposed. Those most commonly used in human medicine are three. The Bispectral Index Scale is an objective scale based on electroencephalography assessment. The Visual Analogue Scale (VAS) where a value between 0 and 10 is given (zero corresponds to the maximum degree of sedation and ten to an awake horse). The Sedation-Agitation Scale (SAS) allocates the patient to a behavioural category ranging from 1 to 7 (Riker *et al* 2001) (Tab. 3).

Score	Category	Description
1	Dangerous agitation	Pulling at endotracheal tube, trying to remove catheters, climbing over bedrail, striking at staff, thrashing side-to-side
2	Very agitated	Does not calm despite frequent verbal reminding of limits, requires physical restraints, biting endotracheal tube
3	Agitated	Anxious or mildly agitated, attempting to sit up, calms down on verbal instructions
4	Calm, cooperative	Calm, easily arousable, follows commands
5	Sedated	Difficult to arouse, awakens to verbal stimuli or gentle shaking but drifts off again, follows simple commands
6	Very sedated	Arouses to physical stimuli but does not communicate or follow commands, may move spontaneously
7	Unarousable	Minimal or no response to noxious stimuli, does not communicate or follow commands

Tab. 3 – Sedation score proposed by Riker *et al*, 2001.

This scoring system was then replaced by the Richmond Agitation-Sedation Scale (Tab. 4) with the scores ranging from -5 to +4. In this system four scores described the state of anxiety or excitement (from 1 restless to 4 aggressive) one to the normal patient (0) and 5 scores described the depth of sedation obtained (from 1 quiet to 5 unresponsive to stimuli). The assessment protocol for this system included visual assessment of the patient, assessment of the response to vocal and noxious stimuli (Rassin *et al* 2007; Khan *et al* 2012; Sessler *et al* 2002).

Score	Term	Description
+4	Combative	Overtly combative or violent; immediate danger to staff
+3	Very agitation	Pulls on or removes tube(s) or catheter(s) or has aggressive behaviour toward staff
+2	Agitated	Frequent non-purposeful movement or patient–ventilator dyssynchrony
+1	Restless	Anxious or apprehensive but movements not aggressive or vigorous
0	Alert and calm	
-1	Drowsy	Not fully alert, but has sustained (more than 10 seconds) awakening, with eye contact, to voice
-2	Light sedation	Briefly (less than 10 seconds) awakens with eye contact to voice
-3	Moderate sedation	Any movement (but no eye contact) to voice
-4	Deep sedation	No response to voice, but any movement to physical stimulation
-5	Unarousable	No response to voice or physical stimulation

1. **Procedure**
   Observe patient. Is patient alert and calm (score 0)? Does patient have behaviour that is consistent with restlessness or agitation (score 1 to 4 using the criteria listed above, under DESCRIPTION)?
2. If patient is not alert, in a loud speaking voice state patient's name and direct patient to open eyes and look at speaker. Repeat once if necessary. Can prompt patient to continue looking at speaker. Patient has eye opening and eye contact, which is sustained for more than 10 seconds (score 1). Patient has eye opening and eye contact, but this is not sustained for 10 seconds (score 2). Patient has any movement in response to voice, excluding eye contact (score 3).
3. If patient does not respond to voice, physically stimulate patient by shaking shoulder and then rubbing sternum if there is no response to shaking shoulder. Patient has any movement to physical stimulation (score 4). Patient has no response to voice or physical stimulation (score 5).

Tab. 4 - Richmond Agitation-Sedation Scale

When a horse is sedated there are different signs that can be noticed: lowering of the head, ptosis of the lower lip, ataxia, sway movement and in geldings/stallions penile exteriorization (Daunt 2002). Frequently the muscle relaxation of the nostrils and larynx produced stridor (Muir 1991).

The height of the head over the ground has previously been used as a measure of the degree of sedation after alpha-2 agonists administration (England *et al* 1992). This method allows an objective assessment of the degree of the sedation, however no importance is given to the quality of the sedation. For this reason different ways of assessing the sedation have been developed.

In another study that assessed the degree of the sedation other parameters were taken into account. These included the degree of muscle relaxation, the ataxia and the ability of completing a certain path assessing the time required to do so (Muir & Hubbell 2006). The height of the head was measured 5 min before the administration and at 5, 25, 45, 90 min after sedation with detomidine (0.02 mg/kg IV). The time required for the horses to complete a circuit with obstacles was the assessed 20 min before sedation and 10, 20, 25, 45, 60 e 90min.

NRS	Postural Instability	Ataxia during motion	Touch	Visual	Acoustic
0	No signs of instability, stable	No signs of instability	Exaggerated reaction after smooth pressing: fast movement of the leg	Undiminished response, animal moves away vigorously	Undiminished response, animal turns vigorously
1	Stable but swaying slightly	No ataxia when straight, slight ataxia when turning	Animal elevates the front leg after normal pressure	Muted response, subdued reaction and movements	Muted response, subdued reaction and movements (turns slowly)
2	Swaying clearly	Ataxia when walking straight	Slightly diminished response with normal to strong pressing	Reaction significantly subdued (elevates head slightly)	No appreciable response, but evidence of hearing (movement of ears)
3	Nearly falling down	Severe ataxia, risk of falling down	No response even with strong pressing	No signs of visual arousal	No sign of noise recognition
4	Horse falling down	Horse falling down			

Tab. 5 - Numerical rating scale (NRS) used to assess postural instability, ataxia during motion, and response to tactile, visual and acoustic stimulation in horses (Ringer *et al* 2013).

A score between 0 and 4 was therefore assigned. With system zero corresponded to a horse able to complete the circuit without any difficulties and four to horses unable to complete the task.

In other studies the heart rate, respiratory rate were also considered (Love *et al* 2011).

One of the most recent studies a VAS was used to measure the height of the head from the ground and also the degree of ataxia when standing, walking and the response after acoustic stimuli (Ringer *et al* 2013). The heart, respiratory rate and temperature were recorded the morning when the study was performed. The scores on the numeric rating scale were then assigned to each patient in the different phases of the study (Tab. 5).

After having assessed all the previous studies we deemed the study of Ringer *et al* the most appropriate in order to evaluate the degree of sedation in the present study.

ALPHA 2-AGONISTS

Alpha-2 agonists, such as xylazine, clonidine, romifidine, detomidine, medetomidine, and dexmedetomidine, are potent analgesic drugs that also induce physiologic and behavioural changes, such as hypertension, bradycardia, atrioventricular block, excessive sedation and ataxia, all of which can potentially limit their systemic use as analgesics in some clinical cases. Therefore it is of utmost importance to know the individual properties of each of the alpha-2 agonists to select the ideal drug for each clinical condition, based on the duration of action of analgesia and behavioural changes.

Since the last review on this subject (Daunt & Steffey 2002), the use of medetomidine and dexmedetomidine has been introduced for equine anaesthesia/analgesia, and although not approved in this species, their increased specificity for alpha-2 receptors may offer some potential advantages over the traditional alpha-2 agonists. Similarly, other routes of administration and benefits of alpha-2 agonists are recognized in the human and laboratory animal literature, which may prove useful in the equine patient if validated in the near future.

Fig. 1 - Adrenergic receptors are subdivided into alpha and beta. Both alpha and beta are further classified into alpha-1, alpha-2, beta-1, and beta-2. Alpha receptors are located post- synaptically (alpha-1 and alpha-2) and presynaptically (alpha-2) at sympathetic neuroeffector junctions of many organs.
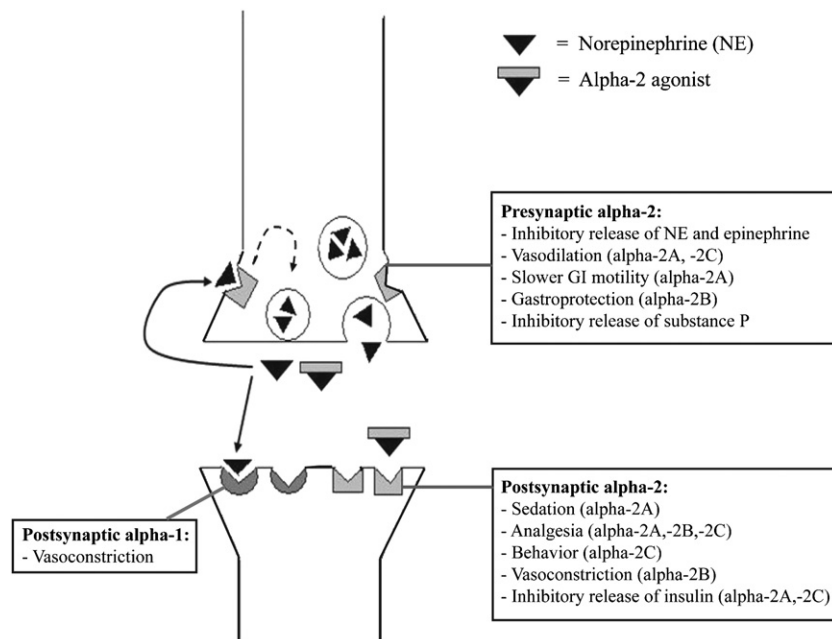
Adrenergic receptors are subdivided into alpha and beta. Both alpha and beta are further classified into alpha-1, alpha-2, beta-1, and beta-2. Alpha receptors are located postsynaptically (alpha-1 and alpha-2) and presynaptically (alpha-2) at sympathetic neuroeffector junctions of many organs (Fig.

1). Beta receptors are located postsynaptically and in general mediate decreased activity of the effector cells (beta-2: vasodilation, bronchodilation, uterine relaxation) or increased activity (beta-1: heart automaticity and contractility). Postsynaptic activation of alpha receptors mediate increased activity of the effector cells.

Activation of the presynaptic alpha-2 receptor inhibits the release of norepinephrine (NE) into the synaptic cleft and autoregulates its actions on the effector cells. The net effect of activation of alpha-2 adrenergic receptors is modulation of sympathetic nervous system activity by inhibition of NE. Manifestations of this response include decreased cardiac output due to decreased inotropy and decreased heart rate, as well as a reduction in systemic vascular resistance. Conversely, the activation of post-synaptic alpha receptors mediates an increase in systemic vascular resistance. Actions at centrally located alpha-2 adrenergic receptors mediate sedation, anxiolysis, analgesia, and hypnosis. It has also been shown that the alpha-1 agonistic activity can reduce the alpha-2 mediated analgesia, and it has been suggested that coadministration of an alpha-1 antagonist (prazosin) with the alpha-2 agonist may enhance analgesic potency (Gil *et al* 2009). The alpha-2/alpha-1 selectivity for alpha-2 agonists is 160 for xylazine, 220 for clonidine, 260 for detomidine, and 1620 for medetomidine or dexmedetomidine (Tab. 6) (Virtanen *et al* 1988), and is unknown for romifidine but higher than for xylazine. As selectivity for alpha-2 receptors increases, the greater specificity results in higher potency, especially at central alpha-2 adrenoceptors. Medetomidine is considered 10 times, 7 times, and 6 times more potent than xylazine, clonidine, and detomidine, respectively (Virtanen & MacDonald 1985). However, other non-adrenergic mechanisms are probably implicated in analgesic actions of alpha-2 agonists because the rank potency order of spinal depressant actions on in vitro preparations is the same as their rank analgesic potencies in vivo: dexmedetomidine (medetomidine) > clonidine > detomidine > xylazine (Faber *et al* 1998), which does not coincide precisely with alpha-2 agonists of intermediate specificity. It has also been suggested that differences between the actions of these ligands are attributable to an action at alpha-1 receptors.

Box 10–2 α_2:α_1-Selectivity	
Drug	α_2:α_1
Xylazine	160:1
Detomidine	260:1
Medetomidine	1620:1
Romifidine	340:1
Clonidine	220:1

Tab. 6 – Alpha 2: Alpha 1 Selectivity

Alpha-2 agonists activate 3 distinct subtypes of alpha-2 adrenergic receptors: alpha-2A, alpha-2B, and alpha-2C. Despite the higher affinity of detomidine and medetomidine for the alpha-2 receptors compared with xylazine, the former drugs cannot discriminate between the different receptor subtypes (Schwartz & Clark 1998) . The alpha-2A adrenergic receptor is the primary mediator involved in alpha-adrenergic spinal analgesia for endogenous NE as well as exogenous adrenergic agonists (Stone *et al* 1997). These receptors are G-protein–coupled receptors that decrease neuronal excitation by several mechanisms, including opening of rectifying potassium channels, decrease of presynaptic calcium influx, and inhibition of adenylyl cyclase (Stone *et al* 1997). The activation of alpha-2 receptors by an agonist induces the receptor to interact with a Gi type of G protein and inhibits the actions of adenylyl cyclase, decreasing the synthesis of cyclic adenosine mono-phosphate from adenosine triphosphate. Besides analgesia, the alpha-2A receptor promotes hypnosis, sedation, inhibition of insulin secretion, neuroprotection, and sympatholysis (Fagerholm *et al* 2008). Alpha-2B receptors are involved in spinal analgesia and vasoconstriction of peripheral arteries (Philipp *et al* 2002). Alpha-2C receptors are involved in pain modulation, mood- and stimulant-induced locomotor activity, regulation of epinephrine outflow from the adrenal medulla, and modulation of cognition.

Receptor type and location	Function
α_1	
Central nervous system	Increase awareness and activity
Heart	Increase force of contraction and sensitization of the myocardium to catecholamines during halothane anesthesia
Smooth muscle	Vasoconstriction
Liver	Glycogenolysis; gluconeogenesis
α_2	
Central nervous system	Decreases norepinephrine and dopamine release causing sedation and cardiopulmonary depression
Sympathetic nerve terminal	Inhibition of norepinephrine release
Cholinergic neurons	Inhibition
Heart	Decrease norepinephrine release
Smooth muscle	Vasoconstriction
Gut	Reduced tone and propulsive activity
Pancreatic islet cells	Inhibition of insulin release
Platelets	Aggregation
Fat	Inhibition of lipolysis (clonidine)

Tab. 7 – Location and function of alpha-$_1$ and -$_2$ adrenoceptors.

Alpha-2 agonists administered intravenously (IV) in clinical doses have elimination half-lives of less than 1.5 hours and relatively small volumes of distribution of 0.5 to 1.6 L/kg. Elimination half-lives are slightly longer when administered intramuscularly (IM) (Grimsroad *et al* 2009).

Based on similar sedative effects, equipotent IV doses of these drugs are: 5 to 10 mg/kg of medetomidine, 3.5 mg/kg of dexmedetomidine, 20 to 40 mg/kg of detomidine, 80 to 120 mg/kg of romifidine, 1 mg/kg of xylazine, and 25 mg/kg of clonidine.

The analgesic effects of alpha-2 agonists result from spinal and supraspinal actions (Tab. 7). Alpha-2 receptors are found on primary afferent terminals in peripheral and spinal nerve endings, at the level of the superficial laminae of the dorsal horn of the spinal cord, and centrally in the brainstem. Therefore, administration of alpha-2 agonists in any of these locations offers the possibility of analgesic actions, which may also be synergistic with other groups of analgesic drugs. There is good evidence for primary analgesic actions as well as opioid-sparing effects; for example, epidural coadministration of alpha-2 agonists in combination with opioids contribute to the potency and efficacy of opioids and allow for administration of lower doses to achieve analgesia (Stone *et al* 1997).

In the horse, systemic and epidural administration is currently the most common routes for alpha-2 agonists (Table 2), but in humans the intra-articular, intercostal infiltration, and intravenous regional routes have also been used (Al-Metwalli *et al* 2008).

The most commonly recognized cardiovascular effects of alpha-2 agonists are a decrease in heart rate, an initial increase in systemic vascular resistance and blood pressure followed by a decrease, an initial decrease in cardiac output and respiratory rate followed by recovery to baseline, and transient decreases in PaO2 (Bettschart-Wolfensberger *et al* 2005). The biphasic effects on blood pressure are caused by initial increases in vascular resistance from postsynaptic alpha-2B receptor stimulation that induces hypertension, followed by decreased sympathetic discharge from presynaptic alpha-2A receptor stimulation that decreases NE release and presynaptic alpha-2C receptor stimulation that decreases epinephrine release from the adrenal glands, resulting in a decrease in vascular resistance and blood pressure (Knaus 2007). The use of higher doses of alpha-2 agonists results in a more prolonged increase in vascular resistance and as a result cardiac output can be more adversely affected, resulting in hypotension despite the increased vascular resistance.

Reported sedative effects of alpha-2 agonists in horses include decreased awareness, ptosis of the head, lower lip, and eyelids, ataxia, and a wide stance, and are mediated through alpha-2A receptors (Knaus 2007).

Both sedative and physiologic effects of alpha-2 agonists have been correlated with plasma concentrations, and have been shown to be of greater magnitude and observed earlier after IV than

after IM administration, although the duration of these changes may be shorter after IV administration (Mama *et al* 2009). It is not clear, however, whether the analgesic effects endure and correlate with the duration of the sedative and physiologic changes. Horses administered clonidine (25 mg/kg, IV) showed peak sedative and analgesic effects at 20 minutes and 30 minutes, respectively. Analgesia was assessed using a heat source to elicit hoof withdrawal reflexes and, despite lowering of the head associated with the sedation for up to 2.5 to 3 hours, significant analgesia was present for a shorter period (45 minutes) and was only considered submaximal at peak effect (Dirikolu *et al* 2006). In another study using a model of nociceptive withdrawal reflex (NWR) that limits the influence of the animal's behaviour over analgesia, administration of equipotent IV doses of xylazine (1 mg/kg), detomidine (20 mg/kg), or romifidine (80 mg/kg) resulted in similar increases in thresholds to elicit the NWR in hind- and forelimbs and therefore equipotent analgesic effects (Rohrbach *et al* 2009). Analgesia peaked at 10 to 40 minutes after injection (first for xylazine and last for romifidine) and lasted 60 minutes for xylazine and 90 minutes for detomidine, and was still present at 120 minutes for romifidine.34 Of note, sedation was significant and present for the duration of action of analgesia for xylazine and detomidine, but shorter (90 minutes) for romifidine (Rohrbach *et al* 2009). It seems from these studies that sedation and analgesia do not correlate, because one study reports shorter duration of analgesia than sedation20 and another study reports similar or more prolonged analgesia than sedation (Rohrbach *et al* 2009). Differences could be due to the properties of the individual alpha-2 agonist, the pain model used and site of nociceptive stimulation, stimulation that decreases NE release and presynaptic alpha-2C receptor stimulation that decreases epinephrine release from the adrenal glands, resulting in a decrease in vascular resistance and blood pressure. The use of higher doses of alpha-2 agonists results in a more prolonged increase in vascular resistance and as a result cardiac output can be more adversely affected, resulting in hypotension despite the increased vascular resistance.

Reported sedative effects of alpha-2 agonists in horses include decreased awareness, ptosis of the head, lower lip, and eyelids, ataxia, and a wide stance, and are mediated through alpha-2A receptors.

Both sedative and physiologic effects of alpha-2 agonists have been correlated with plasma concentrations, and have been shown to be of greater magnitude and observed earlier after IV than after IM administration (Mama *et al* 2009). It is not clear, however, whether the analgesic effects endure and correlate with the duration of the sedative and physiologic changes. Horses administered clonidine (25 mg/kg, IV) showed peak sedative and analgesic effects at 20 minutes and 30 minutes,

respectively. Analgesia was assessed using a heat source to elicit hoof withdrawal reflexes and, despite lowering of the head associated with the sedation for up to 2.5 to 3 hours, significant analgesia was present for a shorter period (45 minutes) and was only considered submaximal at peak effect (Dirikolu et al 2006). In another study using a model of nociceptive withdrawal reflex (NWR) that limits the influence of the animal's behaviour over analgesia, administration of equipotent IV doses of xylazine (1 mg/kg), detomidine (20 mg/kg), or romifidine (80 mg/kg) resulted in similar increases in thresholds to elicit the NWR in hind- and forelimbs and therefore equipotent analgesic effects (Rohrbach et al 2009). Analgesia peaked at 10 to 40 minutes after injection (first for xylazine and last for romifidine) and lasted 60 minutes for xylazine and 90 minutes for detomidine, and was still present at 120 minutes for romifidine (Rohrbach et al 2009). Of note, sedation was significant and present for the duration of action of analgesia for xylazine and detomidine, but shorter (90 minutes) for romifidine (Rohrbach et al 2009). It seems from these studies that sedation and analgesia do not correlate, because one study reports shorter duration of analgesia than sedation20 and another study reports similar or more prolonged analgesia than sedation (Rohrbach et al 2009). Differences could be due to the properties of the individual alpha-2 agonist, the pain model used and site of nociceptive stimulation, alpha-2B receptors induces a gastroprotective effect by regulating gastric acid secretion and by inhibiting chemically (including NSAIDs) and physically (stress) induced gastric mucosal lesions (Gyires et al 2009). These benefits have not been evaluated in horses, and may prove useful in the colic horse that requires analgesia and has the predisposition for ulceration during hospitalization.

Other considerations in horses administered alpha-2 agonists include the effects of these drugs on urine output. Alpha-2 agonists administered for sedation or during anaesthesia increase urine production, due to hyperglycemia from hypoinsulinemia (Valverde et al 2010) mediated through alpha-2A and -2C receptors (Peterhoff et al 2003), and due to a reduced secretion rate of arginine vasopressin (Alexander & Ivrine 2000). Patients should be monitored for urine output, hydration status, and comfort to avoid excessive bladder distension.

Equipotent sedative doses of xylazine, romifidine, and detomidine increase intrauterine pressure for up to 30 minutes, as a result of increased uterine contractions. Electrical activity of the myometrium in the last trimester was observed to increase shortly (3–5 minutes) after IM injection of 20 or 40 mg/kg of detomidine, but with no effect after 60 mg/kg, and lasted for 50 to 70 minutes without any adverse effects, and is therefore considered safe. In a study in which 8 mares during 10 pregnancies were administered detomidine (20 mg/kg, IV) once a week from days 14 to 60 of pregnancy and thereafter once a month until parturition, no side effects could be associated with administration, although 1 case of abortion was reported at 167 days. In a similar study, detomidine (15 mg/kg)

administered to pregnant mares at 3-week intervals during the last trimester of pregnancy to assess maternal and fetal electrocardiographs showed reductions in heart rate in both, but alterations in conduction were detected only in mares, and there were no adverse outcomes of pregnancy. Another study has also demonstrated that despite systemic cardiovascular effects associated with alpha-2 agonists, it appears that based on colour Doppler ultrasonographic examinations local uterine and ovarian perfusion is not affected by detomidine or xylazine (Araujo & Ginther 2009) view of these findings, alpha-2 agonists are safe in pregnant animals, but close monitoring is advised.

ANTAGONISM OF ALPHA-2 ANTAGONIST AGENTS

Even though alpha-2 agonists are safe drugs to use to sedate horses they still carry undesirable effects. These include ataxia, reduced gastrointestinal motility, hyperglycaemia, diuresis, increased uterine tone, sweating, muscle tremors, hypersalivation, penile protrusion (England & Clarke 1996) and tachypnoea in pyrexic horses (Kendall *et al* 2010).

For this reason in past twenty years different drugs have been studied in order to antagonise these adverse effects. The decision to reverse the undesirable effects of α2-agonists could be based on an emergency situation, such as a horse collapsing or accidental overdose or on non-emergency clinically relevant situations such as a need to decrease the degree of ataxia.

Tolazoline, yohimbine and ATZ (both central and peripheral acting α2-antagonists) are available in most countries and have been used to antagonise the α2-agonists in horses (Knych & Stanley 2014). Only tolazoline has been approved by the Food and Drug Administration (FDA) in the United States of America for reversal of xylazine effects in horses (Casbeer & Knych 2013). The Association of Racing Commissioners International (ARCI) (DiMaio Knych et al 2011a) classifies yohimbine as a Class 2 foreign substance. These pointers are important examples of legal considerations the veterinarian should keep in mind when considering administration of an α2-antagonist to a horse. Veterinarians are encouraged to review the legal aspects of drug administration and rules set down by various associations regulating equine related competitions within their country of practice. Despite the highly prevalent off-label use of α2-antagonists in horses, there is a growing body of evidence to demystify their clinical effects as observed in horses.

Tolazoline is a synthetic imidazoline derivative that antagonises α-adrenoceptors non-selectively (Casbeer & Knych 2013) and possesses histaminergic and cholinergic effects (El-Kammar & Gad 2014). Tolazoline administered IV at 4 mg/kg bwt alone has the following pharmacokinetic profile: systemic clearance, steady state volume of distribution and terminal elimination half-life of 0.82 ± 0.18 l/h/kg, 1.68 ± 0.38 l/kg and 2.69 ± 0.21 h, respectively (Casbeer & Knych 2013). The pharmacodynamic profile when tolazoline is administered alone demonstrates minimal behavioural responses; a decreased heart rate (most likely due to the increased sympathetic tone raising blood pressure and stimulating a baroreceptor mediated increase in vagal tone) with second degree atrioventricular blocks; an increase in blood glucose level and packed cell volume (increased sympathetic tone causing splenic contraction) and unchanged head-height-above-ground (measurement used to determine the level of sedation in horses) compared to baseline readings (Casbeer and Knych 2013). Tolazoline, in isoflurane-anaesthetised (Matthews *et al* 1995) or awake sedated ponies (Carroll *et al.* 1997), is effective in transiently antagonising detomidine-induced

bradycardia without inducing arrhythmias or significant alterations to blood pressure. However, Matthews *et al* (1995) reported a significant decrease in the arterial partial pressure of oxygen; thus this must be considered when reversing detomidine- induced bradycardia with tolazoline in isoflurane-anaesthetised patients. Detomidine-induced sedation (assessed by measuring head-height-above-ground that returns to baseline readings) is effectively antagonised with tolazoline (Carroll *et al* 1997). Transient sweating, hypersalivation and gingival hyperaemia may be noticed without overt signs of excitement. Blood glucose, cortisol and free fatty acid levels increase after administering tolazoline which suggests activation of a stress response. This is a clinically significant finding and antagonism of an α2-agonist with tolazoline may perpetuate an inappropriate stress response in horses that have undergone a surgical procedure or are in pain for some other reason (Carroll *et al* 1997). Stress, in the presence of pain, could culminate in a distressed patient which causes significant metabolic derangements not conducive to convalescence (Gaynor & Muir 2009).

Yohimbine is an indole alkaloid derived from several biological sources (bark of the *Pausinystalia yohimbe* tree and root of the *Rauwolfia serpentina* plant) that nonselectively antagonises α-adrenoceptors in the horse (DiMaio Knych *et al* 2011a,b). Yohimbine has been shown at high doses to activate other receptor types including α1-adrenoceptors, dopamine and serotonin receptors and to inhibit monoamine oxidase (DiMaio Knych *et al* 2011b). Yohimbine administered at 0.12 mg/kg bwt IV alone has the following pharmacokinetic profile: systemic clearance, steady state volume of distribution and terminal elimination half-life of 13.6 ± 2.0 ml/min/kg, 3.2 ± 1.1 l/kg and 4.4 ± 0.9 h, respectively, for a 2 compartment model (DiMaio Knych *et al* 2011a). Higher IV doses of yohimbine (0.2 and 0.4 mg/kg bwt) are associated with an increase in the plasma concentration that decreases in a dose-dependent manner, an increased volume of distribution and slower clearance. The elimination half-life remains consistent (DiMaio Knych *et al* 2011b). Detomidine increases the plasma concentration and decreases the clearance and volume of distribution of yohimbine (Knych *et al* 2012). This is clinically significant especially in racehorses where yohimbine is listed as an ARCI Class 2 foreign substance (DiMaio Knych *et al* 2011a); veterinarians would need to allow longe withdrawal periods. Pharmacodynamic effects of yohimbine alone are highly variable among horses, regardless of the dose administered (DiMaio Knych *et al* 2011b). Sedation or excitation (some horses may demonstrate rearing, striking, running in circles, generalised muscle tremors, agitation and exaggerated but mild excitation in horses exposed to sudden environmental stimulation) are typical pharmacodynamic effects that can be expected and usually resolve within 1–2 h after yohimbine administration (DiMaio Knych *et al* 2011b). Transient cardiovascular and gastrointestinal effects such as an increase in heart rate with disappearance of atrioventricular

blocks and an increase in borborygmi, respectively, may be present post yohimbine administration (DiMaio Knych *et al* 2011b). Yohimbine antagonism of detomidine has been studied in horses (DiMaio Knych *et al* 2012). Yohimbine is effective in antagonising detomidine- induced sedation, bradycardia and atrioventricular blocks. However, the pharmacodynamic response is variable among horses which include transient (approximately 10 min) vocalisation, increased arousal and return to normoglycaemia after detomidine-induced hyperglycaemia (DiMaio Knych *et al* 2012).

There is no therapeutic indication or justification to use tolazoline (Casbeer & Knych 2013) or yohimbine (DiMaio Knych *et al* 2011b) as a sole agent to treat unsedated horses, due to their unpredictable pharmacodynamic effects.

Two or more α2-adrenoceptor antagonists have been compared in a single study to antagonise α2-agonists. The antagonistic properties of tolazoline and ATZ have been compared in detomidine-sedated horses (Hubbell & Muir 2006) and donkeys (El-Kammar and Gad 2014). Both agents produce partial antagonism of detomidine-induced sedation and bradycardia. However, tolazoline antagonises detomidine-induced sedation more completely and hastens recovery compared to ATZ. The clinical effects of tolazoline last longer compared to ATZ in detomidine treated horses (Hubbell & Muir 2006; El-Kammar & Gad 2014). Apprehension may be anticipated in tolazoline-treated horses while ATZ does not seem to demonstrate this clinical effect (Hubbell & Muir 2006). Knych and Stanley (2014) have recently reported on the antagonistic effects of tolazoline, yohimbine and ATZ on sublingually administered detomidine. Neither tolazoline nor Yohimbine, is able to completely return the head-height-above-ground measurements, despite the effect being short-lived (5 min). Yohimbine transiently antagonises detomidine-induced bradycardia to baseline values (pre detomidine administration), while tolazoline demonstrates a slight transient increase in heart rate above baseline values. ATZ mildly increases the heart rate without returning it to baseline. Tolazoline increased the packed cell volume, unlike yohimbine and ATZ which had no effect on it. The detomidine-induced hyperglycaemia is antagonised by yohimbine (return to baseline values), worsened by tolazoline (increase in hyperglycaemia) and ATZ demonstrates no effect on blood glucose level (Knych & Stanley 2014). The antagonistic properties of tolazoline and yohimbine have been compared in xylazine sedated horses (Kollias-Baker *et al* 1993). Yohimbine and tolazoline successfully antagonise xylazine-induced bradycardia and sedation. However, yohimbine has a quicker onset and shorter duration of action compared with tolazoline. Yohimbine is also associated with tremors, agitation and excitement, while tolazoline does not demonstrate these behavioural signs in xylazine treated horses. Tolazoline results in a normalisation of the heart rate to baseline readings but is associated with systemic hypertension in the presence of xylazine-induced bradycardia (Kollias-Baker *et al* 1993).

DEXMEDETOMIDINE

Dexmedetomidine is considered to have greater selectivity for the alpha-2 receptor compared with its racemate (Aantaa *et al* 1993) and twice the potency of medetomidine (Savola & Virtanen, 1991; Bettschart-Wolfensberger *et al* 1999a,b; Kuusela *et al* 2000). While dexmedetomidine is associated with potent sedative and analgesic effects, levomedetomidine has been shown to potentiate the bradycardia as well as reduce the magnitude of sedative and analgesic effects from dexmedetomidine but only when levomedetomidine is administered at high doses (Kuusela *et al* 2001). The analgesic effects of dexmedetomidine have not been studied in the horse. A study performed in dogs reports that the analgesia produced by intra-venous dexmedetomidine administration (20 lg/kg) is of similar magnitude but of longer duration (40 min) compared with the one obtained with an equipotent dose of medetomidine (Kuusela *et al* 2000). On the other hand, a study in cats (Slingsby & Taylor 2008) reported that only high doses of dexmedetomidine (40 lg/kg) produced thermal antinociceptive effects, which peaked at 30 min and quickly returned to baseline. A short-lived period of analgesia was also reported in another study after a similar dose of dexmedetomidine was administered to cats (Slingsby *et al* 2010). Recently, a continuous infusion of dexmedetomidine was shown to decrease the minimum alveolar concentration of sevoflurane in ponies by 53% (Gozalo-Marcilla *et al* 2012).

In a study performed in ponies, the cardiovascular effects of dexmedetomidine were considered to be somewhat similar to those caused by other a alpha-2 receptor agonists, but of very short duration. No bradycardia was observed, and stroke volume was reduced only for the first 5 min (Bettschart-Wolfensberger *et al* 2005). This same study also evaluated the pharmacokinetics of dexmedetomidine in ponies and demonstrated a rapid decrease in the drug's plasma concentration, which may favour its use for short procedures as well as a continuous infusion for standing sedation or as an adjunct during general anaesthesia.

The characteristics of dexmedetomidine indicate that the drug has important potential for use in equine anaesthesia; however, the knowledge of its pharmacokinetic and pharmacodynamic effects in the horse is limited.

ATIPAMEZOLE

ATZ is a potent, highly selective α2-adrenoceptor antagonist (Ramseyer et al 1998). There is a lack of pharmacokinetic and pharmacodynamics studies based on ATZ administered alone in horses. ATZ antagonism of detomidine-induced sedation (Ramseyer et al 1998) and bradycardia (Raekallio et al 1990) have been studied in horses. ATZ results in satisfactory but incomplete reversal of detomidine-induced sedation (Raekallio et al 1990; Ramseyer et al 1998). However, the level of ataxia is reduced significantly this allows the horse to ambulate properly (Ramseyer et al 1998). ATZ demonstrates a dose dependant increase in undesirable effects such as sweating, hyperexcitability in horses subjected to external stimuli and head shaking that tended to resolve within 10–15 min of injection in detomidine treated horses. Visual signs of arousal are apparent within 2min of IV administration (Ramseyer et al 1998). ATZ transiently antagonises detomidine-induced bradycardia, decreases the frequency of atrioventricular blocks and normalises the arterial blood pressure (Raekallio et al 1990). ATZ effectively antagonises medetomidine constant rate infusion-induced sedation without causing untoward excitement. However, within 2 min of injection, the horse or pony should raise their head, become alert and may even demonstrate signs of shivering and intolerance to surgical drapes or physiological parameter (electrocardiogram leads, noninvasive blood pressure cuffs, etc) monitoring instrumentation (Bettschart-Wolfensberger et al 1999). ATZ also antagonises medetomidine (Yamashita et al 1996) or xylazine- (Luna et al 1992) induced sedation after a single IV bolus with no apparent undesirable effects.

A novel peripheral α2-adrenoceptor antagonist known as MK-467 (previously L-659'066) has been studied on detomidine sedated horses (Vainionpää et al 2013). MK-467 prevents detomidine-induced bradycardia and gastrointestinal hypomotility without affecting the quality of sedation. In dogs, MK-467 has been investigated more comprehensively and demonstrates cardiovascular sparing effects without compromising on the level of sedation or general anaesthesia (Honkavaara et al 2011; Rolfe et al 2012; Salla et al 2014). Dexmedetomidine induced alterations in glucose, insulin, free fatty acids and lactate levels are attenuated by MK-467 (Restitutti et al 2012).

AIM OF THE STUDY

It is not uncommon for equine clinicians to deal with fractious horses in their everyday practice. A recent survey conducted by BEVA shows that equine veterinarians in the UK carry the highest risk of injury of any civilian occupation (BEVA 2014).

Even though in the majority of the cases physical restrain of the patient is enough to allow a safe execution of the diagnostic analgesia, sometimes this is not enough. In those circumstances performing a nerve block can become a very dangerous procedure for both the patient and the operators/owner. This is exacerbated when the horse is assessed in an unfamiliar environment such as in a veterinary hospital. The lameness investigation can produce a great amount of stress in the patient that could lead to uncontrolled reactions with the potential for personnel to get seriously injured. So far the use of chemical restraint has always been discouraged because of the undesired effects produced by sedative drugs such as ataxia and sedation.

The aim of the present study was to analyse the efficacy and applicability of a sedation protocol with DEX and antagonism with ATZ for use in clinical settings when performing diagnostic analgesia in horses.

MATERIALS AND METHODS

In order to perform this study six healthy horses were recruited (3 males and 3 females) of different breeds (one Arab, two Sella Italiano and three sports horses). The average age was 11.5 ± 6.68 (average ± standard deviation) (range 5-20 year old) and the body weight 455± 67.9 kg (range 366-561 kg).

During the period of the study the horses were stabled in boxes with shavings and fed hay with a minimal grains intake and water ad lib.

Before performing the study each horse was weighted, and clinically examined with particular attention to heart rate (HR), respiratory rate (RR) (lungs sounds were listened too), mucous membranes colour (MM), capillary refill time (CRT), hydration status and rectal temperature (T°).

The main reference for the assessment of the degree of sedation was the height of the lower lip from the ground. During the days preceding the study the height of the lower lip from the ground was measured under normal conditions with the horse conscious. The values obtained were divided by 10 and 3 reference values were marked on the wall of the stable as a reference. The reference height of the head was marked as well as two other values indicating a 10% increase and reduction of the resting value. These two parameters were used to ascertain that the value obtained was representative of the single horse.

The horse was further monitored to make sure that the height of the lip was constantly staying between the marked ranges. Every horse has then been moved in the hallway were a graduated scale corresponding to the one drawn in the stable had previously been drawn on the wall.

The horses were then sedated with DEX IV. The administered dose was adjusted depending on the degree of awareness and behaviour at the time of sedation (range 2.5 to 5µg/kg).

Between 5 and 10 minutes after the sedation was administered, the diagnostic block was performed. This consisted of high plantar nerve block (1 horse) bilateral lateral plantar nerve block in 2 horses and dorsal spinous processes anaesthesia in the other 3 cases.

The local anaesthetic used for all the nerve blocks mepivacaine 2% (Intra epicaine[®]).

For the high plantar nerve block the leg was left weight bearing and two 21G 4cm needles were used (one for each side). The volume of local anaesthetic used was 10 mls in total.

For the lateral plantar nerve block, the limb was positioned on the operator's leg and a using a 23 G x2.5 cm needle used. The volume of local anaesthetic used was 1 ml.

For the block of the interspinous space, a 19 G x 9 cm needle was used. The volume of local anaesthetic used was 5 mls for each interspinous space involved.

The same operator performed the diagnostic analgesia in all the cases.

After 20 minutes the antagonist was administered with an equal volume (ATZ 50 μg/kg IV) to the one of DEX administered.

The following clinical parameters were considered: HR, RR, MM, CRT and quality of the peripheral pulse. Every adverse effect (i.e. arrhythmia) presenting after the administration of DEX was recorded. Degree of stability, ataxia, response to tactile, visual and acoustic stimuli was recorded and used to assess the degree of sedation together with the height of the lip from the ground. The response to the touch of the ear was also assessed.

The assessments were performed at 3, 5, 10 and 15 min after the administration of DEX and 3, 5, 10, 15 min after the administration of atipamezole.

The HR was assessed with a stethoscope whereas the RR was assessed counting the number of excursions of the thorax.

The quality of the pulse was assessed (always by the same operator) at the level of the facial artery.

The degree of sedation was assessed using the VAS previously described by Ringer *et al* 2013. The visual scale was chosen to assess the degree of sedation with a single value that was always given by the same operator. Whereas previously trained operators recorded the NRS scale values.

An umbrella was used in order to assess the response to visual stimuli. This was suddenly opened in front of the animal. The acoustic response was assessed as response to the operator clapping their hands. Response to tactile stimuli was detected by applying increasing pressure to the heel bulb region with a blunt instrument (before the blocks were performed).

Twenty minutes after ATZ administration and after all the previous parameters were collected, lameness investigation was continued. Any adverse reaction or relevant abnormality was recorded. Lameness investigation was performed by the same operator in all cases.

A template of the assessment sheets is reported in the following pages.

SEDATION sheet   HORSE N.

Administered drug: **dose**

Physiologic paramenters

Time	3min	5min	10min	15min	20min
HR					
RR					
Mucous					
Quality of pulse					
Detectable arrhythmias					
Other notes					

Sedation score

Time	3min		5min		10min		15min		20min	
	NRS	VAS	NRS	VAS	NRS	VAS	NRS	VAS	NRS	VAS
Standing ataxia										
Ataxia on movement										
Reaction to touch										
Touch of the ear										
Reaction to visible stimulae										
Reaction to audible stimulae										

Height head from the floor					

Fig. 1 – Positioning of the scale in the stable, in order to assess the basal value.

Fig. 2 – Positioning of the animal next to the sedation scale before administering the sedation.

Fig. 3 – Picture showing the skin sensitivity being tested.

Fig. 4 – Picture showing the height of the lip from the floor after the sedation.

STATISTIC ANALYSIS

The recorded values were analysed with the t-test for paired samples (clinical parameters and height of the head) and non-parametric test of Wilcoxon was used for subjective parameters. Values were considered significant if p was less then 0.05 and highly significant if p less than 0.005.

RESULTS

CLINICAL PARAMETERS

In the present study HR and RR were subjected to statistic assessment, whereas MM, CRT, quality of the pulse and presence of arrhythmia were subjected to clinical evaluation.

The results for the recorded parameters are reported in the following tables.

HEART RATE

Tab. Heart Rate								
Horse	DEX 3min	DEX 5min	DEX 10min	DEX 15min	ATZ 3min	ATZ 5min	ATZ 10min	ATZ 15min
1	32	32	32	32	32	32	32	36
2	20	20	24	24	28	28	26	26
3	28	32	28	28	28	32	28	32
4	28	24	24	24	32	28	24	24
5	24	24	28	32	32	28	28	28
6	16	28	28	20	20	20	24	24

Statistic for paired samples

		Media	N	Standard deviation	Average SD error
Couple N 1	DEX3min	24,667	6	5,8878	2,4037
	ATZ3min	28,667	6	4,6762	1,9090
Couple N 2	DEX5min	26,667	6	4,8442	1,9777
	ATZ5min	28,000	6	4,3818	1,7889
Couple N 3	DEX10min	27,333	6	3,0111	1,2293
	ATZ10min	27,000	6	3,0332	1,2383
Couple N 4	DEX15min	26,667	6	4,8442	1,9777
	ATZ15min	28,333	6	4,8028	1,9607

Test for paired samples

		Difference between couples							
		Average	SD	Average SD error	Inf	Sup	t	df	Sig. (2-code)
					Confidence interval for 95% difference				
Couple 1	DEX3min - ATZ3min	-4,0000	3,5777	1,4606	-7,7546	-,2454	-2,739	5	**,041**
Couple 2	DEX5min - ATZ5min	-1,3333	5,4650	2,2311	-7,0685	4,4019	-,598	5	,576
Couple 3	DEX10min - ATZ10min	,3333	1,9664	,8028	-1,7303	2,3969	,415	5	,695
Couple 4	DEX15min - ATZ15min	-1,6667	3,2042	1,3081	-5,0292	1,6959	-1,274	5	,259

It is possible to appreciate a statically significant difference between the group DEX and ATZ at 3 min. Higher values are recorded for ATZ at 3 min.

RESPIRATORY RATE

Table RR

Horse	DEX 3min	DEX 5min	DEX 10min	DEX 15min	ATZ 3min	ATZ 5min	ATZ 10min	ATZ 15min
1	10	12	12	12	16	16	16	16
2	10	12	10	12	16	16	12	12
3	12	16	10	12	12	16	20	16
4	16	16	16	16	16	20	20	16
5	12	12	20	12	12	12	12	12
6	12	20	20	20	24	24	20	20

Statistic for paired samples

		Average	N	SD	Average SD
Couple 1	DEX3min	12,000	6	2,1909	,8944
	ATZ3min	16,000	6	4,3818	1,7889
Couple 2	DEX5min	14,667	6	3,2660	1,3333
	ATZ5min	17,333	6	4,1312	1,6865
Couple 3	DEX10min	14,667	6	4,6762	1,9090
	ATZ10min	16,667	6	3,9328	1,6055
Couple 4	DEX15min	14,000	6	3,3466	1,3663
	ATZ15min	15,333	6	3,0111	1,2293

Test for paired samples

		Difference between couples							
					Confidence interval for 95% difference				
		Average	SD	Average SD error	Inf	Sup	t	df	Sig. (2-code)
Couple 1	DEX3min - ATZ3min	-4,0000	4,8990	2,0000	-9,1412	1,1412	-2,000	5	,102
Couple 2	DEX5min - ATZ5min	-2,6667	2,0656	,8433	-4,8344	-,4990	-3,162	5	**,025**
Couple 3	DEX10min - ATZ10min	-2,0000	5,9330	2,4221	-8,2263	4,2263	-,826	5	,447
Couple 4	DEX15min - ATZ15min	-1,3333	2,0656	,8433	-3,5010	,8344	-1,581	5	,175

It is possible to appreciate a statically significant difference between the group DEX and ATZ at 5 min. Higher values are recorded for ATZ at 5 min.

SUBJECTIVE PARAMETERS OF THE SEDATION SCALE

The subjective parameters were assessed with two different scales of sedation, NRS and VAS. The results for each parameter are reported below.

Difficulty in maintaining a still posture

Tab. For the ability of maintaining a still posture							
Horse	DEX 3min	DEX 5min	DEX 10min	DEX 15min	ATZ 3min	ATZ 5min	ATZ 10min	ATZ 15min
1	2	1	1	1	0	0	0	0
2	1	1	1	1	0	0	0	0
3	1	1	1	1	0	0	0	0
4	1	1	1	1	0	0	0	0
5	1	1	1	1	0	0	0	0
6	1	1	1	1	0	0	0	0

Report

	DEX3min	DEX5min	DEX10min	DEX15min	ATZ3min	ATZ5min	ATZ10min	ATZ15min
Average	1,167	1,000	1,000	1,000	0,000	0,000	0,000	0,000
SD	,4082	0,0000	0,0000	0,0000	0,0000	0,0000	0,0000	0,0000
E. S. from average	,1667	0,0000	0,0000	0,0000	0,0000	0,0000	0,0000	0,0000

Test[a,c]

			ATZ3min - DEX3min	ATZ5min - DEX5min	ATZ10min - DEX10min	ATZ15min - DEX15min
Z			-2,333[b]	-2,449[b]	-2,449[b]	-2,449[b]
Sig. Asint. a 2 code			,020	,014	,014	,014
Monte Carlo a 2 code Signif	Sig.		**,030**	**,030**	**,030**	**,030**
	Conf interval 99%	Lower Limit	,026	,025	,025	,025
		Upper limit	,035	,034	,034	,034
Monte Carlo a 1 coda signif	Sig.		,016	,017	,017	,017
	Conf interval 99%	Lower Limit	,013	,013	,013	,013
		Upper limit	,019	,020	,020	,020

a. Wilcoxon test

b. Based on positive ranks.

c. Based on 10000 tables of samples 2000000.

This parameter was significant in all groups showing an increased instability amongst the groups DEX compared to the groups ATZ. In the ATZ group no signs of instability were noticed.

ATAXIA

Tab. For ataxia

Horse	DEX 3min	DEX 5min	DEX 10min	DEX 15min	ATZ 3min	ATZ 5min	ATZ 10min	ATZ 15min
1	2	2	2	2	0	0	0	0
2	2	2	2	2	1	1	1	1
3	3	3	2	2	1	1	0	0
4	1	1	1	1	0	0	0	0
5	2	2	2	2	0	0	0	0
6	2	3	2	2	1	1	1	0

Report

	DEX3min	DEX5min	DEX10min	DEX15min	ATZ3min	ATZ5min	ATZ10min	ATZ15min
Average	2,000	2,167	1,833	1,833	,500	,500	,333	,167
SD	,6325	,7528	,4082	,4082	,5477	,5477	,5164	,4082
E. S. from average	,2582	,3073	,1667	,1667	,2236	,2236	,2108	,1667

			ATZ3min - DEX3min	ATZ5min - DEX5min	ATZ10min - DEX10min	ATZ15min - DEX15min
Z			-2,251[b]	-2,271[b]	-2,251[b]	-2,271[b]
Sig. Asint.			,024	,023	,024	,023
Monte Carlo double Signif	Sig.		**,028**	**,030**	**,028**	**,030**
	Conf interval 99%	Lower Limit	,024	,026	,024	,026
		Upper limit	,032	,034	,032	,034
Monte Carlo single code Signif	Sig.		,014	,014	,014	,014
	Conf interval 99%	Lower Limit	,011	,011	,011	,011
		Upper limit	,017	,016	,017	,016

a. Wilcoxon test

b. Based on positive ranks

c. Based on 10000 tables of samples 624387341.

In all the groups the degree of Ataxia was significantly reduced in the group ATZ at 3 minutes.

RESPONSE TO TOUCH

Horse	DEX 3min	DEX 5min	DEX 10min	DEX 15min	ATZ 3min	ATZ 5min	ATZ 10min	ATZ 15min
Tab. Response to the touch								
1	3	3	3	3	1	1	1	1
2	3	3	3	3	1	1	1	1
3	3	3	3	3	3	1	1	1
4	3	3	1	1	3	0	1	0
5	3	3	3	3	2	1	1	1
6	3	3	3	3	3	2	3	1

Report

	DEX3min	DEX5min	DEX10min	DEX15min	ATZ3min	ATZ5min	ATZ10min	ATZ15min
Average	3,000	3,000	2,667	2,667	2,167	1,000	1,333	,833
SD	0,0000	0,0000	,8165	,8165	,9832	,6325	,8165	,4082
E. S. from average	0,0000	0,0000	,3333	,3333	,4014	,2582	,3333	,1667

			ATZ3min - DEX3min	ATZ5min - DEX5min	ATZ10min - DEX10min	ATZ15min - DEX15min
Z			-1,633[b]	-2,264[b]	-2,000[b]	-2,333[b]
Sig. Asint. double			,102	,024	,046	,020
Monte Carlo double Signif	Sig.		,248	**,033**	,124	,032
	Conf interval 99%	Lower Limit	,237	,028	,116	,027
		Upper limit	,259	,037	,133	,036
Monte Carlo single Signif	Sig.		,123	,015	,061	,016
	Conf interval 99%	Lower Limit	,114	,012	,055	,013
		Upper limit	,131	,018	,067	,019

a. Wilcoxon test

b. Based on positive ranks

c. Based on 10000 tables of samples 957002199.

The difference noticed for this parameter was shown to be statistically significant between the two groups at 5 min. Horses in the group DEX were less responsive to stimuli.

RESPONSE TO VISUAL STIMULI

Tab. Response to visual stimuli

Horsee	DEX 3min	DEX 5min	DEX 10min	DEX 15min	ATZ 3min	ATZ 5min	ATZ 10min	ATZ 15min
1	2	3	3	3	0	0	0	0
2	1	1	2	2	1	1	0	0
3	1	1	2	2	0	0	0	0
4	2	2	2	2	1	1	1	0
5	3	2	2	1	0	0	0	0
6	2	1	1	1	0	0	0	0

Report

	DEX3min	DEX5min	DEX10min	DEX15min	ATZ3min	ATZ5min	ATZ10min	ATZ15min
Average	1,833	1,667	2,000	1,833	,333	,333	,167	0,000
SD	,7528	,8165	,6325	,7528	,5164	,5164	,4082	0,0000
E. S. from average	,3073	,3333	,2582	,3073	,2108	,2108	,1667	0,0000

	ATZ3min - DEX3min	ATZ5min - DEX5min	ATZ10min - DEX10min	ATZ15min - DEX15min
			Test[a,c]	
Z	-2,041[b]	-2,060[b]	-2,232[b]	-2,232[b]
Sig. Asint. double	,041	,039	,026	,026
Monte Carlo double Signif — Sig.	,063	,066	**,034**	**,034**
Conf interval 99% — Lower Limit	,057	,060	,029	,029
Upper limit	,070	,073	,039	,039
Monte Carlo single Signif — Sig.	,034	,035	,018	,018
Conf interval 99% — Lower Limit	,029	,030	,015	,015
Upper limit	,039	,040	,022	,022

a. Wilcoxon test

b. Based on positive ranks

c. Based on 10000 tables of samples 92208573.

A statistically significant difference was present between the groups DEX and ATZ at 10 and 15 min. The values are reduced in the groups ATZ suggesting a decreased response to visual stimuli at 10 and 15 min.

RESPONSE TO ACOUSTIC STIMULI

Tab. Response to acoustic stimuli

Horse	DEX 3min	DEX 5min	DEX 10min	DEX 15min	ATZ 3min	ATZ 5min	ATZ 10min	ATZ 15min
1	1	2	2	2	2	0	0	0
2	1	1	1	1	1	1	1	1
3	1	1	2	2	1	1	0	0
4	3	2	1	1	1	0	0	0
5	3	3	3	3	0	0	0	0
6	2	3	1	1	0	0	0	0

Report

	DEX3min	DEX5min	DEX10min	DEX15min	ATZ3min	ATZ5min	ATZ10min	ATZ15min
Average	1,833	2,000	1,667	1,667	,833	,333	,167	,167
SD	,9832	,8944	,8165	,8165	,7528	,5164	,4082	,4082
E. S. from average	,4014	,3651	,3333	,3333	,3073	,2108	,1667	,1667

	ATZ3min - DEX3min	ATZ5min - DEX5min	ATZ10min - DEX10min	ATZ15min - DEX15min
Z	-1,473[b]	-1,857[b]	-2,041[b]	-2,041[b]
Sig. Asint. double	,141	,063	,041	,041
Monte Carlo double Signif — Sig.	,253	,128	,064	,064
Conf interval 99% — Lower Limit	,241	,119	,058	,058
Upper limit	,264	,136	,071	,071
Monte Carlo single Signif — Sig.	,126	,065	,031	,031
Conf interval 99% — Lower Limit	,118	,058	,027	,027
Upper limit	,135	,071	,036	,036

Test[a,c]

a. Wilcoxon test

b. Based on positive ranks

c. Based on 10000 tables of samples 1993510611.

No statistically significant difference was identified.

HEIGHT OF THE HEAD

Tab. Eight of the head							
Horse	DEX 3min	DEX 5min	DEX 10min	DEX 15min	ATZ 3min	ATZ 5min	ATZ 10min	ATZ 15min
1	43	31	28	36	94	103	103	103
2	20	20	25	23	95	105	115	115
3	50	40	45	40	63	80	100	100
4	23	20	33	33	28	84	75	74
5	28	20	32	30	80	100	80	80
6	50	40	30	30	65	75	65	60

Test for paired samples

		Average	N	SD	Average standard error
Couple 1	DEX3min	35,667	6	13,6333	5,5658
	ATZ3min	70,833	6	25,0393	10,2223
Couple 2	DEX5min	28,500	6	9,8742	4,0311
	ATZ5min	91,167	6	13,0141	5,3130
Couple 3	DEX10min	32,167	6	6,9113	2,8215
	ATZ10min	89,667	6	19,2007	7,8387
Couple 4	DEX15min	32,000	6	5,8310	2,3805
	ATZ15min	88,667	6	20,6849	8,4446

	Height of the head (cm)
Average pre-sedation	115,66
50% Average pre-sedation	57,83
Average DEX 3min	35,667*
Average DEX 5 min	28,5*
Average DEX 10min	32,167*
Average DEX 15min	32*
Average ATZ 3min	70,833
Average ATZ 5min	91,167
Average ATZ 10min	89,667
Average ATZ 15min	88,667

*= values lower than the 50% of the average of the height of the head measured before sedation.

Test for paired samples

		Difference in couples							
					Confidence interval for 95% difference				
		Average	SD	Average standard error	Lower	Upper	t	df	Sig. (2-code)
Couple 1	DEX3min - ATZ3min	-35,1667	28,0315	11,4438	-64,5840	-5,7494	-3,073	5	**,028**
Couple 2	DEX5min - ATZ5min	-62,6667	20,8199	8,4997	-84,5158	-40,8176	-7,373	5	**,001**
Couple 3	DEX10min - ATZ10min	-57,5000	21,0024	8,5742	-79,5406	-35,4594	-6,706	5	**,001**
Couple 4	DEX15min - ATZ15min	-56,6667	21,7593	8,8832	-79,5016	-33,8317	-6,379	5	**,001**

For this value it was possible to appreciate a statistically significant difference starting from 3 min suggesting a rapid response to the administration of ATZ.

VAS

VAS scale

VAS								
Horse	DEX 3min	DEX 5min	DEX 10min	DEX 15min	ATZ 3min	ATZ 5min	ATZ 10min	ATZ 15min
1	8	7	7	7	4	4	3	2
2	8	8	8	8	3	2	2	2
3	7	7	7	7	3	2	1	1
4	8	7	7	7	6	2	1	1
5	9	9	8	8	3	1	1	1
6	8	9	9	8	6	4	3	2

	DEX3min	DEX5min	DEX10min	DEX15min	ATZ3min	ATZ5min	ATZ10min	ATZ15min
Average	8,000	7,833	7,667	7,500	4,167	2,500	1,833	1,500
SD	,6325	,9832	,8165	,5477	1,4720	1,2247	,9832	,5477
E. S. from average	,2582	,4014	,3333	,2236	,6009	,5000	,4014	,2236

Test[a,c]

			ATZ3min - DEX3min	ATZ5min - DEX5min	ATZ10min - DEX10min	ATZ15min - DEX15min
Z			-2,214[b]	-2,226[b]	-2,264[b]	-2,264[b]
Sig. Asint. double			,027	,026	,024	,024
Monte Carlo double Signif	Sig.		**,031**	**,032**	**,031**	**,031**
	Conf interval 99%	Lower Limit	,027	,028	,026	,026
		Upper limit	,036	,037	,035	,035
Monte Carlo single Signif	Sig.		,016	,016	,015	,015
	Conf interval 99%	Lower Limit	,012	,013	,012	,012
		Upper limit	,019	,020	,018	,018

a. Wilcoxon test

b. Based on positive ranks

c. Based on 10000 tables of samples 79654295.

For this parameter the results were increased in the group DEX compared to ATZ suggesting a deeper level of sedation. This was also statistically significant.

SUM OF SUBJECTIVE PARAMETERS

Table of the sum							
Horse	DEX 3min	DEX 5min	DEX 10min	DEX 15min	ATZ 3min	ATZ 5min	ATZ 10min	ATZ 15min
1	10	12	11	11	3	1	1	1
2	8	8	9	9	4	3	3	2
3	9	9	10	10	5	3	1	1
4	10	9	6	6	5	1	2	0
5	12	11	11	10	2	1	1	1
6	10	10	8	8	4	3	4	1

This parameter was statistically significant in all the groups with higher values for the group DEX. These results indicate an increased degree of sedation for the group DEX.

Report

	DEX3min	DEX5min	DEX10min	DEX15min	ATZ3min	ATZ5min	ATZ10min	ATZ15min
Average	9,833	9,833	9,167	9,000	3,833	2,000	2,000	1,000
SD	1,3292	1,4720	1,9408	1,7889	1,1690	1,0954	1,2649	,6325
E. S. from average	,5426	,6009	,7923	,7303	,4773	,4472	,5164	,2582

Test[a,c]

			ATZ3min - DEX3min	ATZ5min - DEX5min	ATZ10min - DEX10min	ATZ15min - DEX15min
Z			$-2,207^b$	$-2,201^b$	$-2,214^b$	$-2,214^b$
Sig. Asint.			,027	,028	,027	,027
Monte Carlo double Signif	Sig.		**,031**	**,033**	**,036**	**,032**
	Conf Interval 99%	Lower Limit	,026	,029	,031	,027
		Upper limit	,035	,038	,040	,036
Monte Carlo single Signif	Sig.		,016	,018	,018	,016
	Conf Interval 99%	Lower Limit	,013	,014	,015	,013
		Upper limit	,019	,021	,022	,020

a. Wilcoxon test

b. Based on positive ranks

c. Based on 10000 tables of samples 475497203.

All the horses were able to complete the lameness investigation without requiring further ATZ administration.

DISCUSSION

Although a thorough clinical examination during a lameness investigation is mandatory in every lameness case, there is no question that clinical examination can be misleading (Pilsworth & Dyson 2015). The use of diagnostic analgesia to accurately localise the source of pain is therefore essential (Pilsworth & Dyson 2015). Due to the costs involved in using modern imaging techniques the decision for further diagnostic tests should be based upon the correct localisation of the area with diagnostic analgesia.

Different methods have previously been used in order to restrain fractious horses in order to perform diagnostic analgesia. These include nose twitch, ear or skin twitch. However, sometimes the reaction of the patient to these forms of restraint can be unpredictable with increased risk of injury for the operators involved in the procedure.

Tranquilization is sometimes necessary when clinically assessing lameness in exuberant, nervous or excited horses, in order to avoid masking of clinical signs (López-Sanromán *et al* 2015). However, a consensus on whether use of sedatives should be allowed during lameness investigation has not been achieved yet. It has previously been suggested that sedation of fractious and/or excited horses may lead to a better identification of the lameness (Schumacher *et al* 2013). Furthermore even in cases where sedation was deemed useful and necessary there was not agreement on which drug should be used (Schumacher *et al* 2013, Ross 2012, Kollias-Baker *et al* 1993).

Sedation with detomidine did not change the degree of lameness, but it did alter the general locomotion pattern suggesting that its use could be more beneficial in those cases where a more prolonged duration of effects is required (Buchner *et al* 1999, Ross 2012). The use of xylazine can be advocated in those instances where a shorter duration of action is required. After xylazine administration the majority of accelerometric parameters showed significant differences only until 45 min after injection (López-Sanromán *et al*., 2012). However mild ataxia should be considered as a possible side effect (Buchner *et al* 1999, Schumacher *et al* 2013).

When the intravenous administration of romifidine, detomidine and xylazine were compared it was found that romifidine administration caused the least effect on accelerometric variables even though a statistic significance was not established. However the duration of the effect was significantly

longer (López-Sanromán *et al* 2013). In the study the horses were assessed at walk before and 5, 15, 30, 45, 60, 75, 90, 105, and 120 minutes after each treatment. No difference in the stride length was detected among treatments and between treatments and control group.

The use of a different route of administration for alpha2-agonists has also been considered on the basis that different absorption could have modified the effect of the drug on the musculoskeletal system. When the effect of sublingual administration of detomidine on kinematic parameters was assesses it was shown that significant differences with baseline values were observed 180 minutes after administration, thus indicating a prolonged effect of the drug when administered sublingually (López-Sanromán *et al* 2014). In the former study the prolonged ataxia was attributed to the alteration of the regularity values of the stride that subsequently led to a decrease in stability. It is logic to think that the results obtained in this study at walk would be even more pronounced when the horse is trotted on straight line and particularly on the lunge for a thorough lameness investigation.

In the last decade the use of more recent and specific alpha-2 agonists has greatly increased. In the United States of America the use of DEX has largely replaced the use of less specific drugs (i.e. detomidine) (Rezende *et al* 2014). A pharmacokinetic study showed that after intravenous administration of DEX at the dose of 5μg/kg IV the maximum degree of sedation was noticed between 4 and 10 minutes. Lowering of the head and lack of interest in the surroundings were in fact to be 70% less compared to baseline (Rezende *et al* 2014). Head height gradually returned to normal and was only 20% lower than baseline at 60 min. The ability to ambulate was also assessed. A significant reduction was assessed during the first 60 minutes. Response to different stimuli was also significantly reduced until 30 minutes after administration. The authors concluded that Dexmedetomidine produced intense sedation and severe ataxia as indicated by the decrease in head height and elevation of gait scores. While significant sedation was observed up to 60 min post-drug administration, it was most intense during the initial 20–30 min (albeit still statistically significant, at 45 and 60 min the head height was only 30% and 20% lower than baseline, respectively) (Rezende *et al* 2014). Even though the elimination half life of DEX was 8.03 +/- 0.84 min, it was considered that antagonism of the molecule was appropriate to entirely antagonise the sedation and also to "remove" the analgesic effect provided by DEX.

In veterinary medicine the use of DEX had drastically increased due to its increased specificity for alpha-2 receptor and therefore reduced side effects. Furthermore the availability of a specific antagonist (ATZ) for DEX made its use in the everyday practice even safer (Corletto 2010).

In order to assess the degree of sedation and therefore the effect of the ATZ administration NRS and VAS scores were used. The variables assessed were the same previously used in other studies (Ringer *et al* 2013).

The horses included in this study were admitted to the hospital 2 days before the study was performed in order to allow enough time for acclimatisation. This enabled repeated measurements in order to correctly identify a baseline value for the Head Height Above the Ground (HHAG). In fact, to date this is the best objective measurement of the degree of sedation (Bryant *et al*, 1991; Clarke *et al*, 1991; England *et al*, 1992; Hamm *et al*, 1995; Freeman & England, 1999; Figueiredo *et al*, 2005).

In this study the HHAG showed a statistically significant value at all the points in time indicating a rapid sedative effect of DEX but also a rapid antagonism effect of ATZ.

A statistically significant difference was found for the HR 3 min after sedation and 3 min after antagonism. The ATZ was able to fully reverse the bradycardia induced by the administration of DEX. A statistically significant difference was also found in both groups at T5 suggesting a complete antagonism of the respiratory effects too.

The ability of the horse to stand in a still position was statistically significant in both groups at any point in time indicating a good degree of both sedation and antagonism. After sedation the horses were experiencing a certain degree of sway that completely disappeared after administration of ATZ. The degree of ataxia was markedly increased in horses after the administration of DEX. This finding is supported by previous studies suggesting a marked degree of ataxia after DEX administration (Rezende *et al* 2014). The degree of ataxia was significantly reduced already 3 min after ATZ administration. In this study the same operator subjectively assessed the degree of ataxia. However it is our intention to perform further studies with a more objective measurement of the degree of ataxia using an accelerometer.

The skin sensitivity of horses after sedation was significantly reduced in 5 of the horses included in the study. This is a very important finding because it means that needle positioning will be less likely to cause reaction and therefore reduce the risk of needle breakage. It was deemed essential to assess this parameter before the block was performed in order to evaluate the response of the horse with respect to personnel safety.

In the present study it was possible to perform diagnostic analgesia in all the horses. In two cases a bilateral lateral plantar nerve block was performed. Even though the degree of ataxia was increased after DEX administration it was possible to lift the leg and perform the nerve block. The only inconvenient was the reduced response to lift the leg. However with a second operator performing this procedure the block could easily be performed.

In the other three cases a dorsal spinous ligament block and the high plantar nerve block were performed without any reaction.

It is important to take into account the type of anaesthetic block performed because in some cases 20 min could be too long to guarantee an acceptable specificity of the block (i.e coffin joint and navicular bursa)

The response to visual stimuli was significantly different at 10 and 15 minutes in each group. There was not statically significant difference for the acoustic stimuli. This was an unexpected finding which did not fit the clinical presentation. Even though horses after sedation still presented a response to acoustic stimuli it was markedly reduced compared to horses after administration of antagonist. The noise produced by hand clapping has been shown to be 130 dB (A). Considering that threshold for noise perception in horses is 7dB in a range of frequencies between 33.5 to 55 kHz (Heffner & Heffner 1983) we could speculate that the response in sedated horses with just a head could be considered mild. So it would be appropriate to perform further studies with a known source of noise in order to allow more objective measurements.

When the overall sedation scores are assessed it is possible to appreciate a statistically significant difference between the group DEX and ATZ at any point in time. This can also be clearly appreciated from a clinical point of view.

The protocol presented in this study fulfilled the objectives presented in the aim of the study. However, the clinical applications of this protocol still present some limitations such as costs. One vial of DEX 0.5 mg/ml (10mls) costs in average 67 €. Considering the doses used in our study for an average 500 Kg horse 1 ml every 100Kg is needed costing in total 33.5 €. The cost of the antagonist for the same horse is 27.5€ bringing the total cost to 61€.

Furthermore the use of a single molecule would increase the risk of a sudden arousal. In order to avoid that it is common practice to use a multimodal approach with different drugs. The reason why

this protocol was designed does not allow use of different drugs that could potentially not be reversed.

The "repeatability" of the protocol should also be considered. When the protocol has been administered to a patient it is not possible to repeat it within a short period of time. This gives to the clinician "one shot" to perform the diagnostic analgesia.

CONCLUSIONS

The protocol proposed in this preliminary study was able to provide a good level of sedation and a fast antagonism. It is important to highlight that ataxia was completely antagonised by the administration of ATZ in 4 out of 6 cases. However in the other two cases the recovery was considered satisfactory in order to perform an orthopaedic exam. We would expect that with a greater number of cases and an objective measurement (accelerometer) will allow us to document the degree of ataxia objectively.

The protocol was also safe allowing the clinician to perform the procedure without time pressure and without any injuries occurring. This allows the equine surgeon to perform diagnostic analgesia on fractious horses in a yard setting where transport is not possible.

The reduced number of the sample and the static, suggest that the number should be increased at least 4 folds in order to validate the method for the clinical use.

ACKNOWLEDGMENTS

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