UNIVERSITA' DEGLI STUDI DI MILANO

School of Veterinary Medicine
PhD Course in Veterinary and Animal Science
Class XXXII

From recumbency to standing: improving diagnostic imaging quality trough the evolution of equine anaesthetic protocols

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Academic Year 2018-2019

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Abstract

Magnetic resonance (MRI), computed tomography (CT) and bone scintigraphy have, nowadays, a central role in the diagnosis of equine patient disorders and moreover, appropriate treatment plans for rehabilitation and recovery from musculoskeletal injuries are built on the foundations of an accurate diagnosis and detailed characterization of the pathology. The evolution in the last decades, both in anaesthesia and diagnostic imaging machines has lead clinicians to investigate more deeply on advantages and disadvantages of performing these examination in standing patients.

General anaesthesia in this species is well known to be associated with higher risks due to body mass and anatomical conformation. Thus it is mandatory to evaluate risks, costs and benefits of the diagnostic examination under consideration. The decision of performing second level diagnostic imaging in standing or in general anaesthesia must to be based also considering the ultimate aim of these examinations that is to achieve a correct and accurate diagnosis. Motion artefacts can nullify the diagnostic quality and moreover, even if great improvements have been made in machines and techniques, not all anatomic regions of the horse can be investigated a the standing patient.

Motion-correction software are nowadays available for MRI and bone scintigraphy in the face of mild to moderate swaying motion while for CT examination improvements are still necessary.

Clinicians must be aware of these limitations to correctly plan a diagnostic approach to the pathology under investigation.

In the present thesis, the focus moved to improve standing sedation to avoid patient motions and in cases where standing sedation is not possible, improve balanced anaesthesia in second level diagnostic examinations.

Three studies have been included in this doctorate thesis.

The first study evaluates, during bone scintigraphy, two different sedative opioids combined with detomidine. In this study not only the sedation score was evaluated but also patient immobility; bone scintigraphy is a very sensitive examination but with inherent poor spatial resolution, and thus anatomic detail is lost. Also time needed for acquisition is long with acquisition time for each image of 2 minutes. Therefore, immobility in this diagnostic imaging technique is mandatory. No studies in literature evaluate sedative protocols including

immobility evaluation, thus drugs combination choice is limited to the anaesthesiologist preference and not on evidence based medicine

The second study focused on balanced anaesthesia in horses undergoing MRI in general anaesthesia, also in this case time acquisition is long with examination time balanced between 1 and 3 hours.

Anaesthesia in order to diminish the patient risks should be always considered. Two routes of dexmedetomidine administration, continuous rate infusion (CRI) and subcutaneous, were evaluated during isofluorane general anaesthesia. Cardiopulmonary stability and recovery quality, that have been described to be one of the most problematic and unpredictable phases of an anaesthetic plan for quine patients, were compared. Subcutaneous administration of Dexmedetomidine has not been reported in veterinary medicine, while in human patients this route has begun to be investigated only in the last few years therefore this study represents a scientific novelty regarding this topic.

In the third study an alternative approach to retrobulbar local-anaesthesia was evaluated. Retrobulbar block is widely used in veterinary and human medicine to perform ophthalmic surgeries in general anaesthesia and in the standing horse. For this technique many possible complications and risks have been described, therefore in human and small animals an alternative loco-regional anaesthesia with minimal complications has been described, the peribulbar block. The aim of the work was to evaluate this alternative approach in equine cadavers. Peribulbar blocks were performed by injecting contrast medium and the likelihood of achieving anaesthesia was evaluated thought CT examination. This approach not only could be used as a safer alternative and as a part of balanced general anaesthesia but could also permit surgeons to perform ophthalmic surgeries in the standing horses, reducing costs and eliminating general anaesthesia related risks.

This doctorate thesis consists of studies on different topics but, all aiming to increase safety in equine patients. The purpose of the study were to ameliorate standing sedation in order to achieve high quality images fundamental for a correct diagnosis and moreover an appropriate treatment plan for rehabilitation and recovery. To describe a locoregional technique with fewer and minimal possible complications that could permit avoiding general anaesthesia and in cases in which general anaesthesia is required, the aim was to improve balanced anaesthesia in order to reduce the associated risks, bringing new information into the field of veterinary medicine for an alternative route of administration of dexmedetomidine in equines.

General Introduction

1. Diagnostic imaging:

The days of a simple X-ray being the only imaging option for the assessment of health issues in horses are long gone. Appropriate treatment plans for rehabilitation and recovery from musculoskeletal injuries in horses are built on the foundations of an accurate diagnosis and detailed characterization of the pathology. Radiography and ultrasonography are the cornerstones of the evaluation but, can fall short in the assessment of some conditions. More advanced imaging options such as computed tomography (CT), magnetic resonance imaging (MRI) and nuclear scintigraphy may be beneficial in select cases.

Magnetic resonance imaging (MRI) is becoming the gold standard for diagnosis of musculoskeletal injuries in veterinary medicine, as it has been in human medicine for at least a decade. MR imaging provides excellent visualization of soft tissue and osseous injuries. Several MR systems, both high- and low-field, are available for imaging equine patients; these systems vary from standing low-field strength units that are used to image the horse under sedation, to more powerful high-field units that require general anaesthesia.

Due to the unit configuration, areas that can be imaged in horses are limited. Similar to CT, MRI generates multiple two-dimensional cross-sectional image slices produced using a variety of sequences that uniquely highlight different tissues such as bone, tendon, cartilage, and synovium. When these images are evaluated collectively, they provide an excellent characterization of the area of interest. Subtle soft-tissue abnormalities are more easily recognized with MRI than with other imaging modalities, such as ultrasonography, radiography, nuclear scintigraphy and CT, because of the superior soft-tissue resolution it provides. MRI allows evaluation of the intricate ligaments and tendons contained within the hoof capsule, an area in which even ultrasonographic evaluation of soft tissues is limited at best. Furthermore, MRI allows for thorough evaluation of joints, periarticular structures, and bursas, which are difficult to thoroughly evaluate with other modalities. Another advantage of MRI is that it is the only modality that allows detection of fluid accumulation in bone caused by inflammation, neoplasia, infection, contusions, osteonecrosis, or edema (Balassy et al. 2008) or stress at points of ligamentous attachments (Powell et al. 2010). The use of MRI has transformed veterinary medicine's understanding of injuries to the distal limbs of horses and can be invaluable in the work-up of the equine athlete.

Technological advances have led to the development of spiral (also known as helical) CT scanners, which are in common use today. These scanners allow the patient to move slowly

through the path of a continuously emitted x-ray beam. The radiation transmitted is in the form of a spiral. Spiral CT enables very efficient anatomic coverage during the CT scan, making these studies much more time effective than MRI. CT allows for evaluation of the skull without anatomic superimposition and provides excellent contrast and spatial resolution. For these reasons, CT allows for a more accurate assessment of the extent and physical features of diseases of the skull than conventional radiographs (Tietje et al 1996). Both soft tissues and bone can be imaged with CT; however, the soft-tissue contrast resolution of MRI is superior to that of CT. Contrast enhancement with CT is another technique that can further differentiate normal versus abnormal areas within soft tissues. With this method, iodine-based contrast media is injected intravenously and can help enhance visualization of pathology. Computerized three-dimensional models can also be generated from a study, which further aids in conceptualizing damaged tissues. To prevent gross motion artefact's CT usually requires the horse be placed under general anaesthesia. In the standing horse, CT imaging of the equine head and the distal limbs has been described to a limited extent.

With both modalities, careful case selection is paramount to ensuring that the maximum information is obtained from these studies. Limitations of the use of both modalities in the standing horse are addressed. In standing MRI, decreased image quality resulting from the low field strength of most available open magnets and motion artifact are the most significant limitations. The low availability of facilities equipped to perform CT examinations in the standing horse is the most significant limitation of this modality. With the rapid progression of MRI technology and continued demand for availability of these modalities, it is likely that high-quality standing MR and CT imaging will become available for equine patients in the near future.

Another useful imaging technique is nuclear scintigraphy, which can highlight differences in blood flow patterns, soft tissue inflammation, and active bone modelling. Abnormal bone modelling can occur because of localized injury (bone bruises, stress fractures), degenerative changes (bone spavin, ringbone), excessive stress on certain areas (pedal osteitis), or inflammation and infection. These conditions can be apparent by scintigraphy well before radiographic changes are visible. Scintigraphy also has proven useful in the diagnosis or characterization of acute suspensory desmitis, sacroiliac injuries, soft tissue and osseous conditions of the foot and navicular region, cervical spine osteoarthritis, overriding thoracolumbar spinous processes (kissing spines), bone spavin, stifle injuries, pelvic fractures, radial fractures, high and low ringbone, subchondral bone cysts, and even dental disease. The clinician must weigh the advantages of scintigraphic examination under general anaesthesia

(improved image resolution and better lesion detection) against the cost, time, effort, risk of complications and increased personnel exposure to radiation that is involved with general anaesthesia. Movement of the standing horse can be minimized by appropriate sedation and quiet handling of both the horse and camera. Recently, nuclear medicine software that, is able to correct for motion has become available, and this compensates for the slight swaying of the horse and respiratory movements.

CT, MRI, and nuclear scintigraphy continue to be valuable diagnostic tools that are becoming more widely available to equine patients. They are complementary to a clinical examination and diagnostic analgesia, radiography, and ultrasonography in the diagnosis and characterization of musculoskeletal pathology in the horse.

1.1 Diagnostic imaging in standing

The benefits of imaging the standing equine patient include elimination of general anaesthesia, increased availability of standing imaging, and, in most cases, reduced cost to the client. Arguably the most important benefit of standing diagnostic imaging is that it negates the need for general anaesthesia and eliminates the associated risk to the patient. Anaesthetic complications in horses are well described. The most common perioperative (and perianesthetic) complications include cardiac arrest, fractures in recovery and myopathy. One study performed to evaluate the risk of general anaesthesia in horses undergoing MRI found the mortality rate to be 0.6% (Franci et al. 2006) which is similar to that of healthy horses undergoing nonabdominal surgeries (Johnston et al. 2002, Johnston et al. 2004, Franci et al 2006). Interestingly a greater proportion of horses suffered myopathy after MRI (2.3%) than after surgery (0.8%) (Franci et al. 2006). In a multi- institutional study assessing the risk of general anaesthesia in horses, it was shown that the likelihood of patient death increases if the duration of anaesthesia exceeds 61 minutes (Johnston et al. 1995) Anaesthesia time for an equine patient to undergo a recumbent MR examination of bilateral distal forelimbs in a 1.5-T magnet is approximately 1 to 2 hours. Although sedation is required to perform an MRI or CT examination on a standing horse to limit patient motion and protect the patient and equipment from damage, the need for general anaesthesia is eliminated, thus making standing imaging a lower-risk procedure than recumbent MR or CT imaging.

1.1.1 Magnetic resonance in standing:

In general, high-field (recumbent) MR examinations are often more costly than their low-field counterparts. High-field magnets are built to produce a stronger magnetic field than low-field magnets, thus providing higher-resolution images. However, this benefit comes at a price. High-field magnets are more expensive than low-field magnets to purchase and maintain. Because of the increased strength of their magnetic field, high-field magnets require a large room with conductive material built into the walls, termed a Faraday cage, the purpose of which is to protect the surrounding areas from the stray magnetic field produced by the magnet and to prevent stray radiofrequencies, such as radio waves, from interfering with the function of the magnet and quality of the images produced. The need for the large room to accommodate the size of the magnetic field, and the Faraday cage, adds to the expense of high-field MRI. By eliminating the need for general anaesthesia with standing diagnostic imaging, the overall cost of the procedure is inherently reduced. MR scanners can be in either an open or closed configuration.

Until recently, high-field magnets required a closed (tube-shaped) configuration to produce a stronger magnetic field than low-field magnets. This closed configuration limits the size of the patient that can be imaged. Closed magnets with a wider-bore diameter (71 cm) and magnets with shorter bore lengths (125 cm long) and flared ends have been developed to accommodate larger human patients and claustrophobics. These developments have helped to improve the ease of positioning and to accommodate the larger and more proximally located anatomy of equine patients. However, regardless of bore length or size, general anaesthesia is required to position a horse in the closed bore of a high- field magnet. Open magnets have a C-shaped configuration whereby the anatomy to be imaged is only partially enclosed. It is this open configuration that allows for an MR examination to be performed on horses in the standing position. The open magnets that can be used to image standing horses are of low field strength at present.

The disadvantages of low strength of magnetic field include decreased signal-to-noise ratio, longer scan times, thicker image slices and increased patient motion, all of which result in decreased image resolution and image quality in comparison with high-field images (Patton et al. 1994, Rutt et al. 1996, Tucker et al. 2001). Recently in human medicine, there has been a drive toward improving the image quality of open magnets to better accommodate claustrophobic and bariatric patients and to allow quicker access and ease of patient positioning for orthopaedic imaging. With recent advances, the image quality produced by these magnets

has significantly improved in order for them to become more competitive with their closed, high-field counterparts. Magnets of high field strength with an open configuration are now becoming available for clinical use in humans and, will likely be available for veterinary patients in the near future. This technology will offer the best of both worlds: high-resolution imaging without the risks associated with general anaesthesia.

Imaging techniques such as radiography, ultrasonography and nuclear scintigraphy are often performed before or after MRI, ruling out apparent disease processes that can be diagnosed with these methods and further localizing the source of the lameness. MR examinations are time consuming and should not be used as screening examinations.

Ideally the MR examination is limited to 1 or 2 joints or portions of a limb. However, even with thorough lesion localization, MRI findings do not always correlate with the degree of lameness or suspected lesion location. In such cases, one benefit of standing MRI is the ability to repeat the MR examination on another anatomic site, proximal or distal to the initial site, on the following day. Such a repeat examination becomes more expensive, time consuming, and risky when general anaesthesia is required. With standing MRI, the patient is lightly sedated, and more sedative is administered as needed to maintain an adequate plane of sedation. The region of interest is carefully aligned in the magnet, and images of the affected limb and, often, the contralateral limb are acquired. In routine examinations, a standardized protocol is used to acquire the necessary images in the correct imaging planes. The procedure usually takes anywhere from 1 to 3 hours. Scan times should be kept as short as possible for each sequence acquired to limit artifact associated with patient motion.

Our ability to image certain anatomic regions of the horse, such as the carpus and stifle joint, is limited by the length of the patient's limbs and width of the shoulder or pelvic regions. Using a closed (high-field) magnet with the largest bore size available or a low-field open magnet with general anaesthesia, it is sometimes possible to image as far proximal as the stifle joint in the hindlimb and the distal radius in the forelimb. The region of interest must be positioned as close to the center (termed isocenter) of the magnet to optimize image quality. With an open magnet, images can be obtained from foot distally to as far proximal as the carpus and tarsus. In the open magnet, the ability to image more proximal anatomic regions is limited by magnet size. Larger anatomic regions require longer scanning times, which increases the likelihood of motion and associated artifacts in the non-anesthetized patient. In addition, the more proximal aspects of the extremities are more severely affected by the swaying motion of a sedated horse. Since MRI of the standing horse was first described, numerous articles have described normal anatomy of the equine limb as well as numerous soft-tissue and osseous lesions as observed on

standing MR images. In a clinical situation patient motion has the most significant detrimental effect on the quality of a standing MR image. This factor should be considered when evaluating literature that compares standing and recumbent MRI.

Motion artifact appears as ghosting (the appearance of multiple images) or blurring of the image and can drastically decrease the ability of the examiner to resolve fine details. In severe cases, motion artifact can render the study non diagnostic. Any motion greater than one-half the pixel resolution of the MR image, which corresponds to submillimeter movements, can degrade image quality (McKnight et al 2004). In the standing horse, the distal limb is the least likely to have associated motion artifact. As one images further proximally on the limb, even the subtle swaying motion of a sedated horse becomes a more severe issue. The signal-to-noise ratio is decreased in the low magnetic field strengths used for standing MRI. Decreased signal-to-noise ratio, which translates to degraded image quality, can be improved by increasing the length of time over which the image is acquired.

Unfortunately, the prolonged scan times needed to improve image quality will increase the opportunity for motion artifact to occur. Motion-correction techniques have been developed over recent years and can improve MR image quality in the face of mild to moderate swaying motion. Such methods include faster scanning sequences and retrospective motion-correction techniques (McKnight et al 2004; Zhuo et al. 2006). Some of these techniques have been shown at least to be effective standing horse (McKnight et al. 2004). Dedicated motion-correction software and supportive hardware are necessary to provide motion-correction techniques. Motion can also be limited by providing an adequate plane of sedation while avoiding heavier sedation protocols, which may cause more severe swaying or wobbling. In addition, if the patient is in pain when bearing weight on the limb to be imaged, local analgesia can be provided to promote weight bearing on the affected limb.

1.1.2 Computed tomography in standing:

Until very recently, general anaesthesia was considered mandatory for equine patients undergoing a CT examination. In a few locations across the world today, modifications have been made to allow for CT imaging to be performed on the standing horse. To scan the head of the sedated horse, the CT gantry must be elevated to the level of the horse's neck, which can be achieved by elevating the CT scanner or positioning the horse within a trench to have the

head level with the gantry. In 2002, a peripheral quantitative CT scanner was specifically designed to image the distal equine limb in either the standing or recumbent position (Equine XCT 3000; Norland-Stratec Medical Systems, Fort Atkin- son, WI) (Desbrosse et al. 2008). Images from this CT scanner were considered diagnostic in 89% of cases reported in a retrospective study. With this standing CT, several people may be needed to accompany the horse during the scan to restrain the animal, creating radiation exposure to personnel that would be unnecessary in an anesthetized horse.

As with MRI, the size of the CT gantry limits the ability to image certain anatomic regions. Currently available CT scanners have gantry sizes of up to 85 cm in diameter. The gantry of CT scanners consists of a closed construct, thus limiting the ability to position larger equine anatomy within the gantry. However, whereas most MR scanners have a longer, more tubular shape; CT scanners have more of a simple ring or circular construct, making the positioning of anatomy within the center of the gantry less complicated with CT in comparison with MRI scanners. Similar to that described with standing MRI, the ideal plane of sedation maximally limits patient motion while limiting the swaying or unstable motions of a heavily sedated patient. Because CT image acquisition is rapid, and reformatted images are made by the computer after the data set is obtained from the patient, scan time is significantly quicker with CT than with MRI. The length of sedation needed for a patient is relatively short, with a typical CT scan of a single anatomic region taking approximately 20 minutes.

Sedation duration is lengthened by the time needed to adequately position the standing, sedated patient before initiating the scan. In addition to being time efficient, CT is much more cost efficient than MRI. The lower initial setup and maintenance costs of CT scanners makes them more appealing to practice owners, thus increasing their availability. However, the availability of CT scanners modified for the standing equine patient is still extremely limited. In the standing horse, patient motion is probably the artifact most detrimental to image quality. Patient motion during the CT scan can result in artifacts severe enough to render the reconstructed image non diagnostic.

Motion artifacts on CT images usually appear as shading or streaking of the image. In the standing horse, motion is limited by maintaining an adequate plane of sedation while avoiding sedation so heavy that the patient becomes wobbly. In addition, positioning aids can be used to help stabilize the area of interest within the gantry. Motion-correction techniques such as overscan and underscan modes, which are built into the CT scanner, and software correction can also help to limit motion artifact (Barret et al. 2004). Unfortunately, the standing CT modalities currently in use do not use motion-correction techniques (Desbrosse et al. 2008).

The concept of standing CT is currently in its infancy. However, the benefits of limiting general anaesthesia and recumbency are clinically significant. As standing surgical procedures become more available, the option to limit general anaesthesia altogether with the use of standing advanced imaging becomes even more desirable. Continued development of standing CT scanners is on the horizon. As more standing CT technology is developed, better motion correction/artifact reduction software will likely become more available in the future, thus improving image quality.

1.1.3 Bone scintigraphy in standing:

Nuclear scintigraphy, specifically bone scintigraphy, is one of the mainstays of molecular imaging. It has preserved its relevance in the imaging of acute and chronic trauma, and is particularly useful in the evaluation of athletic injuries. In the first part of the twentieth century, the relationship between musculoskeletal disorders and accumulation of radioactive substance was first recognized (Blum 1924). Since then, nuclear scintigraphy has attained widespread use and availability, with most referral hospitals and universities having g cameras equipped to accommodate horses. Studies continue to be published highlighting the value and use of nuclear scintigraphy despite the availability of other advanced imaging modalities including computed tomography (CT) and magnetic resonance imaging (MRI).

The most common use for nuclear scintigraphy in the horse is as a diagnostic tool to aid in lameness diagnosis (Archer et al. 2007). A major advantage of nuclear scintigraphy is that, unlike modalities such as radiography, this imaging modality allows for evaluation of physiologic function (Weaver et al. 1995). However, the inherent spatial resolution is poor, and thus anatomic detail is lost. Although several radionuclides are available and can diagnose pathologic change in virtually any organ system, the most commonly used radionuclide in horses is technetium 99 m (^{99m}Tc) labeled to tracers (pharmaceuticals) that bind to bone. The most common bone tracers are currently methylene diphosphonate (MDP) and hydroxymethylene diphosphonate (HDP). A bone scan consists of 3 phases after intravenous injection of radiopharmaceutical. The vascular phase (flow phase) occurs within 30 seconds after injection; the soft tissue phase (pool phase), in which the region of interest is imaged 3 to 5 minutes after injection; and the bone phase (delayed phase), with images obtained 2 or more

hours after injection. Two-dimensional images, called planar images, are obtained based on counts or time.

It is the ability of bone scintigraphy to image the skeleton (Dyson 2002; Dyson & Murray 2003, Archer et al. 2007) that makes it an appealing imaging choice for horses. This ability allows detection of unsuspected sites of trauma that are not recognized in other imaging modalities such as radiography (Van der Wall et al. 2010). In addition, nuclear scintigraphy has been reported to be similar in accuracy to MRI for detection of certain bone diseases such as stress fractures in people (Van der Wall et al. 2010).

Despite advances in camera technology and displays, radiotracers still primarily reflect function with limited anatomic resolution (Weaver 1995). Fine anatomic detail is lost, which can be critical to differentiating physiologic from pathologic change. Many advances in nuclear medicine have come in the form of software. Because nuclear scintigraphy for the most part is performed on a standing, sedated horse, acquisition stations are equipped with the ability to account for motion, known as dynamic capture. In addition, region-of-interest calculation, line-profile analysis, algorithms to filter noise, and attenuation correction to account for variation between images are available. Motion correct improves the image quality in examinations of the standing sedated horse. This process obtains images at 1-second to 2-second intervals that are stacked. The outlying images from excessive motion (spatial misregistration) are rejected. The images within the same spatial registration are superimposed on one another, creating an image with better resolution compared with single, static acquisitions. The software correction is particularly useful when imaging the axial skeleton, which is evident in the spine and pelvis where areas of abnormal radiopharmaceutical uptake may not be visible on uncorrected images (Davenport-Goodall 2004). Compared with uncorrected images, motion-corrected images are clearer, and the anatomy is more distinct. However, the technique has limitations when motion is excessive (Dyson et al. 2003a,b).

2. Equine anaesthesia

Mortality in equine anaesthesia has been reported to be approximately 1% in healthy patients but this value ranges depending upon study design between 0.08% and 1.8% (Mitchell 1969; Tevik 1983; Young and Taylor 1990; Johnston et al. 1995; Mee et al. 1998; Bidwell et al. 2007). In emergency patients it is difficult to evaluate the true contribution of anaesthesia to mortality. A number of reports from single clinics report a mortality rate that increases up to 5% if systemically ill animals are included in the study (Mitchell 1969; Tevik 1983; Young & Taylor 1993). Horses may survive emergency anaesthesia and colic surgery only to succumb to post-operative complications such as endotoxaemia, postoperative ileus (Hunt et al. 1986).

Nowadays a incidence of anaesthesia- associated mortality of 1% in healthy horses is considered correct. The same risk in humans is reported to be 0.01-0.001 % (Lunn & Mushin 1982; Irwin & Kong 2014). In dogs is reported a 0.05 % of risk while in cats is 0,11% (Brodbelt et al. 2008). The necessity to stand postoperatively may increase the risk of anaesthesia in horses compared with small animal and humans (Taylor 2002).

The largest study to date evaluating mortality associated to general anaesthesia in equine patients is the "Confidential Enquiry into Perioperative Equine Fatalities" (CEPEF) (Johnston et al. 1995; 2002; 2004). These multicenter studies spanned over 8 years of data collection including 41.824 patients identifying the most common causes of death, as well as risk factors. The information that are collected in these studies gather together different practices, clinics and hospitals with their different caseloads, anaesthesia protocols and both anaesthetic and surgical experience representing a more complete scenario. During general anaesthesia cardiac output can decrease by one-third, 15-30% of pulmonary blood flow may not take part to gas exchange increasing venous admixture shunt. These effects contribute to decreased oxygenation of blood and carbon dioxide retention (Hall et al.1968; Weaver & Walley 1975, Steffey et al. 1978, Aida et al. 1996) The anatomy of adult horse's thoracic cage makes external cardiac or thoracic compression partially ineffective during resuscitation. Small animal and human anatomy allow for more effective cardiac compression in emergency situations.

Hypoventilation and hypoxaemia are common in anesthetized horses (Hubbel 1996).

Body mass can elevate the risk of myopathies and neuropathies due to compressive injury on the table. Despite the use of thick foam padding or inflatable mattress, myopathies can still occur due to inadequate peripheral perfusion (Lindsay et al. 1985; Grandy at al 1987). Risk of fractures may be increased not only by the procedure but also by the patient temperament,

which inclines them to stand soon after anaesthesia, when residual drug effects may produce incoordination.

2.1 History of equine anaesthesia:

The practice of anaesthesia evolved from art to science during the 1800s. Before 1850 (and for a long time thereafter), the practice of equine anaesthesia remained an art overly dependent on herbal remedies (Atropa mandragora, opium, hen- bane, hemlock) and physical restraint ("a heavy hand") (Smithcors 1957; 1971). It was clear that security of the medical staff involved and moreover of the patient was lacking and that physical restraint of the horse "under general anaesthesia" was mandatory. Sir Frederick Hobday published in 1915 the first English text-book totally devoted to veterinary anaesthesia commenting on chloroform, Hobday stated, "*For the horse and dog, chloroform is by far the best general anesthetic both in regard to its utility and cheapness and, also, its safety*." He went on to say, *"It must of course, like all toxic drugs, be used with discretion and in a skillful and proper manner by a careful anesthetist."*. Despite the increasing interest for anaesthesia itself equine anaesthesia emerged from herbalism and physical restraint to a science capable of rendering patients insensible to pain in the 1950s. New monitoring techniques identified hypotension and ventilation abnormalities as significant factors in development of anaesthetic complications in 1980s. Rhabdomyolysis or "tying-up" had been a frequent complication and numerous anaesthetic protocols and padding strategies to prevent it were advised. Myopathy in 1987 was linked by Grandy and colleagues to hypotension identifying as risk factor a mean arterial blood pressure below 70 mm Hg. In the same years arterial blood gas analysis monitoring became prominence leading to the discovery of ventilation-perfusion abnormalities, hypoventilation and hypoxia in anaesthetized horses (Nyman 1989). Despite all the interest in equine anaesthesia the first devoted specifically to the horse was published only in 1991 (Muir & Hubbell 1991). The goal of the text was not only to detail the evolution and use of anaesthesia protocols but also completely deemphasize the need for a heavy hand restraint. One highly significant, but less recognized, advance has been the development of a critical mass of educated and trained individuals skilled in the art and science of equine anaesthesia. Several sources of the America Association of Equine Practitioners (AAEP, established in 1954), the British Equine Veterinary Association (BEVA), veterinary sources and anaesthesia text, revealed that the essential ingredient missing from the practice of

equine anaesthesia before 1970 was not lack of appreciation by earlier practitioners of its importance or use, but a relative absence of knowledge and dedicated equine anesthetist. The science of Veterinary anaesthesia has achieved formal and universal recognition as an independent field of study in 1975 by the formation of the American College of Veterinary Anaesthesia and the European College of Veterinary Anaesthesia in 1993. They are based on the principles of the dissemination of knowledge, the advancement of science and the development and maintenance of minimum standards of care. The self-imposed education and vigilance provided by veterinary anesthetists has significantly impacted the consequences (morbidity) and safety (mortality) of anaesthesia in horses while significantly improving patient well- being and lengthening the duration that equine surgery can be safely performed. What Robert Smith said probably was right, *"There are no safe anesthetic drugs, there are no safe anesthetic techniques; there are only safe anesthetists.*" We cannot change the temperament or the anatomy and physiology of the horse. We can educate and develop skilled individuals to perform anaesthesia to reduce the human error component of anesthetic mortality. We can increase safety by using adequate monitoring and elevated standards of work. Moreover techniques, instruments and machines have improved in the last decades, many procedures that used to require general anaesthesia are now performed in standing sedation. Fractures repair, dental surgery, laparoscopy and many other surgeries are now performed with sedation protocols. Diagnostic imaging that for decades were performed in general anaesthesia with the costs and risk associated are in standing patients. This is possible not only for first level diagnostic imaging such as x-rays and ultrasound but also second level diagnostic imaging, bone scintigraphy, computed tomography and magnetic resonance, investigations that in equine species are fundamental to achieve diagnosis.

2.2 Equine anaesthesia today

As in all areas of veterinary practice, equine anaesthesia and analgesia have progressed rapidly over the last two decades with the introduction of new drugs, user-friendly monitoring devices and new methods of using drugs. Important knowledge has also been gained in identifying the risk factors for equine anaesthesia. There is a growing awareness of the impact of anaesthesia and analgesia on the surgical outcome and, increased comprehension that equine anaesthesia is not just a technical procedure aimed at producing immobilization for the sake of operator

comfort. Many practical issues (e.g., long-term analgesia, hypoxemia, hypotension, recovery quality, drug-related hang- over), and new horizons (e.g., immune system modulation, cytokine pathophysiology) require continued investigation. New ideas, drugs, techniques, and equipment continue to evolve. Equine anaesthesia, unlike human anaesthesia, will continue to require the administration of drugs or techniques that permit control of consciousness with loss of sensation (analgesia) or loss of consciousness and analgesic adjuncts to perform even minor surgical procedures. Although improvements may occur, the next major advance in anaesthesia depends on as yet unrecognized advances in technology and molecular pharmacology. The role of anesthetists in equine surgery will become unquestioned and mandatory. Continued research will be required to provide the qualitative and quantitative data necessary to remove dogma and evaluate new approaches. There is much left to accomplish. The ideal anesthetic drug or combination of drugs has yet to be developed. Monitoring techniques, practices, and equipment remain poorly developed or adapted for use in horses and are difficult to use during recovery. The maintenance and recovery phases of anaesthesia remain major issues in horses as evidenced by the comparatively high mortality rates and the incidence of accidents leading to euthanasia (i.e., fractures). Methods for improving pulmonary gas exchange, limiting ventilation perfusion inequalities and ensuring adequate tissue perfusion need to be developed. These ongoing challenges, combined with an ever-increasing emphasis on prevention and treatment of pain, continue to receive special attention in shaping anesthetic protocols.

2.3 Causes of death

Intraoperative cardiac arrest: The CEPEF studies, in agreement with other, reported that intraoperative cardiac arrest tended to occur in the anaesthetic period, usually within the first 30 minutes. The possible cause was considered resulting from Halothane-induced myocardial sensitization to catecholamines, which may increase the risk for arrhythmias, especially in the absence of premedication and, it was suggested that acepromazine may be protective against such arrhythmias (Johnston et al.1995, 2002; Mee et al. 1998). In several studies including CEPEF-1 and 2 (Johnston et al 1995, 2002; Mee et al. 1998; Bidwell et al. 2007) the most commonly anaesthetic maintenance agent was Halothane which may have influenced the occurrence of adverse intraoperative cardiac events. In CEPEF-3, although overall mortality did not differ between isofluorane and halothane, fewer cardiac arrests occurred, especially in high-risk cases, when anaesthesia was maintained with isofluorane (Johnston et al 2004).

Bidwell and colleagues evaluated the fatality rate of horses undergoing general anaesthesia between 1997-2001 at Rood and Riddle Equine Hospital, n = 17 961 patients were include in the study the casa load was various. The were 42 peri-anaesthetic fatalities, and of these, the fatality directly associated with anaesthesia included 10 cardiac arrests, 8 fatal fractures post-anaesthesia and e fatal myopathy and/or neuropathies in recovery. Is interesting to notice that of the ten cardiac arrest six occurred in horses with severe systemic illness and four horses undergoing elective procedures that were assessed to be healthy at the time of anaesthesia. The record of these patients were inspected in more detail, and all were confirmed to be healthy, athletic animal upon physical examination. Necropsy also was performed, and no pre-existing cardiac disease was noticed. Two of the horses (young Thoroughbreds) during the procedure developed second degree atrioventricular block that initially responded to Atropine administration. Subsequently they re-developed the same dysrhythmia which did not respond to additional doses of atropine or adrenaline and resulted in cardiac arrest. The third case was a Paint horse that developed premature ventricular contractions on the table that responded to lidocaine administration. The dysrhythmia returned in the recovery stall and develop into a tachydysrhythmia (HR > 180 bpm) and eventually arrested despite treatment with lidocaine, adrenaline and thoracic compressions. The fourth horse was a Saddlebred that after orotracheal intubation arrested and died despite resuscitation with adrenaline and thoracic compressions.
Elevated vagal tone is a suggested reason for the higher risk of developing fatal dysrhythmias in athletic horses. In human patients studies involving heart rate variability link increased autonomic tone to athleticism (Iwasaki et al. 2003).

Skeletal fractures: Axial and appendicular fractures have been described as responsible for 26-64% of all anaesthesia related fatalities (Young & Taylor 1993; Johnston et al. 2002, 2004; Bidwell et al. 2007; Dugdale et al 2016). Long bone, cervical or basal skull fractures during recovery have contributed to anaesthesia related mortality through immediate euthanasia or instantaneous death. Horses undergoing fracture fixation are considered at greater risk for the sustaining of further fractures in recovery, but these patients are only a small proportion of the caseload in all reports (Johnston et al. 1995; Bidwell et al 2007; Dugdale et al. 2016). The difference in the incidence of fatal fractures among studies could be explained by the assistance provided to the patients during recovery. There are differences between studies on the necessity of rope-assisted recovery. This kind of recovery was not reported in the CEPEF. In a recent abstract (Chie Niimura et al. 2015) evaluated 5834 recoveries in which rope assistance was

always used. 30 (0,51%) suffered major complications resulting in mortality, 2 (0,03%) suffered fractures and a single horse (0,02%) suffered hock dislocation. Despite this Bidwell et al. (2007) emphasized that rope, head and tail, assistance cannot guarantee successful recovery, but others have been more convinced about its benefits (Wilderjans et al. 2005; Auer & Huber 2013). The Flight instinct in horses preludes them from remaining recumbent throughout recovery, unlike human and small animals. Whether rope assistance or other forms of assistance can reduce the incidence of fractures remains to be unequivocally proven (Kaestner 2010), but rope-assisted recovery techniques appear to be gaining popularity.

Post-anaesthetic myopathy: post-anaesthetic myopathy has been suggested to be a risk factor for the occurrence of fractures during recovery by causing pain, muscular weakness and incoordination. It is well known and demonstrated the importance of intraoperative cardiovascular monitoring and support, particularly the use of dobutamine to maintain mean arterial blood pressure (MAP) above values likely to risk PAM (Grandy et al. 1987; Richey et al. 1990; Young & Taylor 1993; Johnston et al. 2004). Since the earlies 1990s the provision of such support has been excepted practice in most equine hospitals. More recent studies support the conclusion of Duke et al. (2006) that intraoperative treatment of hypotension may not always prevent PAM but can reduce its severity. PAM is considered a form of compartment syndrome (with elements of ischaemia and later reperfusion injury), the occurrence is associated with poor padding and positioning of the anaesthetized patient, intracompartmental muscle pressure, a prolonged duration of anaesthesia, hypotension and venous stasis (Lindsay et al. 1980; 1985, 1989; White & Suarez 1986; Grandy et al. 1987; Heppenstall et al. 1988; Richey et al. 1990; Taylor & Young 1990a,b; Johnston 1993; Raisis 2005 a b). Franci and colleagues in 2006 demonstrated that there was no difference in occurrence of PAM in horses undergoing magnetic resonance investigation or surgery, underling that it is not the type of procedure a risk factors and that in routinely examinations risks are present. It has been shown that although hypoxemia would worsen tissue oxygen delivery already reduced by hypoperfusion/ischaemia, hypoxaemia itself has not been shown to be an independent risk factor (Trim & Wan 1990; Steffey et al. 1992b; Whitehair et al. 1996). It is important to differentiate PAM from other muscular pathologies that have been described in recent years and that often present with prolonged recumbency such as equine polysaccharide storage myopathy, hyperkalaemic periodic paralysis and malignant hyperthermia or hyperpyrexia (Spier 2006; Aleman 2008; Finno et al. 2009; Naylor 2015, Valentine 2005).

Spinal cord malacia/ post-anaesthesia neuropathies: pseudonyms of spinal cord malacia include spinal cord myelopathy, myelomalacia, haematomyelia and poliomyelomalacia. This pathology con be considered as a form of central neuropathy. It is recognized as a non-painful ascending neurological dysfunction which initially affects the tail and pelvic limbs (so that paraplegic horses may appear to "dogsit") and progresses cranially. It is a ischaemic necrosis of the spinal cord, most commonly starting from the thoracolumbar area and is fatal. The recurrence is sporadic and patient of larger breeds undergoing short procedures in dorsal recumbency present greatest risk even in the case of fillies (Blakemore et al. 1984; Brearley et al. 1986) pony (Lam et al. 1995) and following lateral recumbency (Raidal et al. 1997) have been reported. There are no recommended strategies for its prevention since the fact that the aetiology has not been elucidated. There are many theories that suggest that the causes could be: stretch ischaemia of the spinal cord during dorsal recumbency (exacerbated by the haemodynamic consequences of dorsal recumbency and the increase of itra- and peri-spinal cord cerebrospinal fluid pressure), verminous arteritis, embolism and Vitamin E or selenium deficiency (Blakemore et al. 1984; Stolk et al. 1991; Jackson et al. 1995; Raidal et al. 1997; Ragle et al. 2011).

Peripheral neuropathy affecting the limbs, such as femoral nerve injury, especially if bilateral, may prevent animal from standing up. This may impact on postoperative management and ultimately result in euthanasia (Dyson et al. 1988). In addition, as the dysfunction associated with pure neuropathy is usually more of a problem than pain, it is tempting to speculate that predisposition to fractures may increase as proprioception in impaired alongside motor and other sensory dysfunction. Furthermore, neuropathy may accompany myopathy (such as triceps myopathy accompanied by radial neuropathy), in which case pain and lameness or weakness may influence the outcome, as well as potentially increase likelihood of a long bone fracture. Peripheral neuropathy can occur also on the facial area, usually it is unilateral and rarely results in mortality, but morbidities such as impairment of food prehension and/or ocular protection way warrant supportive treatment. Pure peripheral neuropathy usually results from neural trauma or ischaemia (caused by contusion, compression or stretch) and therefore careful patient positioning and padding, as well as good neural oxygen delivery (avoiding hypotension and hypoxaemia), should help to prevent it (Dyson et al. 1988; Johnston 1993). During dorsal recumbency the head and neck position should be considered; over-extension of these areas can cause bilateral recurrent laryngeal nerve paresis or paralysis attributable to the stretching of these nerves. The aetiology of these complications has not been fully determined.

Post-anaesthesia respiratory obstruction: several studies have reported post-anaesthesia respiratory obstruction (PARO) with varying frequencies. In CEPEF (2002) Johnston and colleagues reported a 3,7% of possibility while in other studies the frequency ranged between 0,04% to 1.4% (Thomas et al. 1987; Senior et al. 2013; Dugdale et al. 2016).

Horses differently from small companion animals are obligate nose-breathers and hence nasal mucosal congestion and dorsal displacement of the soft palate after oro-tracheal extubation are common causes of transient upper respiratory tract partial obstruction during recovery from general anaesthesia. PARO is usually easily recognized immediately after extubation by stertor, and most cases are easily remedied by placing a nasopharyngeal or nasotracheal tube, or replacing the orotracheal tube until the congestion resolves. Prophylactic application of topical nasal decongestant such as phenylephrine administered before the horse enters the recovery box are effective, although it has been described that timing of application of the decongestant is important (Lukasik et al 1997). Lethal consequences of PARO may follow severe (complete or near-complete) respiratory obstruction caused by either physical hindrance (e.g. secondary to nasal mucosal congestion or nostril occlusion if a patient becomes awkwardly positioned during recovery), or laryngospasm or bilateral laryngeal paresis or paralysis (Dixon et al. 1993).

Severe respiratory obstruction can cause rapidly (one or two breaths) pulmonary edema by two mechanism: negative intrapulmonary pressure (generated during frantic, stridorous inspiratory efforts), and neurogenic influences (the hyperadrenergic state created by a massive sympathetic response to profound hypoxaemia, hypercapnia and distress results in increase pulmonary capillary pressure and permeability) (Lang et al. 1990; Tute et al. 1996).

To try to prevent fatality immediate treatment should be started as soon as copious pink and frothy fluid is recognized at the mouth and nostril level (usually during or shortly after relief of the obstruction). Many factors have been suggested to be linked to PARO including ischaemia of the recurrent laryngeal nerves (secondary to head and neck over-extension during dorsal recumbency, especially with prolonged general anaesthesia), prolonged anaesthestic duration and hypoxaemia (Thomas et al. 1987; Abrahamsen et al. 1990; Ball & Trim 1996). Although intra-laryngeal nerve damage has been considered unlikely the exact aetiology remains to be determined (Rooney & Delaney 1970; Goulden et al. 1975; Holland et al. 1986; Thomas et al. 1987; Heath et al. 1989; Dixon et al. 1993; Ball & Trim 1996; Bradbury et al. 2008).

It should be notice that any nostril occlusion-type obstruction is likely to be noticed during recovery, and safe intervention is not always possible. On the other hand, respiratory obstruction associated with suspected bilateral recurrent laryngeal nerve paresis or paralysis tends to be delayed in onset and may not be noticed in time to start treatment. Obstruction has

been reported to occur sometime (minutes or hours) after the horse has stood up (uneventfully) and appears to coincide with the need for increased respiratory effort (Southwood et al. 2003; Southwood 2004; Dugdale et al. 2016). The increased respiratory effort probably simply derives from attempts to whinny to horses walking past the recovery box or to vocalize to horses while the patient is being led back to its stable. This situation on the yard often occurs a long way from help, equipment and drugs; hence, treatment may be delayed, with fatal consequences (Dugdale et al. 2016).

2.4 Risk factors associated with mortality

In literature there are many studies of equine anaesthesia-associated mortality that have reported a variety of risks factors which, if amenable to manipulation may help to reduce mortality (Tevik et al. 1983; Young & Taylor 1990; Johnston et al 1995; 2002; 2004; Mee et al. 1998 a , b; Chie Niimura et al. 2015; Dougdale et al. 2016).

American Society of Anesthesiologists (ASA) status: In human, small animal and horses worsening of ASA status has long been associated with an increased risk for mortality (Tevik et al. 1983; Mee et al. 1998; Johnston et al. 2004; Dugdale et al. 2016). Although horses suffering from colic with attendant hypovolemia and endotoxaemia are readily assigned to higher ASA grades Bidwell and colleagues (2006) reported increased mortality in horses presenting with pyrexia and/or increased white blood cell counts. These indicators of ill health may be either misinterpreted (such as pyrexia may be attributed to stress or anxiety) or undiscovered (e.g. if full haematology does not form part of the pre-anaesthestic assessment in animals otherwise perceived as healthy), resulting in the assigning of falsely low ASA grades.

Age: several studies report the association of older age with increasing risk for mortality (Johnston et al. 1995, 2002, 2004; Dugdale et al. 2016). However, CEPEF included sufficient younger animals to suggest that foals, particularly in the first month and if sick were also at increased risk. This was even more clear if anaesthesia induction was performed with a volatile agent; halothane was the most investigated agent (Johnston et al. 1995; 2002; 2004). Older

animals are more likely to suffer from comorbidities and have osteoporosis, especially in old mares (Jones 1989; Glade 1993) and this increases the incidence of fractures during recovery. Age may compound the effects of fatigue in older animals presenting for colic surgery (Johnston et al. 2002; Bidwell et al. 2007). Indeed, horses which suffer fractures in recovery do not all appear to have violent recoveries (Young & Taylor 1993). Hence, underlying muscle weakness or ataxia, or whatever cause, is thought to increase the torque experienced by the long bones which, in turn may result in their structural fracture.

Surgery type and recumbent position: In many studies emergency abdominal surgeries and internal fracture fixation have been associated with greater mortality (Johnston et al. 1995,2002,2004; Dougdale et al. 2016). However, part of this association may reflect the prolonged anaesthesia time required by these invasive surgical procedures. Mortality rate reported by Mee and colleagues (1998) where of 2% in non-colic emergencies and 5,4% in horses undergoing exploratory coeliotomy. The greater mortality affecting horses with colic was considered a result of the probably pre-existing cardiovascular compromise and greater ASA grade. Even if mortality related to colic anaesthesia seems to have recently improved to 1,6% (Dougdale et al. 2016), this improvement may be determined by the earlier referral of cases (fewer cases with high ASA grades), improved anaesthetic techniques, and a greater incidence of intra-operative euthanasia based on increasing evidence regarding long term prognosis (Proudman et al. 2002 a, b; 2006). Colic cases with the poorest prognosis were more likely to be euthanized early in the course of anaesthesia; this would explain the association of non-resection colics and short periods of anaesthesia with increased mortality (Dugdale et al. 2016). Recumbency has been variably linked with mortality and the dorsal position has usually been associated with the worst outcome (Mee et al. 1998 b; Dugdale et al. 2016, Johnston et al. 2016;). Recumbency is, however, a strong covariate of surgery type. Lateral recumbency and prolonged duration of general anaesthesia were associated with increased risk of PAM, but not for mortality, in CEPEF-3 (Johnston et al. 2004) and were considered in detail by Young (1993).

Anaesthesia and surgery duration: Duration of anaesthesia has been associated with higher mortality in several studies [>2 hours (Tevik et al. 1983); 163 minutes versus 74 minutes (Young & Taylor 1990); >2 hours and especially > 4 hours (Johnston et al. 1995)], possibly

because it is linked with more complex surgical interventions. Longer anaesthesia leading to more time during which the concentration of anaesthestic in the brain is within a hypothetical "ataxic range" would promote incoordination during recovery (Young & Taylor 1993). The generally shorter periods of anaesthesia (the majority were < 1 hour) reported by Bidwell ad colleagues (2007) appear to have made significant contribution to the relatively low immediate mortality (0,12%) identified by this group. By contrast with many other species, horses must stand up in the early postoperative period and there does not appear to be one fail-safe method to assist this process. However, during the recent online survey prior to CEPEF-4, a notable 40% of questionnaire respondents recorded the provision of some form of assistance during the recovery process (Wohlfender et al. 2015).

Out-of-hours procedures requiring general anaesthesia: even after adjusting for emergency abdominal procedures such as colic-related interventions and Caesarean sections, mortality remained higher in out-of-hours procedures (Johnston et al. 1995, 2002). The recent attention to developing a "safety culture" in the workplace has refocused attention on human factors. These include the reduction in vigilance, cognitive function and psychomotor skill performance associated with sleep deprivation, circadian rhythm disturbance and fatigue (Williamson & Feyer 2000; Ferguson et al. 2014). Around three-quarters of all incidents in aviation and anaesthesia are caused by human error and fatigue appears to contribute to the majority of these (Howard et al. 2002, Rampersad & Rampensad 2012). Longer-term consequences of shift work and chronic sleep deprivation include both mental and physical illness. Even if most of us have little control over our working days, recognition of one's own chronotype, and awareness of the onset of one's own or others' fatigue can at least warn of the increasing level of risk associated with continued working. Fatigue can be assessed using the Samn Perelli Fatigue Checklist or the Karolinska Sleepiness Scale (Richter et al. 2005), the use of which may also increase the chance that team members will look out for one another (Caldwell et al. 2008; Toff 2010). Tactics to help maintain vigilance are worth investigating and include strategies such as regular intake of healthy meals or snacks, regular intake of water to maintain hydration, intake of caffeine, exercise if possible, napping if possible, the use of bright lights in theatre, the use of checklists and the use of appropriate set, alarmed monitoring devices (Ferguson et al. 2014; Gregory & Edsell 2014). The importance of teamwork and good communication was emphasized in a special issue of the British Journal of Anaesthesia (Hardman & Moppet 2010) devoted to human factors. We should try to accept that we are all humans, embrace modern,

mindful views of "professionalism", and keep in mind this warning from Weinger & Ancoli-Israel (2002): " Physicians must recognize that is neither unprofessional nor weak to admit sleepiness or fatigue when on the job and make efforts to mitigate the potential consequences to patient care".

Anaesthetic agents, techniques and monitoring: Even if most of the patient- and surgery related factors associated with mortality are not subjected to manipulation, anaesthetic-related factors may be. Johnston and colleagues in 1995 reported that lack of premedication was associated with increased mortality, Mitchell already in 1969 suggested that premedication was beneficial. In 2002 Johnston et al. reported that the inclusion of acepromazine reduced mortality in the analysis colic and Caesarean section were excluded. Mortality was also reduced when TIVA was used (Johnston et al. 2002; Bidwell et al. 2007; Dugdale et al. 2016). Although many instances of TIVA were probably have been applied to relatively short and less complicated procedures, there are not universal features and may reflect true benefit of injectable anaesthetic agents. In support of this TIVA have been associated with a reduced stress response (Taylor 1989; 1990; Taylor et al. 1995).

To acepromazine protective effects have been associated such as anxiolytic action which can reduce circulating catecholamines that might otherwise favor development of cardiac dysrhythmias. In addition, its mild sedative effects may reduce anaesthetic induction and maintenance requirements and may contribute to calmer recoveries. Benefit from acepromazine is also apparent when it is included in protocols in which α_2-agonists are used (Marntell et al. 2005). In these circumstances, tissue perfusion in improved through enhanced cardiac output because of reduce systemic vascular resistance and increased heart rate.

This potential increase in tissue oxygen delivery is probably somewhat offset by a reduction in hematocrit caused by the splenic sequestration of erythrocytes (Marntell et al. 2005), but this may improve blood flow as a result of the reduced viscosity (Stone et al. 1968; Spier & Meagher 1989). The reduction in haematocrit is probably attributable to both the acepromazine and α_2-agonist (Parry & Anderson 1983; Kullman 2011). The reduction in systemic vascular resistance, however, may make hypotension more likely during anaesthesia (Parry et al. 1982). Hypotension is well known as a cause factor for PAM and arterial blood pressure monitoring has been associated with a reduction in the PAM severity, therefore the importance of arterial blood pressure monitoring and support cannot be overemphasized (Young & Taylor 1993; Duke et al. 2006). Johnston (et al. 2004) also stated that arterial blood pressure monitoring

reduced mortality caused by intraoperative cardiac arrest, possibly by increasing the vigilance of the haemodynamic status of the patience. Although isofluorane has been associated with a lower incidence of cardiac arrest if compared with halothane, an apparent increase in the number of spinal cord malacia cases with isofluorane (compared with halothane) implies the absence of any overall difference in mortality between these two agents (CEPEF-3 Johnston et al. 2004).

Greater mortality with isofluorane and sevofluorane in comparison with all other maintenance agents was reported by Dugdale and colleagues in 2016. This is probably caused by their more frequent general usage and a preference for their use over other agents in sicker horses undergoing long procedures. For maintenance of prolonged anaesthesia volatile agents are convenient, but the more fat-soluble compounds such as halothane and sevofluorane accumulate in the adipose tissue and can prolong recovery time (i.e. they have context-sensitive decrement times) in a manner somewhat reminiscent to the way in which injectable agents can accumulate (i.e. they have context-sensitive half times). Nevertheless, hepatic metabolism offers an alternative elimination strategy for halothane (~ 20% in metabolized) and sevofluorane (~ 2% in metabolized), which can, to some degree, offset the effect of their greater fat solubility on prolonging recovery from anaesthesia. A negligible effect on recovery is given by other volatile agents that have hepatic metabolism such as isofluorane (~ 0,2%) and desflurane (~ 0,02%). Volatile agents also produce marked dose-related cardiopulmonary depression and related profound stress response.

Despite the fact that the halo-ethers isofluorane and sevofluorane have replaced halothane (a halo-hydrocarbon) for anaesthetic maintenance, isofluorane in particular has been associated with poorer recovery quality compared with halothane (Grosenbaugh & Muir 1998; Donaldson et al. 2000), and the quality of recovery following sevofluorane may not always be superior to that following isofluorane (Leece et al. 2008). The influence of desflurane, another halo-ether but with very low blood and fat solubility, on recovery quality has also been equivocal (Jones et al. 1995; Clarke et al. 1996; Tendillo et al. 1997; Valente et al. 2015). The replacement of halothane with halo-ethers, seems to have reduced the incidence of intra- operative cardiac arrest, producing on the other side more complications during recovery, especially fractures, which currently appear to represent the leading cause of fatality. Currently anaesthesiologists are using partial/supplemental IV anaesthesia, aiming to provide a more balanced anaesthesia and analgesia with a better preservation of cardiopulmonary function and, on the other hand, less marked stress response. Using injectable agents, it is possible to reduce the required dose

of inhalation agents and improving recovery quality (Auckburally & Flaherty 2011; Gozalo-Marcilla et al. 2014,2015). It remains unclear whether this approach will reduce the morbidity and mortality associated with equine anaesthesia and surgery. Young & Taylor (1990) demonstrated that larger volumes of crystalloid fluid administration given intraoperatively were associated with increased mortality, but prolonged duration of anaesthesia was also reported as a risk factor that would have influenced the total volume of fluid administered.

Excessive crystalloid fluid administration in human and feline patients results in widespread tissue congestion and oedema (Holte et al. 2002; Grocott et al. 2005; Cotton et al. 2006; Brodbelt et al. 2007). New guidelines for fluid therapy administration have been published in veterinary medicine (Davis et al. 2013; Fielding & Magdesian 2014) and currently they are under renewed scrutiny people (National Institute for Health and Care Excellence 2013).

Many of the studies on perioperative fluid therapy investigate the interaction of different type of fluid with the endothelial glycocalyx and their immunomodulatoty effect (Boldt 2000; Gosling et al. 2003; Lang et al. 2003; Chappel et al. 2008; Muir 2009; Boldt & Ince 2010). For systemic inflammatory response, although timing of administration is important, useful effect has been seen for colloid, such as hydroxyethyl starches and hypertonic saline (Gosling 2003; Strandvik 2009). It should be kept in mind that recently colloids, especially hydroxyethyl starches, have been blamed for causing nephrotoxicity when used in critically ill human for haemodynamic support, although they were, in these cases, used in huge, and repeated doses (Chan et al. 1983; Allen et al 1986; Mythen 2005; Brandstrup 2006; Lobo et al. 2006; Santry & Alam 2010; Nolan & Mythen 2013). The Pharmacovigilance Risk Assessment Committee (PRAC) of the European Medicine Agency recommends monitoring the renal function when using colloids for the treatment of acute Hypovolemia or oncotic support (Myburgh 2015). Nephrotoxicity may also result from the administration of crystalloids with high concentrations of chloride, partially due to the resultant hyperchloremia/hyperchloremic acidosis causes renal vasoconstriction (Schneider & Bellomo 2013).

Recovery quality: the only study in equine in which it has been reported an association between recovery score and mortality has been written by Young and Taylor in 1990. The reason is probably because horses that die during anaesthesia or never stand up during recovery cannot be assigned a recovery quality score. Furthermore, although it is tempting to presume that the recovery of horses that suffer from catastrophic fractures must have been violent, this is clearly not always the case (Young & Taylor 1993). Only another group (Mee et al. 1998 a,

b) investigated recovery score as a potential factor influencing mortality, but they did not found any association, possibly because the analysis included intraoperative deaths. The same factors that influence mortality (age, ASA physical status, body position, surgery, anaesthesia duration etc.) can influence recovery quality (Young & Taylor 1990; Taylor & Young 1993; Dugdale et al. 2016). Increasing body mass, which for some time has been considered as an important factor(Johnston 1992), has recently been shown to be associated with recovery quality (Franci et al. 2006; Dugdale et al. 2016), as has horse temperament (Leece et al. 2008). The longer the period of anaesthesia maintenance with volatile agents, the less likely the anaesthetic induction agent are to effect the course of anaesthesia and recovery (Taylor & Young 1993). Poorer recoveries were reported in a recent abstract in six horses when midazolam was used in conjunction with ketamine for anaesthesia induction than when Propofol was used with ketamine, before one hour anaesthesia (Jarret et al. 2015). Poorer recovery scores following midazolam-ketamine anaesthesia induction were associated with a higher residual percentage of midazolam in the plasma at the start of recovery compared with propofol but the dose of midazolam used (0,1 mg kg^{-1}) was also higher than is commonly described. It is still to be determined the influence of sedative agents given at the time of premedication on recovery quality. TIVA techniques and sedation in early recovery may improve recovery quality (Santos et al. 2003; Ida et al. 2013; Woodhouse et al. 2013; Dugdale et al 2016).

3. Standing sedation:

The risks associated with equine anaesthesia are well known to any professional involved in equine medical care. The development and practice of sedative protocols that would allow the practitioner to perform diagnostic and surgical procedures with the patient remaining standing would therefore be ideal in certain circumstances. Sedation maintains the physiologic cardiovascular compensatory mechanisms that are commonly depressed during general anaesthesia. Maintaining the horse in a standing position also has the absolute advantage over general anaesthesia of eliminating the detrimental effect of recumbency on gas exchange and muscle perfusion. However, the physiologic advantages of standing sedation are counterbalanced by the inherent difficulties of maintaining appropriate patient restraint for the surgical procedure. Excessive sedation and muscle relaxation would induce tremors, ataxia, or worse, causing the animal to fall. A plane of sedation that is too superficial instead would potentially induce delirium and hypersensitivity to stimulation. In this regard, the use of short-acting sedatives trough continuous infusion seems to be preferable over bolus dosing of longer-acting agents. Infusion of short-acting agents allows the achievement of the wanted effect and a steady state of sedation in a more rapid fashion. Bolus dosing alone instead will likely produce intermittent peaks and troughs of levels of sedation and possibly higher risk of over- or undersedation.

The use of continuous infusions would likely provide a more constant sedative effect once the initial loading bolus has been administered. The combination of drugs with different pharmacologic action allows for reduced doses of individual drugs, thereby decreasing their side effects. A balanced approach by supplementing sedatives and tranquilizers with systemic analgesic or regional anesthetic techniques facilitates standing procedures.

Multimodal analgesia would also provide superior analgesia with potentially fewer side effects than a single- agent approach. Due to the lack of definitive evidence of superiority of one sedative protocol over another, the management of standing sedation in horses is still based on tradition, personal bias, and institutional preference rather than on scientific approaches. Nevertheless, a critical consideration is related to the safety of the staff. The safety of the personnel involved in the procedure represents the most important factor to consider when approaching a procedure under standing sedation in horses. In this regard, the margin of error becomes much narrower than during general anaesthesia and the anesthetist has the

responsibility, not only to ensure the highest level of anesthetic care to the patient but also to provide a safe and protected working condition for the operators involved.

Indications for standing sedation must consider the complexity and duration of the procedure. With appropriate sedation and analgesia, there are several procedures that can be performed in the standing conscious horse. Diagnostic procedures, such as magnetic resonance imaging, scintigraphy and endoscopy, usually require minimal or no analgesia, whereas invasive surgical interventions may require a multimodal approach to control pain. Minor surgical procedures, which are often considered for standing sedation, include tracheostomy, placement of a subpalpebral lavage system, tarsorrhaphy, removal of the nictitating membrane, and cryosurgery for removal of small cutaneous masses. Sinus surgery, excision of large cutaneous masses, fractures repair, thoracoscopy and laparascopy are examples of more invasive indications for standing sedation in which appropriate analgesia is critical for successful results. The combination of analgesic techniques with the sedation protocol would significantly reduce nociceptive stimulation and the risk of unwanted reactions of the patient.

3.1 Phenothiazine Tranquilizers

Acepromazine is commonly used for premedication. It is very effective as an anxiolytic; however, it provides only a mild to moderate degree of sedation (Muir et al. 2009). Phenothiazine tranquilizers are not believed to produce analgesia, but they do enhance the analgesic activity of other drugs such as α-2 agonists and opioids. Phenothiazines activate when given orally, intramuscularly, and intravenously by blocking the action of neurotransmitters (dopamine) centrally and peripherally and causing α adrenergic blockade that can lead to arterial hypotension (Muir et al. 1979). This is of particular concern in excitable horses, horses that have hemorrhaged, or dehydrated horses. Phenothiazines do not significantly affect respiration, but respiratory rate frequently decreases. In stallions and geldings, phenothiazine administration rarely causes persistent penile paralysis (priapism). Onset of action occurs within 15–30 min, but peak effects may not be seen for up to 45 min, a factor that may limit its use as a sole agent in clinical practice. The duration of sedation depends on the dose but may last for 6 –10 h. Acepromazine cannot be relied on to make an aggressive horse a malleable patient. Increasing the dose does not usually produce a greater effect but may increase the duration of effect. Minimal muscle relaxation or ataxia occurs, and therefore, acepromazine can be used to reduce awareness as an aide to breaking and training or to transporting the horse.

The best index of the degree of sedation with acepromazine is the presence of protrusion of the penis in male horses. In addition, the eyelids will droop, and the third eyelid will protrude. Phenothiazine tranquilizers interfere with platelet function, and thus, their use should be avoided if this is a concern (Barr et al. 1992) Hypovolemic horses administered acepromazine may become acutely hypotensive with fainting and may become recumbent. Acepromazine should be avoided if testing for allergens, because the drug has antihistaminic properties. No specific antagonist for phenothiazine tranquilizers is known.

3. 2 Alfa-2 (α2) Agonists

α2-Adrenoreceptor agonists are undoubtedly the main-stem component of any standing sedation in horses. It is realistically impossible to provide a reliable, stable, and profound degree of sedation without using an α2-adrenoreceptor agonist. Xylazine, romifidine, detomidine, and dexmedetomidine are available for use in horses (England et al. 1996). Their peak effect occurs approximately 2 to 5 and 15 to 30 minutes after intravenous and intramuscular administration, respectively (Grimsrud et al. 2009) The intramuscular dose required to produce similar intensity of sedation is approximately double the intravenous dose for all of the agents belonging to this class. All α2-agonists produce reliable, sedative, visceral, and somatic analgesic, and muscle-relaxant effects (Rohrbach et al. 2009). α2-Agonists are characterized by a "ceiling" sedative effect, whereby increasing the dose extends the duration but does not increase the intensity of sedation (Valverde 2010) After the initial bolus, there are 2 options to maintain sedation for prolonged procedures. Supplemental intravenous doses can be given as needed when the sedative effects start decreasing, at approximately one-quarter to one- half of the initial dose. Alternatively, many authors recommend the administration as CRI, avoiding the "peaks and troughs" seen with repeat bolus injections.

Common side effects of all α2-agonists include bradycardia, second-degree atrio-ventricular block, biphasic hypertension followed by hypotension, increased urine production, moderate hyperglycemia, sweating, and decreased gastrointestinal motility (England et al. 1996) Ataxia appears more profound with xylazine compared with romifidine or detomidine (Ringer et al. 2013) α2-Agonists given as a bolus cause a temporary increase in afterload with secondary depression of ventricular function and cause myocardial hypoxia due to coronary vasoconstriction. The magnitude of these effects appears to be largely dose independent and is demonstrated by the near-maximal magnitude of cardiovascular changes occurring even at

microdoses of these agents. Therefore, the use of "low doses" should not be considered safer than high doses. Instead, the preliminary evaluation and appropriate patient selection are the most important factors in determining the safety of the use of any α2-agonist (Yamashita et al 2000). Detomidine is a more specific α-2 receptor agonist than xylazine, and it has an α-2/α-1 specificity of 260:1 compared with 160:1 for xylazine detomidine has 100-fold grater affinity for α-receptors than xylazine and has a duration of action approximately twice as long. Detomidine used alone and in combination with opioids produce standing chemical restraint for a wide variety of procedures. Romifidine is an α-2 adrenoreceptor agonist with an α-2/α-1 selectivity ratio of 340:1, which places its selectivity intermediate among the currently available α-2 agonists. Romifidine causes dose-related sedation when administered intravenously or intra- muscularly, and it has a rapid onset of action and a duration of action similar to detomidine (Freeman et al. 2000). Dexmedetomidine is the most potent agent among the commercially available a2-agonists with a α-2/α-1 specificity of 1.600:1. After bolus administration, dexmedetomidine has been shown to produce cardiopulmonary changes similar to other a2-agonists, but of very short duration. Pharmacokinetic studies of dexmedetomidine in horses showed rapid distribution and rapid clearance. These pharmacokinetic characteristics favor its use as a CRI (Bettschart-Wolfensberger et al. 2005). The cost of the drug, however, still represents the major obstacle to the routine use of dexmedetomidine in equine sedation. α-2 agonists can be administered intravenously, intramuscularly, or orally.

The intensity of the cardiorespiratory side effects after IM injection is reduced, presumably because of the lower plasma concentration of unbound drug. IV administration produces a quicker onset of action and an increased intensity of effect but a shorter duration of effect. Infusions of α-2 agonists, including detomidine and medetomidine, for standing surgery and as adjuncts to IV anaesthesia are gaining popularity (Gozalo-Marcilla et al. 2012). A single bolus administration can be used for short procedures (30 minutes), whereas multiple boluses (ie, as needed) or a constant rate infusion may be necessary for longer procedures. For longer procedures, a loading dose followed by a CRI is probably best because it produces more constant plasma levels and hence more stable sedation levels, and the overall dose used may be lower than with multiple doses. Administering small additional boluses can be used to intensify the level of sedation if required.

3.3 Opioids

The analgesic effect of opiates in horses still represents a major topic of discussion and current investigation. The effect of opioids on somatic and visceral pain has been largely investigated in horses (Walker 2007; Corletto et al. 2005). Opioids have also been shown to provide a significant synergistic effect on sedation produced by α2-agonists, but when they are administered alone to pain-free animals, they can cause nervousness and excitability. The combination of an α2-agonist with an opioid allows a significant reduction of the effective dose of either agent to about half of the dose of each drug used alone (Dyson et al. 1988, Clarke et al. 1991;). The potential side effects of opioids have limited their widespread use in the past. Signs of excitement, such as head shaking and continuous pacing and gastrointestinal hypomotility, are the most feared adverse effects. These complications are rare at analgesic doses, but they can occur at much higher doses (0.5–1 mg/kg morphine) than those used clinically (Combie et al. 1979). However, the debate on the safety of these agents is still open. Opioid-induced histamine release, causing urticaria and hypotension, is possible after rapid intravenous injection. This occurrence is particularly rare; however, opioids should be given by slow injection when administered intravenously.

A single intravenous bolus of butorphanol has a short duration of action, between 30 and 60 minutes. CRIs of butorphanol, used to maintain a steady level of sedation and decrease possible behavioral side effects, have been described (Sellon et al. 2004). The use of butorphanol has been associated with increased head shaking and twitching, so it is not ideal for procedures requiring the head to be static (ie, ocular procedures). When combined with α2-agonists, butorphanol produces a potent synergistic effect.

Morphine at 0.1 to 0.2 mg/kg intravenously produces sedation and analgesia of longer duration than butorphanol. The effect after bolus administration lasts 4 to 6 hours. Morphine has been used successfully as an infusion at 0.03 mg/kg/h, following an initial bolus of 0.05 mg/kg intravenously, in combination with an a2- agonist for standing surgery (Solano et al. 2009). Methadone at 0.15 mg/kg intravenously produces a similar degree of sedation to morphine, but the rate for constant infusion has not been determined at present (Combie et al. 1979).

Buprenorphine has recently been studied in horses and appears to provide satisfactory analgesia for 8 to 12 hours (Taylor et all 2008). The onset of analgesia is slow, and the peak effect occurs at 45 to 60 minutes after bolus administration. Given the long duration of action of buprenorphine, no constant infusion for this agent has been investigated.

3.4 Drug combinations

No single drug produces “ideal” standing chemical restraint in every horse. Although the majority of sedative and analgesic drugs used for restraint are labeled for use as “sole” agents, the majority of equine veterinarians use them in combination with the goal of optimizing the onset, quality and duration of the alteration in mental state while minimizing potentially deleterious side effects. Many combinations are recommended in the literature, but relatively few have been rigorously studied scientifically. α2- agonists alone or in combination with opioids are used most commonly for this purpose (Hubbell et al. 2010). Although such drug combinations in standing sedation are useful and mitigate some of the risks associated with general anaesthesia, they are not free of adverse effects. Dose-dependent respiratory depression, hypertension, bradyarrhythmias, decreases in cardiac output, hyperglycemia, polyuria, and decreased gastrointestinal motility are commonly seen. Whereas α2- agonists are generally considered potent and useful sedatives, some horses sedated with α2- agonists may suddenly react to stimulation (even to touch), may become excited, or may develop aggression (especially with xylazine). Opioids can be used to modify some of these responses and improve the quality of the chemical restraint.

Combining opioids (morphine, butorphanol) with α2- agonists will produce a state referred to as neuroleptanalgesia, in which the level of sedation and analgesia is more profound and greatly improves the chemical restraint. The opioids can be used as a single bolus for short procedures or, for longer procedures, as multiple intermittent boluses or as a loading dose followed by a CRI. Combinations of detomidine and morphine are very useful for procedures expected to be associated with strong noxious stimulation. Morphine should be administered slowly to reduce possible risks of histamine release and to allow for early detection of an excitatory reaction.

A CRI of both drugs can then be instituted (detomidine at 0.005–0.01 mg/kg per hour and morphine at 0.03– 0.05 mg/kg per hour). Alternatively, morphine can be administered as intermittent IV boluses (slowly) as needed throughout the procedure. During the CRI, sedation level can be deepened with additional intravenous boluses of α2- agonists as needed (Guendes 2013).

Butorphanol can be used instead of morphine to improve sedation and analgesia produced by detomidine. It is ad- ministered as an initial intravenous bolus (loading dose) of 0.01–0.02 mg/kg that can be followed by a CRI (0.01–0.02 mg/kg per hour) or intermittent intravenous boluses (0.01–0.02 mg/kg intravenous) as needed. For the CRI, the drugs are best delivered with the use of syringe pumps as it allows for the most accurate dosing. However, drugs can

also be added to a bag of crystalloid fluids and delivered through gravity flow with a drip set. For maximal control of infusion rates, drugs should be given through separate syringe pumps or fluid bags.

In modern equine veterinary practice, surgical and diagnostic procedures in the standing sedated horse are expanding, especially with the growing interest in minimally invasive surgery (Dixon et al. 2005; Coomer et al. 2011; De Linde Henriksen and Brooks 2014; Menzies and Easley 2014). Both in the sedated horse and in the horse under general anaesthesia, the beneficial effects of locoregional techniques (diminished levels of sedation or anaesthesia, prevention of harmful reflexes and pre-emptive analgesic effects) are clearly described (Ong et al. 2005; Oel et al. 2014). Furthermore, in the standing equine patient it is especially important to provide a reliable local anaesthetic block regarding the safety of the horse, the veterinary surgeon and animal handlers.

4. Balanced anaesthesia:

Balanced anaesthesia is a technique of general anaesthesia based on the concept that administration of a mixture of small amounts of several neuronal depressants summates the advantages but not disadvantages of the individual components of the mixture. The objectives of balanced anaesthesia are to calm the patient, minimize pain, and reduce the potential for adverse effects associated with analgesic and anesthetic agents.

All drugs might adversely affect the patient. Some of the most profound negative effects of anaesthesia are caused by inhalant anaesthetics (Steffey et al. 1978). The concept of combining several compounds with different actions, such as amnesia, analgesia, or decrease of autonomic reflexes, was first conceived by George W. Crile in 1903 with a theory called anociassociation. Crile suggested the use of a light general anaesthesia together with local anaesthesia for blocking painful stimuli.

The term balanced anaesthesia was introduced by John S. Lundy in 1926. Lundy's idea was to utilize a balance of agents and techniques (e.g. premedication, regional anaesthesia, general anaesthesia) to achieve the different objectives desired during anaesthesia (i.e. analgesia, amnesia, muscle relaxation, and reduction or elimination of autonomic reflexes while maintaining homeostasis). The concept of balanced anaesthesia today is that, with a combination of different drugs, desired effects are achieved while untoward side effects are minimal.

Balanced anaesthetic techniques for horses aim mainly at maintaining good intra-operative cardiopulmonary function followed by calm and coordinated recoveries. The term balanced anaesthesia is currently mostly used for an inhalational anesthetic-based technique as opposed to techniques that exclude all inhalational anesthetics, which have been termed total intravenous anaesthesia (TIVA). Balanced anaesthesia techniques provide a combination of the cardiorespiratory effects observed during TIVA and volatile anaesthesia. Cardiorespiratory function tends to be better with TIVA techniques that with volatile anaesthesia (Luna et al. 1996; McMurphy et al. 2002).

Usually blood pressure is higher in TIVA due to the use of CRI of α_2-agonists, which increases the systemic vascular resistance; cardiac output is similar between both techniques even when α_2-agonists reduce heart rate, but at the same time can preserve stroke volume, whereas volatile anesthetics maintain heart rate but reduce stroke volume (Luna et al. 1996; Hubbell et al. 2012). The effects on arterial blood gases are also similar between techniques (McMurphy et al. 2002). Balanced anaesthesia preserves cardiovascular function but has the potential to depress

respiratory function to a greater extent than TIVA or volatile anaesthesia. Most studies on balanced anaesthesia have been done in mechanical ventilation therefore the effects on respiratory function have not been well defined. Partial intravenous anaesthesia (PIVA) includes the utilization of both injectable agents in association to volatile anesthetics. It has been well described that anaesthesia performed with the volatile agent alone were associated with violent and traumatic anesthetic inductions and recoveries as well as high incidence of cardiovascular and respiratory system depression or arrest (Henderson 1847).

Today many associations have been described with different induction protocols that include a dissociative agent such as ketamine with a muscle relaxant such as benzodiazepines. Maintenance is then achieved with inhaled agents in association with CRI. The most widely used volatile inhaled anesthetic in equine anaesthesia is isofluorane, in part because it is also the least expensive. Is the most potent as showed by the lowest MAC (minimum alveolar concentration) value 1,3%, but its greater solubility both delays anesthetic equilibration between the alveoli and central nervous system and delay anesthetic elimination drug recovery (Brosnan 2013). The others agents are sevoflurane, half as potent as isoflurane and desflurane the least potent. All agents cause dose dependent hypoventilation.

As a species, horses are more sensitive to the respiratory-depressant effects of volatile anesthetics than are dogs, cats, or humans. All agents also cause dose-dependent hypotension. To achieve balanced anaesthesia to these volatile inhaled agents' injectable drugs are associated. In this way central nervous system depression is reduced together with anesthetic depth and therefore decrease volatile anaesthetic requirements. It is also possible benefit from the sedative and analgesic effects of these injectable agents in the postoperative period during recovery. The adjustment in doses is based, among other factors, on the requirements for volatile anaesthesia, required duration of anaesthesia, expected degree of pain, and preference/experience of the anesthetist with the different groups included in the balanced technique. Many studies investigate different drugs and concentrations for CRI.

Most of the balanced anaesthesia techniques include the use of an α_2-agonists (xylazine, romifidine, detomidine, medetomidine or dexmedetomidine) due to their potent sedative and analgesic effect. The addition of an injectable drug is also known as partial intravenous anaesthesia (PIVA). Side effects following bolus administration include bradycardia, arrhythmias, decreases in cardiac output (CO) and increases in systemic vascular resistance, respiratory depression, transient decreases in arterial partial pressure of oxygen(PaO2) and ataxia (England & Clarke 1996; Yamashita et al. 2000). Reported sedative effects α2-agonists in horses include decreased awareness, ptosis of the head, lower lip and eyelids, ataxia and a

wide stance (England & Clarke 1996; Valverde 2010), all of which are mediated through α2-receptors (Knauset al. 2007). Intestinal motility has been shown to decrease in horses after intravenous administration of different α2-agonists (Adams et al.1984; Freeman & England 2001). These effects may vary with respect to their administration to the conscious or the anesthetized patient (Bettschard-Wolfensberger et al.2005; Solano et al. 2009; Valverde 2010; Schauvliege et al. 2011; Gozalo-Marcilla 2012). These potent effects can be exacerbated in the presence of volatile anaesthetics; however, it seems that isofluorane and sevofluorane in combination with α_2-agonists results in less cardiorespiratory depression that halothane.

The sparing effect (decrease in MAC of volatile agents) induced dose-dependently (Steffey et al. 2000 – 1991) by α_2-agonists allows for similar or better cardiovascular function at equipotent doses that the volatile anaesthetics alone (Yamashita et al. 2000). Horses administered CRIs of α_2-agonists tend to produce vast amounts of urine therefore, it is recommended that urine output be monitored and, catheterize the bladder to prevent the horse from retaining urine and voiding it the recovery stall and/or causing discomfort that affects the quality of recovery (Valverde 2010). Although ideally the bolus dose and subsequent CRI should consist of the same α_2-agonists, soma anaesthetists do not necessary use the same α_2-agonists for both purposes (Valverde 2010; Kempchen et al. 2012). Currently, xylazine, detomidine and romifidine are approved for use in horses in Europe.

Medetomidine and dexmedetomidine are licensed for small animals only, although both drugs have been investigated in horses (Bryantet al. 1991; Bettschart-Wolfensberger et al. 1999; 2005; Marcilla et al. 2012) α2-agonists reduce the MAC of volatile agents, most likely by providing extra sedation and analgesia, with a more stable maintenance of anaesthesia and better recovery qualities. However, their impact on cardiovascular function should be considered. It is worth mentioning that all the studies reporting the use ofa2-agonists were performed in healthy horses. The use of these drugs in compromised (e.g. colic) horses remains controversial.

Other drugs have been used in CRI for balanced anaesthesia. Traditionally Lidocaine has been used as a local anaesthetic agent and for treatment of premature ventricular contractions. Today it is used due to its benefits such as analgesia, minimal cardiovascular effects and anti-inflammatory action (Doherty et al. 2010). In horse's lidocaine gained widespread popularity in the last decade. It is used as an adjunct to inhalation anaesthesia to reduce the requirement for the volatile agent but also perioperatively in order to improve gut motility or to provide analgesia. As lidocaine is metabolized by the liver and has a very short half-life (Engelking et

al. 1987) it must be administered by CRI. Horses have been demonstrated to be less sensitive to adverse cardiovascular effects elicited by lidocaine in other species (Sinclair et al. 2009). Moreover concurrent administration of lidocaine with α_2-agonists that have depressive effects on intestinal motility, which may predispose to ileus (Merrit et al. 1998; Sutton et al. 2002), may prove useful in high-risk patients such as colics, owing to the prokinetic effects of lidocaine in this subpopulation (Malone et al. 2006; Torfs et al. 2009; Cook & Blikslager 2009).

Ketamine is a dissociative agent which, in systemically healthy horses, induces analgesia, amnesia, and immobility without depressing cardiovascular function. These properties make ketamine an ideal agent for balanced anaesthesia in the horse. Anaesthetic doses usually preserve adequate cardiovascular function including blood pressure, heart rate, and cardiac output (Mama et al. 2005; Wagner et al. 2012) moreover many studies describe a significant reduction in MAC of anaesthetic volatile agents with ketamine CRI (Muir & Sams 1992) and there is a reduced requirement for other anaesthetic agents with known cardiovascular depressant effects, which in turn contributes to the overall improvement of cardiovascular function (Muir et al 1992). It was also described that ketamine CRI decrease nociception in isofluorane anaesthesia in a more pronounced fashion than when the inhalant anaesthetic was used alone (Hall et al. 2000; Spadavecchia et al. 2006). Minimal respiratory depression with only mild hypercapnia were described (Persson et al. 1999).

While in human anaesthesia, short-acting opioids are the intraoperatively analgesics most commonly used for balanced anaesthesia in horses they have not yet gained widespread popularity, as their effect is debated by many authors.

Morphine is the natural reference opioid and has been used in veterinary medicine for many years in order to produce analgesia, different degrees of sedation and reduction of the minimum alveolar concentration(MAC), and/or the clinical requirements of inhalant anaesthetics in many species. Opioids have been associated with ‘excitement and unpredictable’ reactions in horses (Tobin 1981) and it has been suggested by Kamerling et al. (1989) that the behavioural and cardiovascular effects of morphine are stronger than its analgesic effects.

The different behavioural reactions to opioids compared with other species such as dogs may be related to the binding and distribution of opioid receptors in the brain (Hellyer et al. 2003). With respect to their volatile anaesthetic sparing effects, opioids do not consistently alter the MAC in the equine. Behavioural effects associated with the use of opioids in horses may affect recovery. Steffey and colleagues (2003) noticed individuals recovering violently with signs of CNS excitement when high doses (2.0 mg kg^{-1}) of morphine were administered intravenously in a MAC study in six adult horses, whereas horses that received the low dose (0.25 mg kg1)

recovered well, with little evidence of ataxia. In an experimental study, testing different concentrations of fentanyl, undesirable and excitatory behaviours were observed during recovery phase. The authors concluded that the routine use of fentanyl is not justified (Knych et al. 2009).

Other side effects that should be taken into consideration when using opioids in the equine include risks of reduction of gastrointestinal motility and postoperative colic (Roger et al. 1985; Sellonet al. 2004; Boscan et al. 2006) and respiratory depression that could lead to a rapid increase in arterial partial pressure of carbon dioxide during general anaesthesia in spontaneously breathing horses (Steffey et al. 2003). Steffey and colleagues (2003) also showed undesirable residual CNS stimulating locomotor effects after administration of morphine. Clinical evidence seems to suggest that opioids enhance the sedative and analgesic effects ofa2-agonists. Morphine, methadone and butorphanol improved the sedative effects α2-agonists, decreasing the responses to external stimuli, with butorphanol producing the most reliable response (Clarke &Paton 1988).

In conclusion, opioids may be added when using intravenous balanced anaesthetic techniques in horses providing additional analgesia and sedation. However, their inconsistent effects on the MAC, CNS stimulation, reduced gastrointestinal motility and other side effects may limit its use. Combination with other drugs such as α2-agonists may enhance their sedative and analgesic properties, providing a multimodal analgesic approach while reducing their potential excitatory effects.

4.1 Locoregional anaesthesia

In modern equine veterinary practice, surgical and diagnostic procedures in the standing sedated horse are expanding, especially with the growing interest in minimally invasive surgery (Dixon et al. 2005; De Linde Henriksen and Brooks 2014; Menzies and Easley 2014). Both in the sedated horse and in the horse under general anaesthesia, the beneficial effects of locoregional techniques (diminished levels of sedation or anaesthesia, prevention of harmful reflexes and pre-emptive analgesic effects) are clearly described (Ong et al. 2005; Oel et al. 2014). Furthermore, in the standing equine patient it is especially important to provide a reliable local anaesthetic block regarding the safety of the horse, the veterinary surgeon and animal handlers. The use of local anaesthetics for the diagnosis of lameness in horses is commonplace; the same techniques and drugs can also be used for the provision of effective perioperative analgesia.

The recent improvement in understanding pain, in terms of perception, transmission, modulation, and the development of a wide spectrum of analgesic compounds has led scientists and clinicians to suggest that effective pain control can be achieved only with multimodal analgesia. Multimodal analgesia consists of using several drugs, with different mechanism of action, interfering with perception, transmission, and modulation of pain and locoregional anaesthesia. Local anaesthetics stop the transmission of the pain signal by binding to and blocking Na^+ channels when they are in the open or inactivated state, for this reason nerves of high firing frequency are most likely to be blocked. One limitation of the local anaesthetics is their reduced performance in infected tissues, this is due to the altered pH of the tissue affecting the fraction of unionised drug (Ueno et al. 2008). The use of nerve blocks to facilitate dental and ophthalmic procedures in the standing and anaesthetised horse is well described (Harding et al. 2012). Infiltration of local anaesthetic into the testicle, cord and subcutaneously before castration in standing, sedated horses is routine; this technique is also useful in anaesthetised horses and may reduce the incidence of movement as well as requirement for supplemental doses of anaesthetics (Portier et al. 2009).

Subcutaneous infiltration of local anaesthetic is easy to perform before wound closure. Increasingly, orthopaedic surgery is being performed in standing, sedated horses and nerve blocks can improve surgical conditions and reduce the requirements for additional chemical restraint. In standing, sedated horse's distal limb blocks should be performed before arthroscopy or fracture repair, with specific nerve blocks often being used alongside a ring block.

Desensitization of the limb being operated on with local anaesthesia can also be considered in horses having the procedures done under general anaesthesia. Careful thought should be given to the individual case as ataxia and effects on control of the limb are potential hazards during recovery from anaesthesia. Intraarticular injection following arthroscopic procedures can contribute to postoperative analgesia (van Loon et al. 2010). Paravertebral thoracolumbar anaesthesia in horses having standing laparoscopic procedures produces good to excellent anaesthesia in 80% of cases (Moon & Suter, 1993). Epidural anaesthesia was first described in the horse almost 100 years ago (Pape & Pitzschk, 1925). Use of both a single injection and repeated dosing via an epidural catheter are options for both intra-operative and post-operative analgesia.

The use of multimodal analgesia utilizing drugs from different classes and given both systemically and locoregionally will improve outcomes and the welfare of patients achieving balanced and multimodal anaesthesia.

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Aim of the study

The topics addressed in the introduction of the present thesis deal with second level diagnostic imaging in equine patients and their unavoidable correlation with equine anaesthesia and standing sedation. Magnetic resonance (MRI), computed tomography (CT) and bone scintigraphy have nowadays a central role in the diagnosis of equine patient disorders and moreover, appropriate treatment plans for rehabilitation and recovery from musculoskeletal injuries are built on the foundations of an accurate diagnosis and detailed pathology characterization. The evolution in the last decades, both in anaesthesia and diagnostic imaging machines has led clinicians to investigate more deeply on advantages and disadvantages of performing these examinations in standing patients. General anaesthesia in this species is well known to be associated to higher risks due to body mass and anatomical conformation. The decision of performing second level diagnostic imaging in standing should evaluate risks and also costs, but obviously it has to be taken in account also that the ultimate aim of these examinations is to achieve high quality images for a correct and accurate diagnosis. Standing sedation is surely an excellent alternative to general anaesthesia but it is associated to motion artifacts that can drastically reduce the image quality and therefore diminish the diagnostic quality. Motion-correction techniques have been developed over recent years, and can improve image quality in the face of mild to moderate swaying motion. In severe cases, motion artifact can render the study non diagnostic. Moreover, sedation still comes with some limitations regarding the anatomical region of interest that can be examined. Using a closed (high-field) magnet with the largest bore size available or a low-field open magnet with general anaesthesia, it is sometimes possible to image as far proximal as the stifle joint in the hindlimb and the distal radius in the forelimb. With an open magnet used in standing patients, images can be obtained only from the foot distally to as far proximal as the carpus and tarsus, excluding the possibility of investigating higher anatomical regions.

The aim of the present thesis is therefore to improve standing sedation, to avoid patient motions and when anaesthesia is needed, improve balanced anaesthesia in second level diagnostic examinations.

Three studies have been included in this doctorate thesis.

The first concerns the comparison of two different sedative opioids administered intravenously in equine patients undergoing bone scintigraphy. The objective of the work was to compare butorphanol, a κ- agonist μ- antagonist, and morphine a pure μ-agonist combined with detomidine, not only considering patient sedation but also patient immobility, through the evaluation of the number of re-acquisitions required to achieve a high diagnostic image quality. As undelined in the introduction part of the thesis, motion artifact can lead to non-diagnostic

examination therefore patient immobility should be part of the method used to evaluate a standing sedation protocol in diagnostic examinations.

The second study focused on dexmedetomidine (DEX) administration in patient undergoing MRI in general anaesthesia. The aim of the study was to evaluate two routes of administration of dexmedetomidine the most selective sedative agent among the commercially available α2-agonists with a α-2/α-1 specificity of 1.600:1 (Giovannitti et al.2015). DEX was administered in horses during isofluorane general anaesthesia with a continuous rate infusion or with a subcutaneous administration as a part of a balanced anaesthesia, cardiopulmonary function and recovery quality were evaluated. As widely pointed out in the introductive part of this thesis, achieving balance anaesthesia in equine patients is mandatory to reduce the risks associated to this species during general anaesthesia. Subcutaneous administration of Dexmedetomidine has not been reported in horses, while in human patients this route has begun to be investigated only in the last few years therefore this study represents a scientific novelty regarding this topic.

In the third study an alternative approach to retrobulbar local anaesthesia was evaluated. Retrobulbar block has widely been described in veterinary medicine, also in equine patient. An alternative approach called per peribulbar block has been described in humans, dogs and cats. No studies have evaluated this technique in equine. Aim of the work was to establish the feasibility of four different techniques to perform peribulbar block in equine cadavers. Locoregional blocks were performed with contrast medium and likelihood of achieving regional anaesthesia assessed by computer tomography investigation. Since retrobulbar block is described with many and in some cases major complications peribulbar approach that has been recommended in human medicine as a safer alternative could replace retrobulbar technique in equine practice. This approach not only could be used in general anaesthesia a part of a multimodal approach to analgesia, but also in standing patient during ophthalmic surgeries avoiding in this way the need of general anaaesthesia.

This doctorate thesis consists of studies on different topics, but all aiming to increase safety in equine patients. The purpose of the study were to ameliorate standing sedation in order to achieve high quality images fundamental for a correct diagnosis and moreover an appropriate treatment plan for rehabilitation and recovery. To Describe a locoregional technique with fewer and minimal possible complications that could permit avoiding general anaesthesia and in cases in which general anaesthesia is required, the aim was to improve balanced anaesthesia in order to reduce the associated risks, bringing new information into the scientific veterinary of dexmedetomidine administration in equines.

Research papers

Paper ready for submission to an international journal

Comparison of acepromazine and detomidine combined with either morphine or butorphanol for standing sedation in horses undergoing bone scintigraphy

Objective: To compare sedative proprieties of morphine or butorphanol combined with detomidine in horses undergoing standing bone scintigraphy

Study design: Blinded, prospective, randomized clinical pilot study.

Animals: Fifty-four adult, healthy, client-owned, non-food producing horses referred to perform full body or sectorial nuclear bone scintigraphy

Methods: Horses received 0.03 mg kg $^{-1}$ acepromazine intravenously (IV) followed by detomidine 10 µg kg^{-1} IV. Then butorphanol 0.01 mg kg^{-1} (BTF group, *n= 24)* or morphine 0.25 mg kg^{-1} (MOR group, *n= 24)* was administered IV. Adjunctive detomidine boluses were given if needed to maintain and adjust sedation. Heart rate (HR), respiratory frequency (*fR)*, sedation score (SDS) (Taylor et al. 2014), total dose of detomidine and time needed to perform the exam were recorded together with number of retakes necessary to acquire high diagnostic image quality. Image quality was evaluated by the same radiologist blind to the protocol given. Acquisitions were labeled as G1 (simple) average number of retakes per image <1 and G2 (complex) acquisitions with average number of retakes ≥1. Data normal distribution were tested with the Kolmogorov-Smirnoff test and subsequently *t*-test or the Mann-Whitney test was used to assess significant differences between groups ($p < 0.05$).

Results: Heart Rate was higher in MOR group ($p = 0.004$), while *f*R was lower ($p = 0.044$), no difference in time between groups was detected (HR, $p = 0.68$; *fR,* $p = 0.9$). Time and number of detomidine boluses and total dose of detomidine were not significant between groups. In full body examination time was higher in BTF group ($p = 0.03$). Average sedation score during the procedure was higher in MOR group ($p=0.009$) while standard deviations were not different ($p=0.07$).

Both G1 and G2 retakes were statistically higher in BTF group.

Conclusions and clinical relevance: The minor number of retakes found in MOR group has led to a reduction in time for scintigraphy examination, together with a reduction in sedation

time and a reduction in time of radiation exposure of involved personnel. The higher sedation score has also allowed to obtain a higher image quality in MOR group

Introduction:

Standing sedation for diagnostic or surgical procedures is routinely used (Vigani et al. 2014) to avoid risks and costs related to general anaesthesia (Johnston et al. 2002; Franci et al 2006; Bidwell et al 2007). The disadvantage of standing techniques is strictly related to movement artefacts. Software for motion correction have become available for nuclear medicine and this compensates for the slight swaying of the horse and respiratory movements (McKnight et al 2004; Zhuo et al. 2006). Chemical restraint in horses is often achieved by using drug combinations. Alpha$_2$ agonists are routinely used alone or in combination with other drugs (Clarke et al. 1991; Hubbel et al. 2010). Different $\alpha 2$-agonists, including detomidine, produce sedation and analgesia through the alpha2-adrenoreceptors (Valverde 2010). Inclusion of an opioid provides a multimodal analgesic approach, enhancing sedation and analgesia (Clarke & Paton 1988; Schatzmann et al. 2001). Opioids play an important role in other species whereas in equine their use in pain-free animals is limited by the risk of behavioural side effects such as central nervous system excitement (Szoke et al. 1998; Carregaro et al. 2006; Sanchez et al. 2007), increasing of spontaneous locomotor activity and muscle tone (Carregaro et al. 2007) and reduction of gastrointestinal motility (Senior et al. 2004; Boscan et al. 2006). The dose, route, and duration of opioid treatment likely influence whether adverse effects develop and the severity of those effects (Tobin et al. 1979; Combie et al. 1981; Boscan et al. 2006). Butorphanol induced more ataxia and behavioral changes, including head jerks, muzzle tremor, head pressing and raised tail for up to 50 min, rarely seen with morphine in one study (Clarke and Paton 1988). Ataxia, shivering and restlessness have been reported in horses that received a single IV injection of 0.1 mg kg^{-1} butorphanol (Kalpravidh et al. 1984; Sellon et al. 2001). Nevertheless butorphanol has been more investigated than morphine for standing sedation (Cruz et al. 2004; van Hoogmoed and Galuppo 2005; Russell and Maclean 2006). The most important factor limiting the use of morphine in horses is the fear of undesirable behavioural effects, that have been described even with low doses such as 0,1 mg kg^{-1} (Combie et al. 1979). In order to limit the previously described side effects, opioids are administered to horses in conjunction with an adrenoceptor agonist or phenothiazine tranquilizer (Combie et al. 1981; Gozalo-Marcilla et al. 2018 a,b). Detomidine is an α_2 agonist well described in equine patients, adverse effects are described occurring after IV administration such as ataxia,

bradycardia, arrhythmias, increased systemic vascular resistance and reduction in cardiac output, respiratory rate (fR), arterial oxygen tension and intestinal motility (Yamashita et al 2000; Valverde 2010). Inclusion of an opioid provides a multimodal analgesic approach, enhancing sedation and analgesia and potentially reducing side effects (Potter et al 2016; Gozalo-Marcilla et al. 2017). The study aimed to evaluate the effectiveness of a multimodal sedation protocol using butorphanol and morphine combined with acepromazine and detomidine for standing bone scintigraphy in horses, in order to reduce motion artifacts.

Material and methods:

Animals: Client-owned, healthy adult, non-food producing horses referred at the Veterinary Teaching Hospital of the University of Milan to perform full or sectorial bone scintigraphy were enrolled in the study. The present study complies with ethical standards and was conducted under the approval of the Ethical Committee of the Università degli Studi di Milano (OPBA_13_2018). Owner written consent was obtained.

Instrumentation and study design: Horses were fasted for 8-12 hours with water *ad libitum,* and weighted prior nuclear scintigraphy examination. Patients were considered healthy ASA 1 or 2 (American Society of Anaesthesiologists) based on physical examination and complete blood work. Horse were randomly (www.randomizer.org) assigned to one of two treatments group (BTF or MOR). Horses received acepromazine maleate 0.03 mg kg^{-1} intravenously (IV), after fifteen minutes patients were subjected to warm up by longing for 20 minutes on both reins to avoid cold limb syndrome. After clipping and skin disinfection a 14 G angiocatheter was positioned following desensitization of the insertion site with 1 mL of Lidocaine 2%. Intravenous administration of 1 GBq/100 kg bwt 99^{m} technetium methylene diphosphonate labeled hydroxymethylene diphosphonate (HDP) was performed. To avoid limb contamination due to patient urine the lower limbs up to the fetlock and feet were protected with bandages and plastic bags. Ninety minutes prior imaging all patients received furosemide at 1 mg^{-1} kg^{-1} IV. All horse received 10 μg kg^{-1} of detomidine IV followed by either butorphanol (BTF) at 0.01 mg/kg $^{-1}$ or morphine (MOR) at 0.25 mg/kg^{-1}. Opioids were diluted with saline 0.9% to a volume of 20 ml and given within 10 minutes by slow IV injection. Horses were then placed in the stock, bandages and bags were removed, cotton was placed in the hears and blinders were positioned over the head. All static bone phase acquisitions were obtained using a single head gamma camera (Picker Prism 2000XP) with a general purpose collimator. Each acquisition was taken for 120 seconds. A lead shield was placed between the forelimbs for acquisition of lateral images and between the forelimbs and hindlimbs for acquisition of dorsal images.

Images were analyzed using a dedicated software (Hermes Nuclear Diagnostic, Gravesend, Kent, UK). Baseline assessment was recorded prior sedation. Time of administration of the initial doses of detomidine was designated T0; further assessments were recorded five (T5) and fifteen (T15) minutes later and then fifteen minutes thereafter. Image acquisition started at the end of the opioid administration in both groups. The investigator recorded HR and *f*R and, evaluated the depth of sedation using a partial simple descriptive scale (SDS) created from Taylor et al. 2014. Sedation scores were recorded with a scale from 0 to 3, with 0 indicating no sedation, animal alert, with normal posture and response to contact with assessor, and, score 3 indicating marked sedation, attempts to or become recumbent. No response to intervention (Taylor et al. 2014).

In case of patient sedation scored 0 or 1, adjunctive boluses of 2 μg kg^{-1} of detomidine were given IV. Total amount of detomidine, number of boluses and time of the administration were recorded. Furthermore, number of retakes necessary to achieve a high-quality diagnostic image were recorded. During the acquisition the diagnostic quality of the images were assessed blindly by an expert radiologist. Time needed to perform full body or sectorial nuclear bone scintigraphy was recorded. At the end of the exam cotton and blinder were removed and the patient returned in the isolated dedicated stable. A muzzle was placed for two hours. Due to Italian laws on nuclear safety and radiation protection patients were hospitalized for 48 hours during which they were monitored for signs of box-walking and abdominal pain or discomfort. Only authorised personnel were admitted to the isolated dedicated stable.

Statistical analysis:

Statistical analysis was performed using PASW 18.0 (SPSS Inc, Chicago, USA).

Data normal distribution were tested with the Kolmogorov-Smirnoff test and subsequently *t*-test or the Mann-Whitney test was used to assess significant differences between groups. Sedation score, HR and *f*R means and standard deviation were evaluated between groups (BTF, MOR) in time during examination up to 120 minutes trough Mann-Whitney test. On the basis of the mean number of retakes, acquisitions were labeled as G1 (simple acquisitions) when average number of retakes per image was <1, or as G2 (complex acquisitions) when the average number of retakes was ≥1. The number of retakes was averaged for each horse on G1 and G2, respectively. Differences across the two sedation protocols were evaluated both on G1 and on G2.

Significance was considered when $p < 0.05$.

Results:

A total of fifty-four horses were included in the study (seventeen mares, fifteen stallions and twenty-two gelding). Patients were randomly assigned to BTF (14 sectorial, 12 full body) or MOR (11 sectorial, 17 full body). There was no significant difference in age (BTF 8.8 ± 3.7; MOR 8.8 ± 3.0 years) and body weight (BTF 567 ± 58; MOR 542 ± 50 Kg; p = 0.08). Heart Rate was significantly higher in MOR group (p = 0.004) at all times; while, *f*R was higher in BTF group (p = 0.044); in both groups no statistically difference was found in HR and *fR* when considering the distribution of standard deviations suggesting equal stability in time (p=0.68 and p=0.9 respectively) (Fig 1,2). No difference in times of detomidine administration were detected; number of detomidine adjunctive administration and total dose of detomidine in time was not significant (Tab.1).

The median sedation score during the procedure was higher in MOR group (p=0.009) while standard deviations were not statistically different (p=0.07) suggesting comparable stability trough time (fig.3,4)

Both for G1 and G2, statistical differences were found across the two sedation protocols ($p<10^{-4}$). Median number of retakes for G1 where in group MOR = 0.16 ± 0.19 while in group BTF = 0.77 ± 0.81; for G2 median retakes where in group MOR = 0.81 ± 0.65 while in group BTF = 1.96 ±1.11, boxplot of the four groups are displayed in Fig 5. Considering only patients that underwent full body examination BTF = 12 horses; MOR = 17 horses: time was significative higher in group BTF 137 ± 14 min, MOR 122 ± 21 min (p = 0.03), total dose of detomidine in time was not significative different MOR 2.39 ± 0.66, BTF 2.33 ± 0.49 $\mu g^{-1}Kg^{-1}$

Discussion:

Standing sedation in horses using α2-agonists constant rate infusion (CRI) alone (Bettschart-Wolfensberg et al. 1999) or in combination with opioids (Benredouane et al. 2011; Medeiros et al 2017) is well described. The use of CRI provides a constant target concentration and prevents wide fluctuations in plasma concentrations associated with episodes of excessive or inadequate sedation and ataxia that usually occur using intermittent boluses. Side effects may be less likely to occur with use of CRI because total administered dose of the drug is less over time (Ringer et al. 2012 a; Ringer et al. 2012 b;). In this study the administration of detomidine in CRI it was not permitted owing to safety problems related to continuous shifts of the gamma camera around the horse during scanning procedures. Timing of boluses administration was evaluated based on Taylor's partial SDS scale (2014), by an expert anesthesiologist blind to the treatment

given, this approach resulted in a lack of episodes of inadequate or excessive sedation during scintigraphy examination

The total dose of detomidine given together with the number and timing of boluses administrations in the two groups was similar, whereas the degree of sedation was better in the MOR group.

It is popularly believed that butorphanol provides better sedation than morphine in horses, an unexpected finding of this study was greater sedation in the morphine group. The degree of sedation in experimental horses is usually assessed by the height of the head above the ground (Ringer et al. 2012 a, b). However, this is not assessable when the horse's head must be supported in a fixed position for diagnostic imaging procedures. For clinical studies, a simple descriptive scale (SDS) has been described in which sedation, ataxia and overall outcome were graded within four possible scores to evaluate the sedation (Taylor et al. 2014). The degree of ataxia or instability is usually evaluated by observing the spontaneous posture of the standing horse and/or describing the degree of leaning on the stocks (England et al. 1992; Figueiredo et al. 2005; Rohrbach et al. 2009; Müller et al. 2017). Alternatively, the horse can be pushed to detect swaying (Gozalo-Marcilla et al. 2017; Medeiros et al. 2017) or observed at walk on a flat surface (Ringer et al. 2013). In this work a partial SDS from Taylor (2014) was used; ataxia was not evaluated due to examination condition the significantly minor number of retakes in MOR group can partially reflect a minor movement and increased posture stability of these patients. Following opiate/sedative combinations an animal is claimed to be very stable, standing rigidly with all four legs square (Taylor 1985), moreover morphine was shown to result in less ataxia and behavioral changes in horses sedated with detomidine than other opioids including butorphanol (Clarke and Paton 1988); our findings are in accordance with literature. The mean sedation score during the procedure was higher in MOR group ($p=0.009$) while standard deviations were not statistically different ($p=0.07$) suggesting comparable stability trough time.

Both for G1 images and G2 images in group MOR the number of retakes was significantly lesser. G1 includes images acquired at the horse's pelvic region, indicating that even in this area, that is more subject to patient swinging and to respiratory movements morphine resulted in a shake reduction. A possible explanation for this finding is that the synergistic effect of morphine at the dosage used in combination with detomidine is greater than that combined with butorphanol. Moreover morphine evaluation in standing sedation protocols has been described but with lower morphine dosages (0.1 mg^{-1}/k^{-1}) (Clarke & Paton 1988; Corletto et al. 2005; Potter et al. 2016) if compared to the dose evaluated in our study (0.25 mg^{-1}/k^{-1}). The

pharmacodynamics of morphine in horses have not been completely evaluated, therefore the clinical action and also duration is yet to be determined.

Administration of opioids in horses has been reported to cause increased locomotor activity, muscle twitches, restlessness, dysphoria, excitation and cardiovascular stimulation, all these effects are dose-related. Butorphanol and morphine have been reported to induce these effects at higher dosages compared than those used in this study (Robertson et al.1981; Kalpravidh et al.1984; Boscan et al. 2006) and moreover these effects are seen when opioids are used as a single drug. The Administration of α-2 agonist drugs is believed to reduce behavioral side effects caused by opioid (Combie et al. 1979, 1981) and proved effective in the current study. Significant behavioral side effects attributable to opioid drugs did not occur in any patient either with butorphanol and morphine therefor in this study the dose of detomidine used was adequate to prevent opiate induced excitement entirely. The good sedation quality, minor number of retakes of MOR group are associated to a reduction in time needed to perform the examination.

Radiation dose to technologists working with human patients undergoing bone scintigraphy was measured and found to be only 0.5 μSv. The potential radiation exposure dose to nuclear medicine technologists and other personnel working with horses is higher than when scanning human patients. A radiation dose rate of 3 μSv/h has been documented at a distance of 2 m from the horse 2–3-h post injection (Attenburrow et al.1989) A dose rate of 50 μSv/h at 0.5 m from the surface of a horse immediately post injection of 5-MBq ^{99m}Tc-MDP has been documented, and the conclusion made that a worker spending 2 h/week for 50 weeks in a year could receive a dose of 5 mSv (Voute et al. 1995). In light of this, the reduction in time for scintigraphic examination is important in terms of cost and top-flight for radiation exposure reduction of involved personnel.

Both α-2-agonists and opioids can reduce intestinal motility (Clark et al. 1988; Sellon et al. 2004; Boscan et al. 2006; Valverde 2010) moreover with opioids in horses central nervous system excitement with increase behavioural changes still remains a concern (Bennet & Steffey 2002; Clutton 2010). At the doses used in our study no clinical signs of colic such as abdominal pain or discomfort together whit behavioural effects such as box walking, were observed in the 48 hours following Gamma-scan examination with both opioids.

The research presents some limitation. The ataxia that could have been evaluated trough Taylors sedation score system (2014), due to examination condition was not considered, in fact patient immobility and absence of swinging was evaluated by the number of image retakes that represent patient moving, swaying or change of limbs in resting position. Another limitation is

the lack of a continuous rate infusion of detomidine, CRI it was not permitted owing to safety problems related to continuous shifts of the gamma camera around the horse during scanning procedures.The CRI administration is favoured during long sedations to avoid wide fluctuations in plasma concentrations associated with intermittent boluses that result in episodes of excessive or inadequate sedation and ataxia. However, in this study timing of boluses administration was evaluated by an expert anesthesiologist blind to the treatment given, this approach resulted in a lack of episodes of inadequate or excessive sedation during scintigraphy examination

Conclusion

Detomidine did always overcome opioid induced excitement adverse effect of morphine or butorphanol at dosages used. The combination detomidine morphine reached a better sedation quality and patient immobility with a minor number of retakes and reducing examination time and staff radiation exposure time. Morphine-detomidine could be successfully used for different diagnostic standing procedures, further investigations are required.

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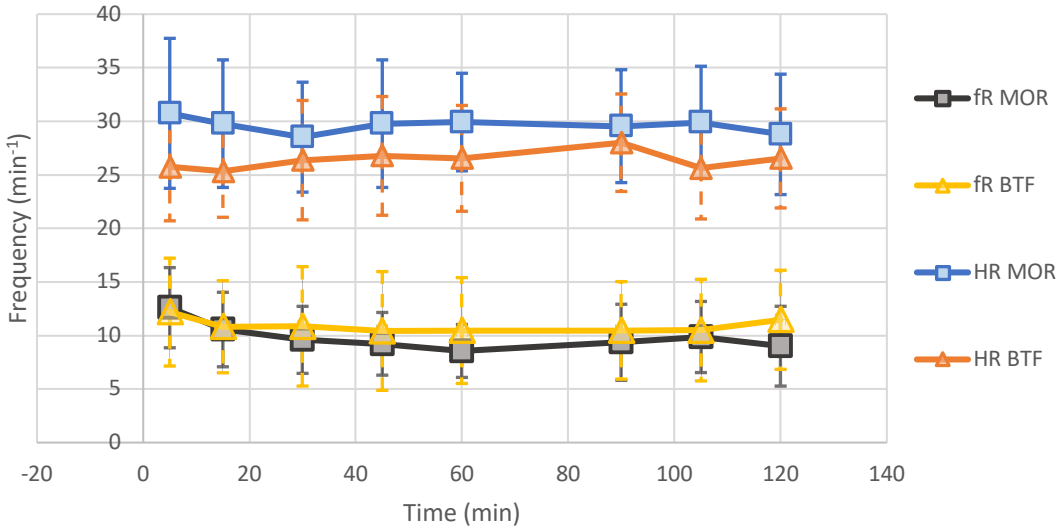
Tab.1 Mean ± standard deviation (SD) of variables of horses treated with either butorphanol or morphine in combination with detomidine for standing bone scintigraphy. All times were recorded from T0

	BTF	MOR	P value
Total administration DTM $\mu g^{-1}Kg^{-1}$	2.3 ± 0.5	3.5 ± 1.1	0.4
1° DTM minutes	31 ± 13	28 ± 16	0.4
2° DTM minutes	58 ± 15	56 ± 17	0.9
3° DTM minutes	81 ± 17	77 ± 14	0.6
Total detomidine/time $\mu g^{-1}Kg^{-1}$/exam	1.9 ± 0.5	2.1 ± 0.6	0.1

BTF, butorphanol; MOR, morphine; DTM, detomidine. Significance was considered when $p < 0.05$.

Fig 1 Heart and respiratory rates (mean and standard deviation) in time in horses treated with either butorphanol or morphine in combination with detomidine for standing bone scintigraphy.



*f*R MOR, HR MOR: respiratory rate and heart rate group morphine; *f*R BTF, HR BTF: respiratory rate and heart rate group butorphanol.

Fig. 2 Heart and respiratory rates mean and standard deviation of groups in horses treated with either butorphanol (BTF)or morphine (MOR) in combination with detomidine for standing bone scintigraphy

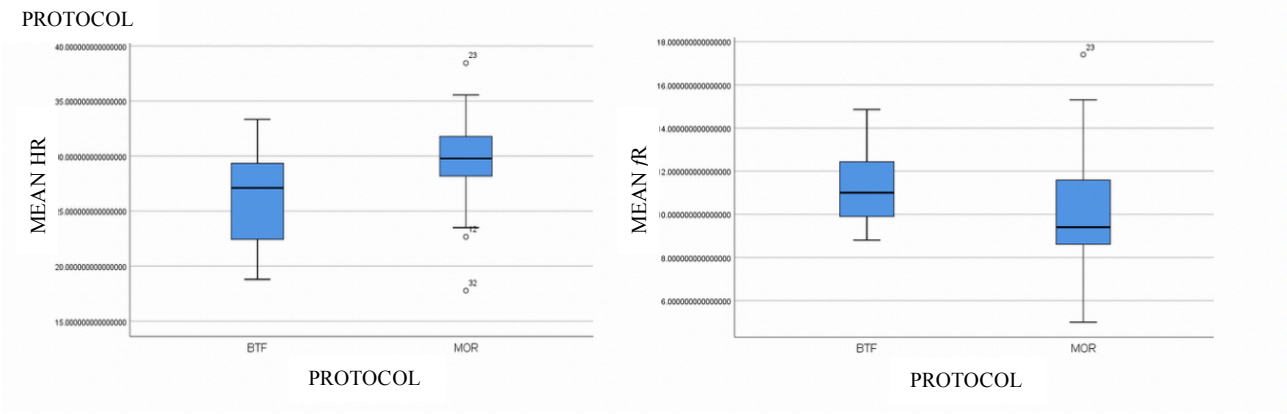


Fig 3 Sedation score (mean ± standard deviation) in time in horses treated with either butorphanol (BTF) or morphine (MOR) in combination with detomidine for standing bone scintigraphy.

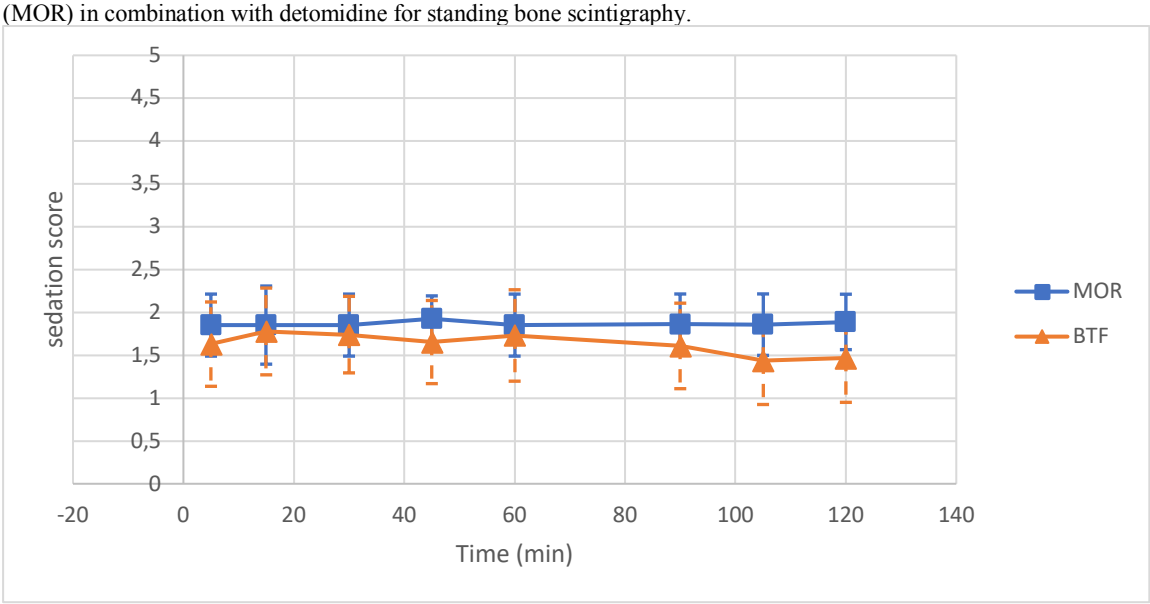


Fig. 4 Sedation score mean and standard deviation of groups in horses treated with either butorphanol (BTF) or morphine (MOR) in combination with detomidine for standing bone scintigraphy

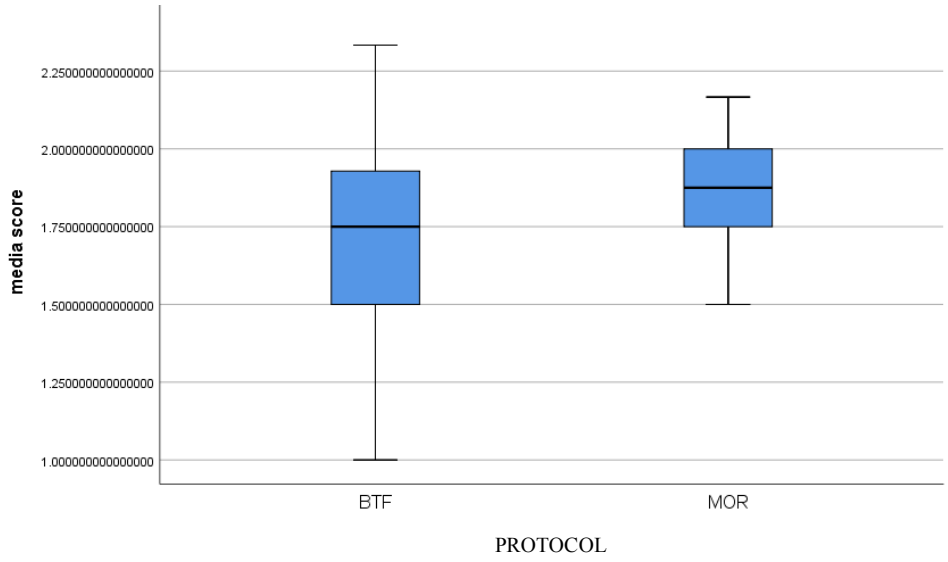


Fig. 5 Number of retakes that had to be performed on G1 and G2 for MOR and BTF groups, respectively, normalized by the number of acquisition types in each protocol. G1 was defined as the acquisition group where the amount of retakes across patients for the specific acquisition <1 on average, while G2 was the group of complex acquisition (average #retakes≥1)

Paper ready for submission to an international journal

Clinical comparison of continuous rate infusion or subcutaneous administration of dexmedetomidine in isoflurane anesthetized horses.

Abstract

Objective: To compare the effects of two administration routes of dexmedetomidine, Continuous rate infusion (CRI) and subcutaneous (SC) on cardiopulmonary function and recovery in horses

Study design: Prospective, blinded, randomized clinical study.

Animals: Thirty healthy adult horses undergoing magnetic resonance in general anaesthesia

Methods: Horses were sedated with acepromazine 0.03 mg Kg^{-1} and detomidine 10 μg kg^{-1} intravenously. General anaesthesia was induced by intravenous (IV) combination of ketamine/diazepam and maintained with isoflurane in oxygen/air. Fifteen horses each received IV dexmedetomidine by continuous rate infusion 1 μg $kg^{-1}hour^{-1}$ (CRI group) or 2 μg kg^{-1} by subcutaneous administration (SC group). Mechanical ventilation preserved end-tidal expired carbon dioxide pressures at 40-50 mmHg. Heart rate, invasive arterial blood pressure, inspired and expired gas and arterial blood gases were measured. Ringer's lactate (2 mL kg^{-1} $hour^{-1}$) and dobutamine were administered to maintain mean invasive blood pressure ≥ 70 mmHg. Recovery quality was assessed using Young and Taylor's simple descriptive scale. Data normal distribution were tested with the Kolmogorov-Smirnoff test. Student's *t test* or the Mann-Whitney and two-way ANOVA with repeated measures in one factor were applied.

Results: No significant differences in age (CRI 9 ± 2 years; SC 9 ± 3 years), body weight (CRI 523 ± 65 Kg; SC 503 ± 66 Kg) and anaesthesia time (CRI 131.9 ± 19.1 minutes; SC 137.2 ± 23.4 minutes) between groups were detected. There was significant difference between groups in dobutamine infusion rate (CRI: 0.79 ± 0.38 25 μg $kg^{-1}minute^{-1}$; SC: 0.56 ± 0.2 μg $kg^{-1}minute^{-1}$; p = 0.03). Urine output was higher in group SC (CRI: 6.7 ± 2.5 ml Kg^{-1} $hour^{-1}$; SC 8.8 ± 2.8 ml Kg^{-1} $hour^{-1}$; p = 0.02). Score system recovery quality was better with SC (4.7 ± 0.4) compared to CRI (4.2 ± 0.9) (p = 0.01). No horses required additional bolus of alpha2-agonist for recovery.

Conclusions and clinical relevance: in comparison to CRI, subcutaneous administration of dexmedetomidine results in lesser dobutamine administration and better recovery score. CRI and SC administration of dexmedetomidine at indicated dosage can be useful in balanced anaesthesia during isoflurane anaesthesia in horses undergoing magnetic resonance examination.

Introduction

General anaesthesia in equine patients carries higher risk of mortality if compared with humans and small animals (Johnston et al. 2002). A balanced anaesthetic protocol with partial intravenous anaesthesia (PIVA) using two or more ancillary agents is a common concept in modern equine general anaesthesia (Gozalo-Marcilla et al. 2014, 2015). It allows to reduce the amount of inhalant agents and therefore keeps undesirable effects to a minimum. Constant rate infusions (CRIs) of different α2-adrenergic agonists in combination with isoflurane have been used in anaesthetized horses for this purpose (Ringer et al. 2007; Devisscher et al. 2010; Schauvliege et al. 2011; Marcilla et al. 2012; Poppel et al. 2015). Furthermore, the analgesic effects of these drugs can improve recovery qualities (Bettschart-Wolfensberger & Larenza 2007; Gozalo-Marcilla et al. 2015).

Alpha2-agonists produce sedation and analgesia in all species and have been shown to reduce inhalational anaesthetic requirements when administered as a bolus (Steffey et al. 2000) or as a constant rate infusion in horses (Wagner et al. 1992; Neges et al. 2003; Kuhn et al. 2004), although clear minimum alveolar concentration (MAC) reductions were not always reported (Devisscher et al. 2010; Schauvliege et al. 2011; Marcilla et al. 2012). Classic side effects of α2-agonists in horses, include bradycardia, arrhythmias, a decrease in cardiac output and an increase in vascular resistance (England & Clarke 1996; Yamashita et al. 2000), but despite these effects, these agents have been accepted for use in balanced anaesthetic protocols.

Dexmedetomidine, the dextrorotary, active enantiomer of medetomidine has a beneficial pharmacological profile, including a shorter half-life compared to medetomidine in dogs (Kuusela et al. 2000) and both horses and ponies (Bettschart-Wolfensberger et al. 2005, Grimsrud et al. 2009). Moreover, in ponies, dexmedetomidine was shown to be redistributed more rapidly than in humans due to a larger volume of distribution. A dexmedetomidine bolus also had shorter lasting cardiopulmonary effects without a decrease in heart rate (HR) compared with medetomidine (Bettschart-Wolfensberger et al.1999b, 2005). Consequently, dexmedetomidine has been suggested to be an ideal agent for CRIs in equine anaesthesia (Bettschart-Wolfensberger et al. 2005; Marcilla et al. 2010; Marcilla et al.2012; Gozalo-

Marcilla et al. 2013a; Gozalo-Marcilla et al. 2013b, Gozalo-Marcilla et al. 2015). The use of a dexmedetomidine CRI at a rate of 1.75μg $kg^{-1}hour^{-1}$in isoflurane-anaesthetized horses under clinical circumstances produced limited cardiopulmonary effects, significantly improving recovery qualities compared to isoflurane alone (Marcilla et al. 2012).

In human medicine subcutaneous (SC) injections have been extensively used as a route of administration for drugs with short duration of action, due to ease administration, constant rate of drug absorption, better patient acceptance and better hemodynamic profile when compared to the intravenous (IV) route (McLennan et al. 2005). Slower rate of absorption of subcutaneously administered drugs, which results in a slow rise in plasma concentration of the drug in contrast to the IV route thereby resulting in much less hemodynamic instability and prolonged duration of analgesia could be attributed to the longer half-life of subcutaneously administered drugs. Preliminary investigation on pediatric patients suggest that dexmedetomidine is rapidly and efficiently absorbed after SC administration (Tobias 2008; Uusalo et al. 2018; (Srinivas & Lakshminarasimhaiah G.). The main purpose of the study was two compare two different routes of administration of dexmedetomidine (CRI and SC) in horses undergoing magnetic resonance to assess the effects on cardiopulmonary parameters and recovery phase. The hypothesis of the present study was that both routes were expected to be similarly efficacious, without expected side effects after either CRI or subcutaneous administration. Moreover, based on available pharmacokinetic data and previous clinical reports few and short-lasting cardiopulmonary effects were hypothesized during dexmedetomidine administration. Smooth recoveries were expected with both routes.

Materials and methods

Animals

Thirty client-owned, non-food-producing horses of various breeds presented at the Veterinary Teaching Hospital of the University of Milan for magnetic resonance examination in general anaesthesia were included in the study. Inclusion criteria were body weight (>350 Kg), age (4- 20 years), physical status American Society of Anesthesiologists 1 or 2 based on physical examination and blood work. The present study complies with ethical standards and was conducted under the approval of the Ethical Committee of the Università degli Studi di Milano (OPBA_12_2018). Owner written consent was obtained.

Instrumentation and study design

Horses were fasted for 12-16 hours prior anaesthesia with water *ad libitum.* Each patient at the beginning of premedication was randomly (www.randomizer.org) assigned to either group CRI (dexmedetomidine continuous rate infusion) or SC (dexmedetomidine administered subcutaneously). Demographic data and body condition score (BCS), adapted from Carroll & Huntington (1988) were recorded. All anaesthetic procedures were performed by the same experienced anaesthetist while the recovery phase was evaluated and scored by another experienced anaesthetist who was unaware of the treatment. After clipping and skin disinfection a 14 G jugular catheter was placed following desensitization of the insertion site with 1 mL of Lidocaine 2%. Each horse received acepromazine 0.03 mg kg^{-1} intravenously (IV) thirty minutes before anaesthesia induction. After 15 minutes from acepromazine administration horses were sedated with detomidine at 10 μg kg^{-1} IV. One third of the dose was given as a bolus in the stable, then the mouth was rinsed, horseshoes were removed and the horse walked into the induction area, where the remaining two-thirds of detomidine were given. The patient was positioned within the induction room and after five minutes depth of sedation was assessed. Sedation was considered adequate when criteria adapted from Taylor et al. (2014) were fulfilled. These criteria included head height lower than withers, lower lip atonic and no reaction to stimulation with a pen, when touching the inside of the ears. If one of the conditions were not present a supplemental dose of detomidine 2 μg kg^{-1} was administered IV. Sedation was then re-evaluated after five minutes and, if necessary, this procedure was repeated. Total dose of detomidine administered and time needed to achieve an adequate sedation was recorded. Anesthesia was induced with IV diazepam 0.06 mg kg^{-1} followed by IV ketamine 2.2 mg kg^{-1}.

Once the horse was recumbent and orotracheal intubation achieved (silicon tubes, internal diameter 24-28 mm) they were hoisted onto a padded MRI-dedicated table and moved into the faraday cage. All patients were in lateral left or right recumbency depending on the limb under investigation. Patients were attached to a large animal ventilator (SAMED, elettromedicali s.r.l. (LO) Italy) and mechanical ventilation was started immediately. Anaesthesia was maintained with isoflurane delivered in oxygen and air so as to maintain the inspired O_2 fraction (FiO_2) between 80 - 90%.

Every five minutes depth of anaesthesia, HR, MAP, inspired and expired gases (CO_2, O_2 and isofluorane), and temperature were evaluated and isoflurane vaporized settings were adjusted to maintain an end-tidal of 1.3-1.4 % (Datex Ohmeda S5). Intermittent positive pressure ventilation was applied, peak inspiratory pressure was set at 30 cmH_2O and positive end-

expiratory pressure at 10 cmH_2O, inspiratory to expiratory ratio was 1:2 and a tidal volume of 10 mL/kg. Respiratory rate was set at 5 breaths/min and adjusted if hypercapnia was detected ($PaCO_2$ > 60 mmHg). All parameters were adapted to maintain $PaCO_2$ between 45 and 50 mmHg.

Invasive blood pressures: systolic arterial pressure (SAP), diastolic arterial pressure (DAP) and mean arterial pressure (MAP), were recorded via catheter inserted into the transverse facial artery. The catheter was connected to a pressure transducer, placed at the level of the heart with the scapulohumeral joint as a reference point and zeroed to ambient pressure. Collection of arterial blood samples was achieved by a catheter placed in the metatarsal artery; samples (approx. 2.5 mL each) were anaerobically withdrawn into plastic heparinized syringes before dexmedetomidine administration then at t0 (administration time), t10, t50, t90, and immediately analyzed. Analysis included pH, arterial oxygen and carbon dioxide partial pressures (PaCO2, PaO2), base excess (BE) and electrolytes (Na^+, K^+, Ca^{2+}, Cl^-) as well as arterial lactate (Lac) glucose and total haemoglobin.

After collecting the white, basal arterial blood sample, that was considered as t0, an IV continuous rate infusion of dexmedetomidine at 1 $\mu g\ kg^{-1}hour^{-1}$ was delivered by an infusion pump in group CRI.

Prior to Dexmedetomidine administrations the patients bladder was catheterized and emptied, catheter was left in position to evaluate urinary output. Group SC received dexmedetomidine 2 $\mu g\ kg^{-1}$ subcutaneously every hour, the injections were performed at the level of the pectoral muscles.

Ringer's lactated solution at 2 mL $kg^{-1}hour^{-1}$ and dobutamine were administered IV. Dobutamine was administered by and infusion pump at a starting dose of 1.25 $\mu g\ kg^{-1}minute^{-1}$ that was adjusted as needed to maintain MAP ≥ 70 mm Hg. If nystagmus or fighting against the ventilator occurred, ketamine at 0.1 $mg^{-1}\ kg^{-1}$ IV was delivered. In case of sudden movements, thiopental 0.5 $mg^{-1}\ kg^{-1}$ IV was administered.

The mean cumulative dose of ketamine, thiopental and dobutamine administered to horses, together with urinary output was finally calculated over the total anaesthesia time.

The incidence of cardiac arrhythmias were also recorded. At the end of anaesthesia, dexmedetomidine CRI was interrupted and in both groups mechanical ventilation was stopped; horses were moved to a padded recovery box, in a quiet and soft lights environment. From the moment of the lateral positioning of the horse, the recovery timing started. Ventilation was assisted using a demand valve (3 breaths per minute in 100% O_2) until the horses started to

breathe spontaneously. If horses either moved or showed excessive nystagmus and were in danger of an untimely recovery, dexmedetomidine 1 μg kg^{-1} was administered IV. Oxygen flow-by was given trough the endotracheal tube. The trachea was extubated once the horses either started to show nystagmus or regained their swallow reflex. Anesthesiologist evaluated the degree of respiratory effort and if considered excessive a nasal tube was passed. Horses were allowed to recover without assistance. Time to extubation, time to sternal recumbency, and time to standing were recorded; also number of attempts to achieve sternal recumbency and standing position were noted.

An experienced blind anaesthetist assessed the recovery quality. It was graded on a standard scoring 5-point scale Young and Taylor's simple descriptive scale. A score of 5 represented a recovery with no ataxia, no struggling, standing up at first attempt as fully conscious; while, a score of 0 was used for a very violent (wall of death), self-inflicted injury, prolonged struggling or unable to stand 2 hours after the end of anaesthesia (Young & Taylor 1993).

Statistical analysis

Statistical analysis was performed using PASW 18.0 (SPSS Inc, Chicago, USA).

Data normal distribution was tested with the Kolmorgov-Smirnoff test and subsequently *t-test* or the Mann-Whitney test was used to assess significant differences between groups in terms of weight, age, anaesthesia duration, recovery times, attempts and scores.

HR, SAP, DAP, MAP and blood gas analysis parameters were analyzed using a two-way repeated-measures analysis of variance (ANOVA) for differences between groups and over time. All recorded parameters were compared at defined time points t0, t10, t25, t30, t60, t85, t90. Significance was considered when $p < 0.05$.

Results

Thirty horses of different breeds were included in the study: fifteen horses (2 stallions; 7 geldings and 6 mares) received CRI treatment; fifteen patients (one stallion; 4 geldings and 10 mares) received SC. In CRI group 10 horses and in SC group 8 horses did not fulfil the sedation criteria according to the adapted Taylor's et al. scale (2014) and required one supplemental dose of 2 μg kg^{-1} of detomidine. No significant differences in age (CRI 9 ± 2 years; SC 9 ± 3 years), body weight (CRI 523 ± 65 Kg; SC 503 ± 66 Kg), BCS, type of recumbency total duration of anaesthesia (CRI 131.9 ± 19.1 minutes; SC 137.2 ± 23.4 minutes) were identified between groups. Dexmedetomidine CRI duration was 106 ± 22 min while number of subcutaneous administration were 2.2 ± 0.5; time from the last subcutaneous administration

and end of anaesthesia was 28 ± 16 minutes. Table 1 displays cardiopulmonary parameters emphasizing no significative difference in HR between group and within groups in time. A significative higher MAP was recorded in SC group at t25, t85, t90 ($p = 0.01$). A group-independent decrease over time in SAP; DAP, MAP in both groups was noted (Fig 1).

The were no significance difference between groups at all times in $PaCO_2$ (mmHg), PaO^2 mmHg), PO_2/FIO_2, pH, lactate ($mmol/L^{-1}$) and also in electrolytes such as $Na^+(mmol/L^{-1})$, $Ca^{2+}(mmol/L^{-1})$ and $Mg^{2+}(mmol/L^{-1})$. Significance between groups at different times was found in Glucose ($mmol/L^{-1}$), Hb (g/dL^{-1}), Be ($mmol/L^{-1}$), HCO_3^- ($mmol/L^{-1}$), and electrolytes such as K^+ ($mmol/L^{-1}$) and Cl^- ($mmol/L^{-1}$). Results are summarized in table 2.

Hypercapnia was never detected therefore, respiratory rate was not modified during general anaesthesia in all horses.

There was significant difference between groups in the dobutamine infusion rate (CRI: 0,8 ± 0.4 $\mu g\ kg^{-1}minute^{-1}$; SC: 0.6 ± 0.2 $\mu g\ kg^{-1}minute^{-1}$; $p = 0.03$). Five horses in CRI group required adjunctive ketamine during general anaesthesia while in SC group only 2 horses.

Urine output was higher in group SC (CRI: 6.7 ± 2.5 ml Kg^{-1} $hour^{-1}$; SC 8.8 ± 2.8 ml Kg^{-1} $hour^{-1}$; $p = 0.02$). In 2 horses of CRI group and in 6 horses of SC arrhythmias were recorded.

Recovery times did not differ significantly between groups; also number of attempts to sternal recumbency did not differ among groups (Tab 3). Attempts to standing were higher with CRI (1.8 ± 0.8 n° attempts) compared to SC (1.4 ± 0.6 n° attempts) ($p = 0.03$). Based on Young and Taylor's score recovery quality were better with SC (4.7 ± 0.5) compared to CRI (4.2 ± 1) ($p = 0.01$). (Tab 3). No horses required additional bolus of alpha2-agonist for recovery.

Discussion:

The present study aimed to evaluate cardiopulmonary parameters and recovery quality of two different administration routes of Dexmedetomidine in isoflurane anesthetized horses undergoing magnetic resonance. Cardiopulmonary function was maintained within clinically acceptable range during general anaesthesia with both groups.

For both administration routes, typical alpha2-adrenergic agonist associated cardiopulmonary effects were recorded following induction of anaesthesia (Bettschart-Wolfensberger et al. 1999a; Kuusela et al. 2000), but cardiovascular function was well maintained with the support of dobutamine CRI and fluid substitution.

Within groups both CRI and SC showed a decrease over time of SAP DAP and MAP. These findings are in accordance with Marcilla and colleagues (2012) study on dexmedetomidine even

if in our study isoflurane was maintained with a constant end-tidal of 1.3-1.4% for the entire anaesthesia duration without modifying isoflurane to effect.

In other studies arterial blood pressure and cardiac output increased with time during prolonged constant-dose anaesthesia compared with baseline values (Steffey et al. 1987). In the present study decreasing of arterial pressure could be correlated to gradually diminishing effect of the detomidine dose used for premedication. Moreover the lack of a correct fluid supplementation based on urinary output that it is known to be increased by alpha2-agonist administration (Tranquilli et al. 1984; Steffey & Pascoe 2002) could have contribute in arterial pressure decrease in time.

The cardiovascular effects of ketamine used for induction of anaesthesia should also be considered, as these may again have influenced measured values, especially the ones used as baseline. Although studies conducted on in vitro hearts of guinea pig have showed that ketamine´s direct effect on the heart is depressant (Graf et al. 1995), it has been shown to increase the sympathetic efferent activity, hereby increasing HR, arterial blood pressure (Wong & Jenkins 1974). Nevertheless, these stimulating effects may have been counteracted in the present study by the detomidine sedation or even the administration of diazepam. MAP was always higher in SC group from t25 moreover, at times points t25, t85, t90 it was statistically higher with SC administration. Also the dobutamine infusion rate was significantly lower in SC group; this latter finding could be explained by the higher dose of dexmedetomidine administered to this group.

Interestingly statistical significative higher MAP was detected after both subcutaneous injections after 25 minutes from dexmedetomidine administration (t25, t85). These significative increases could be explained by the delay in subcutaneous adsorption of dexmedetomidine. The authors decision of two different dexmedetomidine dosages was based on dexmedetomidine short half-life and quick establish of a steady-state plasma levels when administered intravenously (Bettschart-Wolfenberger et al. 2005*;* Grimsrud et al. 2012; Rezende et al. 2015; Ranheim et al. 2015). The authors aim was to administer dexmedetomidine subcutaneously every hour. Due to the lack of studies on pharmacokinetic of subcutaneous dexmedetomidine administration in veterinary medicine a subcutaneous dosage lower than 2 μg kg^{-1} $hour^{-1}$ was hypotized not to be effective to produce effects lasting one hour and to reach a steady state of plasma concentration. In human medicine subcutaneous administration of drugs with short duration of action have been used extensively due to the constant rate of drug absorption, and better hemodynamic profile when compared to the IV route (McLennan et al. 2005).

In human pediatric patients, preliminary investigations have suggest that dexmedetomidine is rapidly and efficiently absorbed after SC administration (Tobias 2008; Uusalo 2018; Srinivas et al. 2019). Our results seem to be in accordance with what described in pediatric patients with a probably absorption peak after 25 minutes from injection, demonstrated by an already mentioned significative higher MAP in SC group at t25 and t85 and a good hemodynamic profile.

Further studies on subcutaneous pharmacokinetic are necessary in order to evaluate the exact differences of SC or CRI and the subsequent effects on the cardiopulmonary system.

Cardiovascular parameters resulted to be better and with less dobutamine requirement with subcutaneous administration suggesting that SC dexmedetomidine can probably be efficiently absorbed and capable to reach a steady-state plasma level producing effects that are comparable with a continuous rate infusion administration.

Blood gas analysis showed no significative difference among groups in blood glucose concentration at all times, while within groups there was a significative increase in glucose level at t50 and t90 for CRI group and t10, t50 and t90 in SC group; hyperglycemia has been widely described after α-2 agonist administration caused from hypoinsulinaemia (Gasthuys et al. 1986, 1987) mediated through α_{2a} and α_{2c}-receptors (Peterhoff et al. 2003) and additionally due to a reduced arginine vasopressin secretion (Alexander & Irvine 2000). Urine production was significantly higher in group SC. Alpha-2 agonists are known to increase urine output in awake (Thurmon et al. 1984) and anaesthetized healthy equids (Tranquilli et al. 1984; Steffey & Pascoe 2002), mainly due to hyperglycemia. The difference among groups in hyperglycemia together with urine output is probably related to the higher alpha-2 agonist dose administered in SC group.

Recovery from inhalant anaesthesia in the horse is a critical and difficult period to manage (Young and Taylor 1993; Johnston et al.1995). Alpha-2 adrenoceptor agonists have been used to improve recovery from anaesthesia in horses after inhalant anaesthesia. In our study recoveries were more successful at the first attempt in horses administered subcutaneous dexmedetomidine compared to CRI. An improvement in recovery quality was seen when comparing dexmedetomidine with saline (Marcilla et al. 2012). Santos and colleagues (2003) compared recovery in isoflurane anesthetized horses after administration of xylazine, romifidine and detomidine in the recovery stall. Interestingly in our study times were longer for both groups, even if compared with detomidine administration that reached in Santos study the longer times for extubation, sternal recumbency and standing. Recovery time of our treatments

were prolonged but within times suggested in literature (Clark-Price 2013) CRI 51,7 ± 14,3 minutes and SC 57,5 ± 10,3 minutes. No significative difference in time to standing was found between groups even if time from the last subcutaneous administration and end of anaesthesia was 28 ± 16 minutes. Limits of the study may be the authors decision of setting a standard isoflurane end-tidal at 1,3-1,4% without adjusting the vaporization settings based on anaesthesia depth. The authors decision was based on one of the study aims; to evaluate the dobutamine infusion isoflurane that has been extensively described to produce dose dependent hypotension in equine patients (Brosnan 2013) was maintained whit a preset end-tidal.

The other limit of the study is the utilization of a different apha-2 agonist, in this case detomidine, for patient premedication before induction. It has been described the possibility of using two different alpha-2 agonists for premedication and maintenance with isoflurane in general anaesthesia (Valverde 2013); this could be a variable to considerer when evaluating the first anaesthesia period during which detomidine effects could still be present. Nevertheless effects of detomidine after IV administration were reported to be short-lasting with a elimination half-life of 30 minutes (Grimsrud et al. 2009). Consequently, the effects of detomidine at the dose administered as premedication in the present study should have been minimal at t25 when significative higher cardiovascular parameters were noticed in SC group.

Conclusion

In conclusion cardiopulmonary function during general anaesthesia was well maintained within acceptable physiological range in both groups but better in SC group and with less dobutamine required. Recoveries from general anaesthesia were more successful at the first attempt and of better quality in horses administered dexmedetomidine subcutaneously compared to those treated with CRI, without any differences in recovery-related times. Moreover subcutaneous administration seems to produce effects that are comparable with a continuous rate infusion administration.

CRI and SC administration of dexmedetomidine at indicated dosage can be useful in balanced anaesthesia during isoflurane anaesthesia in healthy horses undergoing magnetic resonance examination.

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Tab 1 Heart rates and systolic arterial pressure (SAP), diastolic arterial pressure (DAP) and mean arterial pressures (MAP) in horses during isoflurane anaesthesia; group CRI (continuous rate infusion dexmedetomidine) and group SC (subcutaneous dexmedetomidine) (mean ± Standard Deviation SD). Significance $p \leq 0,05$

	groups	**time point**							*p value within group*	
		Basal t0	t10	t25	t30	t60	t85	t90		
heart rate (bpm)	CRI	29 ± 4	29 ± 4	29 ± 3	29 ± 3	30 ± 6	29 ± 6	29 ± 6	t10 = 0.34 t25 = 0.50 t30 = 0.34	t60 = 0.11 t85 = 0.20 t90 = 0.17
	SC	28 ± 4	27 ± 6	27 ± 4	28 ± 5	29 ± 4	28 ± 5	29 ± 5	t10 = 0.21 t25 = 0.38 t30 = 0.42	t60 = 0.10 t85 = 0.33 t90 = 0.20
	P value ***intergroups***	0.44	0.08	0.20	0.29	0.16	0.32	0.42		
DAP (mmHg)	CRI	93 ± 10	89 ± 8	80 ± 8	80 ± 8	80 ± 8	76 ± 4	76 ± 5	t10 = 0.32 **t25 = 0.02** **t30 = 0.03**	**t60 = 0.05** **t85 = 0.005** **t90 = 0.01**
	SC	90 ± 7	90 ± 8	86 ± 8	84 ± 7	81 ± 7	81 ± 6	80 ± 4	t10 = 0.17 t25 = 0.12 **t30 = 0.04**	**t60 = 0.002** **t85 = 0.01** **t90=0.0002**
	P value intergroups	0.19	0.47	**0.01**	0.053	0.45	**0.003**	**0.01**		
SAP (mmHg)	CRI	114 ± 10	109 ± 8	100 ± 8	101 ± 9	97 ± 6	95 ± 5	95 ± 5	t10 = 0.17 **t25 = 0.007** **t30 = 0.02**	**t60 = 0.003** **t85 = 0.004** **t90 = 0.004**
	SC	108 ± 8	106 ± 8	105 ± 7	102 ± 8	100 ± 9	99 ± 7	97 ± 7	t10 = 0.10 t25 = 0.15 **t30 = 0.04**	**t60 = 0.007** **t85 = 0.01** **t90 =0.0005**
	P value intergroups	0.058	0.25	**0.04**	0.30	0.14	**0.03**	0.08		
MAP (mmHg)	CRI	100 ± 10	96 ± 8	87 ± 8	87 ± 8	86 ± 7	82 ± 4	82 ± 4	t10 = 0,26 **t25 = 0,01** **t30 = 0,03**	**t60 = 0,02** **t85 = 0,004** **t90 = 0,005**
	SC	96 ± 7	95 ± 8	93 ± 7	90 ± 7	87 ± 7	87 ± 6	86 ± 5	t10 = 0,13 t25 = 0,12 **t30 = 0,03**	**t60 = 0,002** **t85 = 0,01** **t90 =0,0002**
	P value intergroups	0.13	0.35	**0.01**	0.12	0.31	**0.01**	**0.01**		

Tab 2 Mean ± standard deviation (SD) of arterial blood-gas values, arterial oxygen and carbon dioxide partial pressures ($PACO_2$, PAO_2), ratio of arterial partial pressure of oxygen to fraction of inspired oxygen (pO_2/FIO_2), arterial pH, arterial lactate and glucose concentration, arterial base excess and electrolytes in in horses during isoflurane anaesthesia; group CRI (continuous rate infusion dexmedetomidine) and group SC (subcutaneous dexmedetomidine)

parameter	**group**	**time point**			
		Basal t0	t10	t50	t90
$PaCO_2$ (mmHg)	CRI	40,98 ± 6,82	38,80 ± 7,34	38,39 ± 7,29	41,20 ± 7,82
	SC	37,39 ± 3,83	37,37 ± 5,44	37,82 ± 3,50	36,61 ± 4,22
	P value	0,09	0,31	0,41	0,09
PaO_2 (mmHg)	CRI	222,53 ± 96,30	257,58 ± 99,74	282,94 ± 97,33	320, 37 ± 106,52
	SC	229,71 ± 87,93	265,33 ± 78,43	335,61 ± 73,89	345,79 ± 80,80
	P value	0,43	0,42	0,08	0,29
pO_2/FIO_2	CRI	501,65 ± 216,37	587,55 ± 241,31	683,97 ± 282,20	812,12 ± 263,51
	SC	618,42 ± 161,69	662,90 ± 196,17	848,39 ± 185,12	843,44 ± 192,58
	P value	0,10	0,23	0,06	0,39
ph	CRI	7,42 ± 0,33	7,44 ± 0,06	7,46 ± 0,05	7,44 ± 0,06
	SC	7,45 ± 0,03	7,47 ± 0,03	7,47 ± 0,04	7,47 ± 0,03
	P value	0,09	0,13	0,35	0,08
Lactate (mmol/L^{-1})	CRI	1,10 ± 0,43	1,06 ± 0,28	1,26 ± 0,47	1,23 ± 0,52
	SC	1,42 ± 1,33	1,86 ± 1,94	1,16 ± 0,42	1,44 ± 1,03
	P value	0,24	0,11	0,29	0,29
Glucose (mg/dL)	CRI	125,89 ± 20,87	129,67 ± 29,24	183,00 ± 41,36	221,50 ± 46,67
	SC	133,00 ± 26,36	145,86 ± 23,82	199,56 ± 39,52	226,43 ± 39,63
	P value between groups	0,28	0,13	0,20	0,42
	P value within group	CRI	t10 = 0,19	**t50 = 0,0005**	**t90 = 0,0005**
	P value within group	SC	**t10 = 0,02**	**t50 = 0,0001**	**t90 = 0,002**
Hb (g/dL^{-1})	CRI	11,21 ± 1,55	10,79 ± 1,64	10,05 ± 1,03	9,72 ± 1,12

	SC	10,56 ± 1,14	10,59 ± 1,30	11,14 ± 1,03	10,70 ± 1,16
	P value	0,15	0,38	**0,01**	**0,04**
BE (mmol/L^{-1})	CRI	2,21 ± 2,32	1,97 ± 2,03	2,68 ± 1,54	3,51 ± 1,98
	SC	1,09 ± 4,53	-0,78 ± 3,13	-0,19 ± 2,85	1,33 ± 2,49
	P value between groups	0,25	**0,02**	**0,005**	**0,04**
HCO_3^- (mmol/L^{-1})	CRI	26,85 ± 2,41	26,29 ± 2,19	26,97 ± 2,06	28,09 ± 2,41
	SC	23,87 ± 2,26	23,14 ± 3,41	24,12 ± 2,94	25,40 ± 2,59
	P value between groups	**0,01**	**0,02**	**0,01**	**0,03**
Na^+ (mmol/L^{-1})	CRI	134,46 ± 1,74	135,05 ± 1,47	135,53 ± 2,17	136,41 ± 3,16
	SC	134,46 ± 2,02	134,80 ± 2,27	135,97 ± 2,00	136,60 ± 1,89
	P value	0,50	0,39	0,31	0,44
K^+ (mmol/L^{-1})	CRI	3,60 ± 0,23	3,46 ± 0,28	3,51 ± 0,27	3,62 ± 0,61
	SC	3,75 ± 0,26	3,71 ± 0,35	3,60 ± 0,26	3,70 ± 0,27
	P value	0,09	**0,04**	0,24	0,36
Ca^{2+} (mmol/L^{-1})	CRI	1,43 ± 0,04	1,40 ± 0,06	1,39 ± 0,05	1,37 ± 0,07
	SC	1,45 ± 0,08	1,43 ± 0,05	1,42 ± 0,05	1,40 ± 0,05
	P value	0,22	0,13	0,09	0,15
Cl^- (mmol/L^{-1})	CRI	102,51 ± 1,76	103,14 ± 2,38	103,05 ± 2,05	102,47 ± 3,45
	SC	105,51 ± 2,84	105,16 ± 2,74	104,66 ± 1,86	104,11 ± 1,52
	P value	**0,01**	**0,04**	**0,03**	0,10
Mg^{2+} (mmol/L^{-1})	CRI	0,59 ± 0,13	0,54 ± 0,12	0,55 ± 0,14	0,57 ± 0,14
	SC	0,67 ± 0,17	0,70 ± 0,24	0,72 ± 0,41	0,67 ± 0,18
	P value	0,15	0,06	0,15	0,12

Tab 3 Mean ± standard deviation (SD) of times (in minutes) to spontaneous breathing, to extubation, from spontaneous breathing to extubation and to sternal recumbency and standing; number of attempts to sternal recumbency and standing. Final recovery score evaluated with Young and Taylor's simple descriptive scale. A score of 5 represented a recovery with no ataxia, no struggling, standing up at first attempt as fully conscious; while, a score of 0 was used for a very violent (wall of death), self-inflicted injury, prolonged struggling or unable to stand 2 hours after the end of anaesthesia (Young & Taylor 1993). Parameters were recorded during the recovery phase in horses during isoflurane anaesthesia; group CRI (continuous rate infusion dexmedetomidine) and group SC (subcutaneous dexmedetomidine)

Parameter	**CRI**		**SC**		*P value*
	Mean	S.D.	Mean	S.D.	
Time spontaneous breathing (min)	5,7	3,6	4,4	1,9	0,09
Extubation time (min)	10,6	4,3	9,4	2,9	0,17
Time spontaneous ventilation/extubation (min)	4,9	2,1	5,1	1,9	0,43
Time to Sternal recumbency (min)	40,2	13,6	47,0	9,8	0,09
Attempts to sternal recumbency (number)	1,4	0,6	1,3	0,6	0,34
Time to Standing (min)	51,7	14,4	57,5	10,3	0,12
Attempts to stand (number)	1,8	0,8	1,4	0,6	**0,03**
Final recovery score (YT)	4,2	1,0	4,7	0,5	**0,01**

Paper submitted at the Veterinary Anaesthesia and Analgesia Journal on the 3rd December 2019

A comparison of four peribulbar anaesthetic techniques: a preliminary study in equine cadavers.

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Conflict of interest: the authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Abstract

Objective Compare peribulbar injectate distribution and regional anaesthesia likelihood of four peribulbar anaesthetic techniques in an equine cadaveric study,

Study Design Prospective randomized masked study.

Animals Twelve equine isolated heads.

Methods Twenty-four orbits underwent one of four injection techniques (six orbits each) with a mixture of contrast medium (CM) 15% and saline (1:4): ventrolateral peribulbar (PB) injection (V-20) (20 mL), dorsolateral PB injection (D-20) (20 mL), or ventrolateral and dorsolateral PB injections (10 mL each, VD-20; 20 mL each, VD-40). Needle position, injectate

distribution score at the base and within the extraocular muscle cone (EOMC), and around the optic nerve before and after pressure application on the periorbital area were assessed by computed tomography.

To asses distribution of CM scores among injection techniques Kruskal-Wallis analysis of variance was used. Mann-Whitney U test was used for post-hoc comparisons between treatments group when global test was significant.

Injectate distribution evaluation of likelihood to provide regional anaesthesia were presented with a 95% binomial confidence interval. Significance set at 0.05.

Results

CM distribution at the EOMC base was considered likely to provide regional anaesthesia before pressure application in 50% (V-20), 0% (D-20), 33% (VD-20), 100% (VD-40). After pressure application in 66% (V-20), 16% (D-20), 50 % (VD-20), 100% (VD-40). Difference in injectate distribution within the EOMC was not detected among groups. Mean optic nerve circumference was significantly higher in VD-40 and V-20. Injectate distribution considered likely to result in regional anaesthesia within the EOMC was never achieved before or after pressure application.

Conclusion and clinical relevance Peribulbar block (PBB) seems to be more likely to achieve complete regional anaesthesia using the VD-40 technique, even if authors advise caution for large volume used due to potential severe complications. Our results seem encouraging but future studies on equine PBB will be necessary to evaluate in vivo technique.

Keywords horse, local anaesthesia, nerve block, peribulbar anaesthesia, retrobulbar anaesthesia, computed tomography.

Introduction

In equine surgery, eye block techniques have been proved to be useful for standing procedures in order to avoid risks and costs of general anaesthesia and nondepolarizing neuromuscular-blocking agents that require positive pressure ventilation (Accolla et al. 2006; Hazra et al. 2008),

Local blocks have the advantage to provide multimodal analgesia and to increase patient comfort in the post-operative period (Robertson 2004). Additionally, eye block techniques reduce the risk of tissue damage by providing akinesia and help facilitate ophthalmic examinations and/or procedures (De Linde Heriksen & Brooks 2014).

Akinesia and anaesthesia of the eyeball are achieved by the blockade of the optic (II), oculomotor (III), trochlear (IV) abducens (VI) nerves, and of the maxillary and ophthalmic branches of the trigeminal nerve (V) (Miller Michau 2005). Nerves and vessels enter the orbit from the cranial cavity by way of the orbital foramina. The oculomotor and trochlear nerves, the ophthalmic branch of the trigeminal nerve and the abducens nerve travel through the orbital foramen to reach the orbit. The optic nerve and the internal ophthalmic artery enter the orbit through the optic foramen, while the maxillary branch of the trigeminal nerve passes through the foramen rotundum (Samuelson 1999; Carastro 2004).

In veterinary literature several techniques have been described to provide analgesia for ocular surgery. These techniques can be divided in intraconal (within the extraocular muscle cone EOMC) and extraconal (outside the EOMC). Human studies have found that the injectate distribution is similar with both techniques (Ripart et al. 2001).

Several approaches for retrobulbar (intraconal) administration of local anaesthetics have been described in dogs (Accola et al 2006; Giuliano 2008; Myrna et al. 2010; Shilo-Benjamini et al. 2018), cats (Shilo-Benjamini et al. 2013) and horses (Raffa et al. 1986; Miller Machau 2005; Brooks 2006; Morath et al. 2013). In each technique, a needle is inserted, blindly or under ultrasound guidance, behind the globe inside the muscle cone formed by the four recti muscles and the superior and inferior oblique muscles.

Complications of needle placement are rare but potentially devastating (Robertson 2004; Luyet et al. 2008). Possible complications of RBB include retrobulbar haemorrhage, intravenous injection of anaesthetic, globe perforation, optic and other neuropathies, extraocular muscle damage and intrathecal injection, which can induce seizures and cardiorespiratory arrest; prevalence of complications in animals is still unknown (Rubin 1995; Accola et al. 2006; Skarda & Tranquilli 2007). RBB has been widely used in human medicine, in 1986, Davis and Mendel described for the first time the peribulbar block (PBB), as a safer alternative. Nowadays many anaesthesiologists in human medicine favour the PBB that relies on local anaesthetic diffusion throughout the orbit, across the connective tissue, into the muscle that forms the EOMC and including the eyelids (Alhassan et al. 2011). The needle is introduced within the extraconal space (i.e. outside the EOMC); therefore, it is also called extraconal block.

To perform PBB a short needle is used and the local anaesthetic is injected around the globe avoiding entering the muscle cone. In comparison to the RBB, fewer complications are described due to the risk reduction of injuries of the intraconal structures, especially the optic nerve.

Classically, PBB requires two injections, one ventrolateral and a second dorsomedial; however,

some studies in humans have proven that the local anaesthetic distribution is similar performing only one injection, which may also be safer (Demirok et al. 1997; Nouvellon et al. 2010a). In veterinary medicine, few studies describe the PBB technique in dogs (Ahn J 2013; Wagatsuma et al. 2014; Shilo-Benjamini et al. 2017) and cats (Shilo-Benjamini et al. 2013, 2014) and in the last years popularity has been gained by ultrasonography for extraconal blocks (Morath et al. 2013, Wagatsuma et al. 2014; Viscasillas et al. 2019). To the author's knowledge, no studies have been published describing PBB in the equine patients.

The aim is to compare, in an equine isolated heads, injectate distribution and likelihood to reach a complete regional anaesthesia of four peribulbar anaesthetic techniques

Materials and methods

Study design:

Twelve heads from horses humanely euthanized for reasons not related to the study at the Department of Veterinary Medicine (University of the study of Milan), were used.

None had pathologies of the head region. All heads were removed from the neck immediately after death and peribulbar injections were immediately performed at room temperature of 21 °C. Each head was positioned on the computed tomography table, oriented with the nostrils towards the CT-gantry and the occipital bone in the opposite direction, in order to reproduce standing position in clinical surgical procedures.

A 1:4 mixture (by volume) of iodinated contrast agent 15% (Iomeron® 150 mg/mL, Bracco Imaging Italia S.r.l., Milan, Italy) and saline formed the injectate. The mixture distribution was evaluated trough a computed tomography scanner (CT scan). Each of the twenty-four orbits were randomly (www.randomizer.org) assigned to one of the four injections groups.

Injection techniques:

The PBB injections were performed using techniques similar to those described in human (Nouvellon et al.2010; Ripart et al. 2001) and veterinary medicine (Shilo-Benjamini 2013,2017). All peribulbar blocks were performed with a 21-gauge x 40 mm hypodermic needle (Artsana Group S.p.A, Como, Italy). The volume of injected solution was established through a comparison with the amount used in human and veterinary medicine (Nouvellon et al. 2010a Shilo-Benjamini et al. 2018) and the different dimension between human mean orbital volume 31.66 ± 1.91 cm^3 (Mustafa et al. 2015) and equine 186 cm^3 (Samuelson 1999). Moreover, the

different volumes described in human medicine between RBB and PBB (Murdoch 1990) were considered. Four injection techniques were tested (each on 6 orbits): two techniques were performed with a single 20 mL mixture injection, at the ventrolateral site (V-20) or at the dorsolateral site (D-20). The other two techniques were performed with a double injection ventrolateral and dorsolateral; six orbits were injected with a total amount of 20 mL equally divided (10 ml for each injection site VD-20) and six orbits with 40 mL equally divided (20 ml for each injection site VD-40). The ventrolateral injections were performed trans-conjunctively, at the junction between the medial and the middle third of the inferior orbital ridge and directed with a 90° angle to the surface of the skin, along the orbital floor tangent to the eyeball. The dorsolateral injections were performed trough the upper eyelid with the needle inserted laterally to the supraorbital foramen along the upper orbital wall directed with a 90° angle to the skin surface. In both injection sites the needle was always inserted blindly and with the bevel oriented towards the globe; it was advanced for is entire length in close proximity of the orbit wall. Negative pressure was obtained on aspiration prior to all injections then the mixture was slowly injected by a connected syringe; slight forward pressure was applied to the needle during injection to ensure that it remained in the desired location. A ten-minute application of 30 mmHg (Atkinson et al. 1948; Jay et al. 1985) pressure on the periorbital area surface exerted through an oculopressor (Honan balloon) carefully fixed around the head of the horse was applied. All injections were performed by an experienced veterinary anaesthesiologist.

Injectate distribution assessment

After needle placement and before each injection, a basal CT scan was performed in order to visualise the needle path needle tip position (Fig 3). On basal CT images the needle was considered as correctly positioned when the ocular bulb was not contacted, and the needle tip did not exceed the orbital rim.

Distance between the needle tip and the optic nerve were measured. A second CT scan was performed immediately after injection to evaluate the injectate distribution. The third CT scan evaluated the local diffusion of the injected mixture after pressure application. The extent of diffusion of CM mixture was evaluated by an experienced veterinary radiologist who was blinded to the injection technique.

Computed tomography was performed using a 16-slice GE Brightspeed Elite (General Electric Co., WI, USA). All images were obtained in helical mode with typical raw data acquisition at 280 mA, 140 KV, 1.25 mm slice thickness with 0.938:1 pitch. All CT examinations were

acquired using an edge-enhancing (bone) algorithm (WL=600, WW=3600). The field of view (FOV) lines were established cranially, by the second molar, caudally, by the temporomandibular joint and, centrally, by the orbit. Images (DICOM: digital imaging and communication in medicine) were reviewed on a dedicated workstation using a certified medical imaging software (OsiriX MD, Pixmeo SARL GVA). Image data sets were reformatted as necessary to view the region of interest in different anatomic planes.

Based on the Shilo-Benjamini study of 2017, the injectate volume distributions at the base of the EOMC (where the optic nerve emerges) and within the EOMC (intraconally) were each assigned a score (0=none; 1=moderate; 2=large). Additionally, the optic nerve circumference contacted by the injectate was evaluated (rated in degrees of arc 0°, 90°, 180°, 270°, 360°).

The likelihood of achieving regional anaesthesia was evaluated by two criteria formulated by Shilo-Benjamini (2017), both concluding "likely," "possible," or "unlikely." The first definition used both intraconal distribution score and optic nerve circumference contacted by the CM concluding "likely," when the contacted optic nerve was 270-360° and intraconal distribution score was 2; "possible" when the extent of optic nerve contact was 90-180° and intraconal distribution score was 1; and "unlikely," when the contacted optic nerve was 0° and intraconal distribution score was 0. The second definition for estimating likelihood of regional anaesthesia used only the injectate distribution score at the base of the EOMC: likely, with a distribution score of 2; possible, when the score was 1; and unlikely, with a score of 0. Injectate distribution around the orbital foramina such as optic foramen and foramen rotundum was recorded but not evaluated in this study.

Statistical analysis

Statistical analysis was performed using PASW 18.0 (SPSS Inc, Chicago, USA).

To globally asses the distribution of the injectate scores among technique groups Kruskal-Wallis of variance was used. The Mann-Whitney U test was used for post-hoc comparison between techniques groups when global test was considered significant.

The proportion of orbits and their injectate distribution evaluation of likelihood to provide regional anaesthesia were presented with a 95% binomial confidence interval (CI). For all statistical analysis p values < 0,05 were considered significant.

Results

Twelve fresh isolated cadaver heads were used (11 mares, 3 gelding and 1 stallion); mean age was 10.4 ± 3.9 years, and body weight 567 ± 58 Kg. Twenty-four orbits were used and each injection technique was performed successfully by the same expert anaesthesiologist in all of the six orbits of each group.

Regarding the single injection techniques, the mean minimum distance between needle tip and optic nerve were not significant between groups (listed in mm ± SD): V-20: 22.3 ± 2, D-20: 21.9 ± 3, In the double injection approaches, VD-20: dorsal needle 22.3 ± 3.3; ventral needle 22.3 ± 4.5, in VD-40: dorsal needle 24.1 ± 4.3; ventral needle 21.7 ± 4.3.

In all 24 orbits the needle positioning was considered correct after CT basal acquisition (FIG 1).

After CM mixture injection, 1 out of 6 needle positioning in D-20 group and 2 out of 6 needle positionings in VD-20 group resulted in an incorrect diffusion of CM, since there was no CM diffusion within or at the EOMC base and neither the optic nerve circumference was involved. Scores for injectate volume distribution at the base and within the EOMC, and around the optic nerve had variable ranges with all techniques and also before and after oculopressor application (Table 1).

There was no significative difference before and after pressure application within groups in CM distribution within the EOMC. Between groups, the distribution around the optic nerve was significative higher before and after pressure application in VD-40 compared to D-20 (before: $p = 0.0001$; After: $p = 0.002$) but no significative difference was with V-20 and VD-20 (before: $p = 0.6$; after: $p = 0.4$). None of the techniques, either before or after pressure application, were able to achieve “likely” regional anaesthesia when evaluated with both intraconal distribution score and optic nerve distribution criteria (Table 2).

The CM distribution at EOMC base was considered “likely” to provide regional anaesthesia in all techniques after pressure application while it was considered as “possible” in D-20 before the oculopressure application. In the VD-40 group the distribution considered “likely” was achieved in all orbits without pressure application, but severe conjunctival chemosis was noted in all eyes immediately during the second injection. Mild conjunctival chemosis was noted immediately after the peribulbar injection V-20, D-20 and VD-20 which resolved after pressure application. After pressure application, only 1 orbit in V-20 group, 1 orbit in D-20 group and 2 orbits in VD-20 group were considered “unlikely” to provide analgesia (Table 3).

In all injection techniques, CM distribution around the orbital foramina was detected. Interestingly, CM diffusion in this area was not constant (9 out of 24 injections); of these 4 orbits VD-20, 2 orbits D-20 and 3 orbits VD-40 were defined as "unlikely" or "possible" to achieve regional anaesthesia. During the injection, minimal resistance was appreciated in all the orbits with the exception in the VD-40 group in which during the second injection mild resistance was detected.

Based on these results, CM was observed in the expected peribulbar area moreover, no CM was detected within the lumen of blood vessels in any of the investigated specimens. Contrast medium diffusion, caudally and ventrally, was noted along with CM collection below the orbit. No intraocular, intravascular, intrathecal or intramural CM spread was observed in any CT scan image.

Discussion

To our knowledge, this is the first equine cadaveric study to develop and compare, injectate distribution and likelihood of regional anaesthesia of four peribulbar techniques.

In the present study the likelihood of achieving regional anaesthesia was evaluated with two different criteria. By using the first intraconal definition, the distribution of the CM was evaluated within the EOMC and around the optic nerve circumference. Based on this evaluation, none of the injection techniques reliably delivered CM distribution scores "likely" to produce regional anaesthesia. Of the 24 PBB's evaluated, none were scored as "likely" either before or after pressure application. After pressure application, 3 out of 6 (50%) injection distributions in the V-20 and VD-40 groups were evaluated as "possible" to produce anaesthesia. All injection distributions (100%) in the VD-20 group and one (16%) in the D-20 group rated as "unlikely" to produce regional anaesthesia. The reason for these results is easily explained by the fact that in our cases intraconal distribution was low or absent, in fact only 7 seven out of 24 (29%) of injection after pressure application showed intraconal distribution scored as 1. Even if no intraconal distribution was observed, the average circumference of CM around the optic nerve, after pressure application, was still rated "likely" or "possible" to achieve analgesia.

Intraconal distribution is well described and achieved through PBB in human (Nouvellon et al. 2010b) and in cats (Shilo-Benjamini et al. 2014). These studies demonstrate that peribulbar injection achieves an excellent intraconal diffusion by injection of 4 ml of bupivacaine and CM.

Different studies proved that in dogs it is difficult to obtain an intraconal diffusion using PBB (Nouvellon et al. 2010b, Shilo-Benjamini et al. 2017), even when using large injectate volume (Shilo-Benjamini et al. 2017). The same results have been observed in this study, in which intraconal distribution has been found in only in 7 out of 24 eyes with a score of 1 and using a double injection of 20 ml of CM (VD-40).

These differences in intraconal diffusion between species; dogs, cats, humans and horses may be due to anatomical variations. In humans there is no intramuscular membrane separating the extraconal and the intraconal spaces, thus permitting a continuous space where the local anaesthetic can passively spread (Ripart et al. 2001). In domestic mammals, the muscles that are responsible for the eyeball movements are four rectus muscles, two oblique muscles and the retractor bulbi, which is not present in humans. The presence of the retractor bulbi could explain the altered injectate distribution in dogs, although the presence of this muscle does not explain the difference in intraconal distribution between dogs and cats, as this muscle is also present in the latter species. In horses the retractor bulbi muscle is one of the largest extraocular muscle, it originates from the posterior orbital wall and inserts into the posterior sclera (Carastro 2004). This could influence the lack of distribution from the extraconal to the intraconal space as observed in this study. Additionally, a caudal ventral diffusion and a collection of CM below the orbit and not behind the globe was observed and noted to be increased by the ocular pressure applied. This finding was also encountered in cats by Shilo-Benjamini (2014) when performing ventrolateral injection. Anatomical differences could explain this CM spread. Compared to humans, cats have a smaller skeletal component of the inferior orbital floor (Dyce et al. 2010), while the equine orbital floor is formed by soft tissue consisting mostly of fat and resting on the pterygoid muscle (Carastro 2004).

Distribution of CM at the foramina level such as optic foramen, through which the optic nerve and the internal ophthalmic artery enter the orbit, and foramen rotundum, that gives passage to the maxillary branch of the trigeminal nerve was observed in 9/24 cases (not evaluated) (Fig 2). In human and veterinary studies on peribulbar locoregional anaesthesia this finding was never reported. It is highly possible that the distribution of anaesthetic drug within the foramina could increase the possibility of achieving regional anaesthesia since from these foramina nerves and vessels enter from the cranial cavity to the orbit. Nevertheless, based on this ex-vivo study, it is not possible to affirm that a regional anaesthesia can be reached when distribution is detected only at the foramina level even with a lack of distribution within the EOMC and around the optic nerve circumference. Moreover, it is not possible to speculate on the volume needed to achieve anaesthesia if only this region is target to be reached by the injectate.

Based on Shilo-Benjamini study (2017) the likelihood of achieving adequate regional analgesia was also evaluated at the EOMC base. At this level, the ophthalmic branch of the trigeminal nerve emerges from the orbital fissure which is adjacent to the optic nerve as it emerges from the optic foramen (Evans & Kitchell 1993). Evaluation of CM diffusion of at the EOMC base after pressure application showed an increase likelihood to achieve analgesia. The use of an oculopressor is well described in human medicine (30 mmHg for 5–10 minutes) (Meenakashi et al. 2015) and allows an improvement of the injectate distribution, avoiding elevated periorbital/peribulbar pressure and chemosis. In our study, the pressure application after the injection promoted a better CM distribution at the EOMC base, increasing the percentage of injections to be considered "possible" and "likely".

In humans, a single needle technique is generally preferred due to the less anatomical distortion and reduced risk of complications; in some selected cases a second injection should be performed, when the first injection has failed to provide effective anesthesia (Demirok et al. 1997; Nouvellon et al. 2010a).

In this study, a ventral (V-20) or dorsal (D-20) single injection and a double injection with different volumes (VD-20 and VD-40) were evaluated. The single ventral injection was significantly more effective than the dorsal approach, based on CM distribution at the EOMC base. The single ventral approach also appeared to be more effective than the double approach using 20 ml of CM, however this was non-significant. The double injection with 40 ml of CM was the technique that most frequently score as "likely" to provide anaesthesia probably due to the higher volume injected. Despite the satisfactory distribution it should be kept in mind that a severe conjunctival chemosis was noted immediately during the second injection.

Possible complications described following PPB include exophthalmos, chemosis and ecchymosis due to small blood vessels damage caused by rostral spread of the injected volume (Shilo-Benjamini 2019). As reported in dogs (Wagatsuma et al. 2014), cats (Shilo-Benjamini et al. 2014, 2016) and in human medicine (Alhassan et al. 2011), these complications usually resolve spontaneously and do not interfere with surgery. In cats (Shilo-Benjamini 2019) a transient but clinically increase in intraocular pressure (IOP) was observed. In human ophthalmic anaesthesia the Honan balloon has been routinely used as a compression device after injection of local anaesthesia, not only to help injectate diffusion but also to reduce IOP (Atkinson et al. 1948; Rodriguez et al. 2003). Increased IOP is not a concern when enucleation is planned; however PPB should be used with caution or not used at all in patients whit globes at risk of rupture or glaucoma (Shilo-Benjamini et al. 2014; Allgoewer 2018).

It should be considered that the present work is an ex-vivo study and that the distribution should be negatively influenced by the viscosity of CM. Even in dilution, CM is a viscous liquid compared with local anaesthetic solution. In this study CM was maintained at room temperature 21 °C. In human medicine studies have focused on the evaluation of CM viscosity at different temperatures, showing a decrease in viscosity with higher temperatures (Kok et al. 2014). Furthermore, vascular circulation, important for the injectate distribution, was not present. These two factors; the contrast high viscosity and the absence of normal blood flow, would likely impede the distribution of CM. This in turn, may have result in an underestimation of *in vivo* spread of local anaesthetic.

In human medicine, advantages and disadvantages of the RBB versus PBB are still controversial (Murdoch 1990; Ripart et al. 2001; Alhassan et al. 2011). Although retrobulbar anaesthesia is classically assumed to be more efficacious than peribulbar, it appears that, by using a larger volume of local anaesthetic for PBB, both techniques have a similar efficacy. Further studies on PBB should focus on the use of smaller amounts of injectate and on calibrating these volumes to the equine head dimensions. (Mustafa et al. 2015).

Although we did not perform ocular examination or dissections following the injection, we did not observe any intraocular, intravascular, intrathecal or intramural injectate on CT images. Additionally, the mean minimum distance measured between the tip of the needle and the optic nerve was above 22 mm for all injection techniques, indicating that the risk of puncturing the optic nerve or the ophthalmic artery that passes next to it was very low.

The study has several limitations such as the small sample size and the average room temperature that, as previously described, could influence the CM distribution. Also the techniques and the volume of CM injected are based on human and small animal literature, where anatomy is quite different from the equine anatomy. The likelihood of achieving regional anaesthesia was based on CT images and may not correlate to effectiveness of locoregional anaesthesia in living animals. In humans (Ghali & El Btarny 2010; Bowman et al. 2013), cats (Shilo-Benjamini et al. 2013) and dogs (Wagatsuma et al. 2014) increase intacocular pressure (IOP) after PBB injection has been reported. Usually this increase does not persist beyond 10 minutes. In the present study this parameter was not evaluated, since no literature was present on the technique in equine species. The authors, instead, focused on the feasibility and the injectate distribution. Further studies should include the evaluation of IOP during PBB.

Conclusion

In conclusion PBB seems to be more likely to achieve regional anaesthesia with the VD-40 technique. Although the authors advise caution for large volume use due to potential severe complications associated with it. The V-20 technique was the most likely to produce anaesthesia even if in D-20 technique 66% of injections were evaluated as “possible” to achieve analgesia.

These techniques need to be evaluated in clinical trials to assess their feasibility and effectiveness in locoregional anaesthesia. Further investigations on equine PBB are needed. Our results seem to be encouraging but future studies will be necessary to evaluate this technique in vivo.

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Table 1: median distribution scores following peribulbar injections of contrast medium at 15% in 24 orbits before and after pressure application. V-20 (6 orbits): ventrolateral single injection of 20 ml; D-20 (6 orbits):dorsolateral single injection of 20 ml; VD-20 (6 orbits): double injection, ventrolateral and dorsolateral of 20 ml equally divided; VD-40 (6 orbits): double injection, ventrolateral and dorsolateral of 20 ml equally divided. One radiologist evaluated injectate distribution at the base of the extraocular muscle cone (EOMC) and within (0 = none; 1 = moderate; 2 = large) and at the circumference of the optic nerve with which injectate came in contact (degree of arc 0°, 90°, 180°, 270°, 360°).

Group	Volume Distribution score within the EOMC (0-2)		Optic nerve circunference contacted (%)		Volume distribution score EOMC base (0-2)	
	pre	post	pre	post	pre	post
V-20	0,1	0,5	165	240	1,2	1,5
D-20	0	0,1	105	150	0,6	1,1
VD-20	0	0	120	180	0,8	1,1
VD-40	0,5	0,5	345	345	2	2

Table 2 Number (percentage) of orbits in which regional anaesthesia was considered "unlikely", possible or "likely" to achieve regional anaesthesia. Contrast medium (at 15%) distribution was evaluated thought computed tomography. Distribution scores of intraconal and optic nerve circumference prior and after pressure application were considered "likely," contacted optic nerve 270-360° intraconal distribution 2; "possible" contacted optic nerve 90-280° intraconal distribution score 1; "unlikely," contacted optic nerve 0° intraconal distribution.
V-20 (6 orbits): ventrolateral single injection of 20 ml; D-20 (6 orbits):dorsolateral single injection of 20 ml; VD-20 (6 orbits): double injection, ventrolateral and dorsolateral of 20 ml equally divided; VD-40 (6 orbits): double injection, ventrolateral and dorsolateral of 20 ml equally divided. CI, confidence interval 95%.

Intraconal distribution and optic nerve circumference before pressure						
Group	Unlikely	C I	Possible	C I	Likely	C I
V-20	5 (83%)	33-99%	1 (16%)	3-56%	0 (0%)	0-39%
D-20	6 (100%)	51-100%	0 (0%)	0-39%	0 (0%)	0-39%
VD-20	6 (100%)	51-100%	0 (0%)	0-39%	0 (0%)	0-39%
VD-40	3 (50%)	18-81%	3 (50%)	18-81%	0 (0%)	0-39%
Intraconal distribution and optic nerve circumference after pressure						
Group	Unlikely	C I	Possible	C I	Likely	C I
V-20	3 (50%)	18-81%	3 (50%)	18-81%	0 (0%)	0-39%
D-20	5 (83%)	33-99%	1 (16%)	3-56%	0 (0%)	0-39%
VD-20	6 (100%)	51-100%	0 (0%)	0-39%	0 (0%)	0-39%
VD-40	3 (50%)	18-81%	3 (50%)	18-81%	0 (0%)	0-39%

Table 3 Number (percentage) of orbits in which regional anaesthesia was considered unlikely, possible or likely to achieve regional anaesthesia. Contrast medium (at 15%) distribution was evaluated through computed tomography. Distribution scores at the extraocular muscle cone base were considered likely with a distribution score of 2, possible when the score was 1 and unlikely with a score of 0

V-20 (6 orbits): ventrolateral single injection of 20 ml; D-20 (6 orbits): dorsolateral single injection of 20 ml; VD-20 (6 orbits): double injection, ventrolateral and dorsolateral of 20 ml equally divided; VD-40 (6 orbits): double injection, ventrolateral and dorsolateral of 20 ml equally divided. CI, confidence interval 95%.

Extraconal base distribution before pressure						
Group	unlikely	IC	possible	IC	likely	IC
V-20	2 (33%)	6-75%	1 (16%)	3-56%	3 (50%)	18-81%
D-20	2 (33%)	6-75%	4 (66%)	24-94%	0 (0%)	0-39%
VD-20	3 (50%)	18-81%	1 (16%)	3-56%	2 (33%)	6-75%
VD-40	0 (100%)	0-39%	0 (0%)	0-39%	6 (100%)	51-100%
Extraconal base distribution after pressure						
Group	unlikely	IC	possible	IC	likely	IC
V-20	1 (16%)	3-56%	1 (16%)	3-56%	4 (66%)	24-94%
D-20	2 (33%)	6-75%	3 (50%)	18-81%	1 (16%)	3-56%
VD-20	2 (33%)	6-75%	1 (16%)	3-56%	3 (50%)	18-81%
VD-40	0 (0%)	0-39%	0 (0%)	0-39%	6 (100%)	51-100%

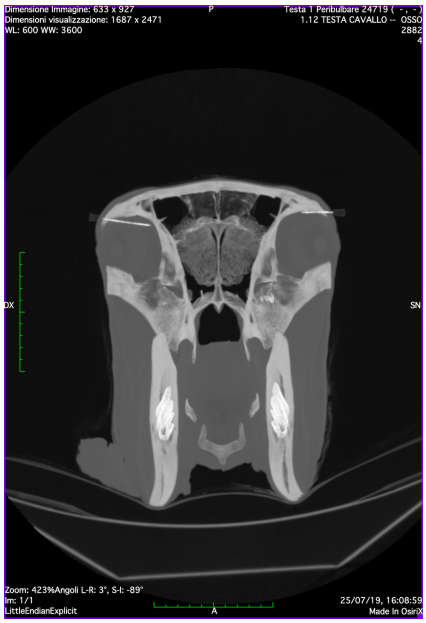

FIG 1: Dorsolateral Group. Transverse oblique MIP (maximum intensity projection) view, parallel to the needle, showing the distance from the needle tip and the optic nerve

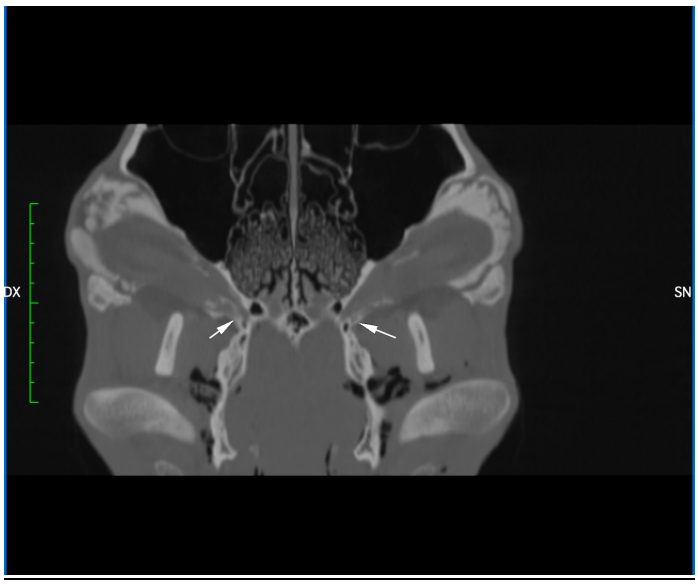

FIG 2: dorsal view at the level of the optic nerve emergence. Note the contrast agent within the foramen, bilaterally (white arrow). Images were acquired after ventrolateral and dorsolateral injection of 20 mL of contrast agent

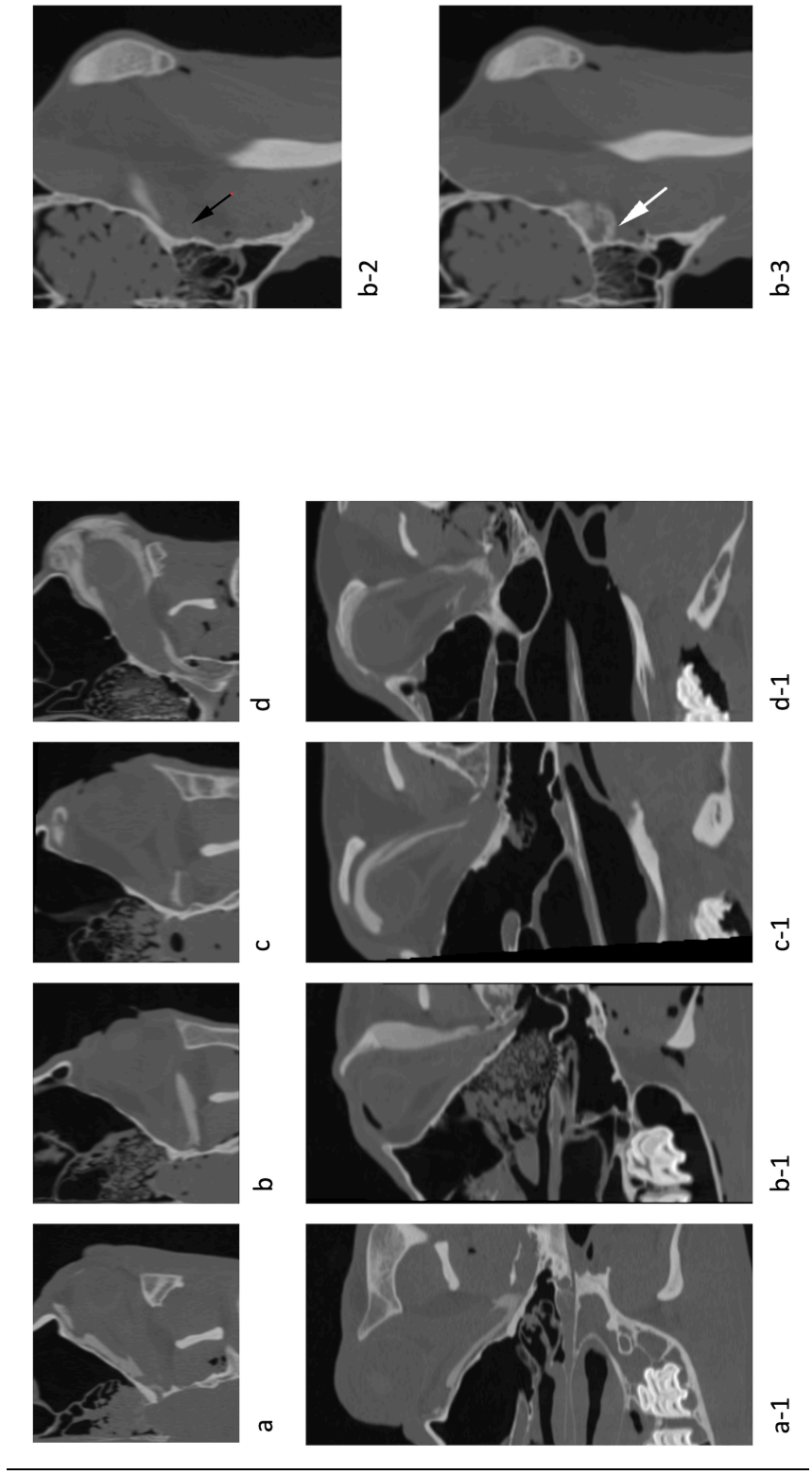

FIG 3
Dorsal plane (a,b,c,d), reformatted sagittal oblique plane (a-1, b-1, c-1, d-1) and transverse plane (a-2, a-3) computed tomography images of the left orbit of four horse isolated heads followings injections of iodinated contrast agent. (a) Injection of 20 ml of contrast agent via a single ventrolateral peribulbar injection and (b) via a single dorsolateral injection. Contrast

agent was injected via a double approach, dorsolateral and ventrolateral, using 10 ml per site (c) and 20 ml per site (d), respectively.
The same orbit before (b-2) and after (b-3) pressure application: note the lack of contrast agent at the EOMC base (black arrow) before pressure application. After pressure, the contrast reach the EOMC base and it is seen within the foramina (white arrow).

General discussion

This doctorate thesis deals with risks and benefits that must be considered wen performing diagnostic imaging in equine patients. It has been extensively described that the unique body mass and anatomical conformation of horses makes this species more at risk during general anaesthesia.

There has been an incredible technological improvement over the last decades in veterinary medicine. Diagnostic imaging techniques have improved with the aim of avoiding general anaesthesia by performing examination in standing patients. MRI and CT systems have been modified for horses allowing investigations of many anatomical regions without general anaesthesia. Motion correction software have been created to partially eliminate artifacts caused by patient movement that can diminish and, in some cases nullify the diagnostic quality of images acquired. Clinicians are well aware that appropriate treatment plans for rehabilitation and recovery from musculoskeletal injuries in horses are built on the foundations of an accurate diagnosis and detailed characterization of the pathology. Despite the increasing interest and effort in imaging techniques there are still some limitations that render general anaesthesia mandatory. Not all anatomical regions that can be examined in general anaesthesia can be investigated in standing and moreover not all medical structures have access to adapted standing systems especially for CT. Furthermore, not all standing systems such as MRI have the same image quality if compared to those that require general anaesthesia. Together with the evolution in the diagnostic field, also in anaesthesia there has been an increasing interest first of all in ameliorating general anaesthesia, but also in standing sedation protocols and in locoregional techniques. Improvements have been made in cardiovascular support and monitoring, together with drugs and protocols aiming a balanced anaesthesia and risks reduction; despite this we are still far away from mortality rates associated to general anaesthesia reported in human and small animals.

Standing sedation is becoming increasingly popular in equine medicine, most importantly it allows to avoid general anaesthesia and it also decreases cost making procedures such as surgeries and second level diagnostic imaging more affordable for owners. Despite the numerous studies in literature, the evaluation of sedative protocols based on different procedures performed (i.e. ophthalmic, laparoscopic, orthopaedic surgeries, MRI, CT, bone scintigraphy) is lacking. Even today the choice of which sedative protocol administer is still based on anaesthesiologist preferences and not on the evidence-based medicine. Also, with regards to diagnostic imaging no studies evaluate sedation protocol with a “radiologist” point of view. Drugs have been studied focusing on ataxia, sedation, and analgesia but evaluation on

patient movements is still missing. Standing diagnostic imaging certainly carries benefits but still some considerations should be kept in mind. MRI is a long procedure with long acquisition time; therefore, sedation needs to be maintained stable for a long period. Bone scintigraphy is a very sensible technique but on the other side spatial resolution is very poor, therefore minimal patient movement can strongly decrease imagine quality. For these two techniques motion correction software has been created but they're able to correct slight movements. For CT these software are still not available, and another consideration for this investigation is the patient safety, during standing CT of the head region the horses head is within the gantry making this examination more dangerous for the patient.

In all standing techniques finally, medical personnel are more involved, a sedated horse needs to be handled for the entire procedure therefore not only patient safety should be considered but also security of medical personnel and more importantly during CT and bone scintigraphy radiation exposure should be kept in mind.

Finally, new approaches to locoregional anaesthesia have permitted to perform surgeries that twenty year ago were done with the patient in general anaesthesia in standing. This is an important improvement if we think that some lower limb fractures repair is now performed in standing horses eliminating not only the risk during general anaesthesia but moreover during the recovery phase that is critical in these procedures.

As previously mentioned, the present doctorate thesis contains several researches on different topics but all under the same aim of improve horse's safety. The purpose of clinicians should be to permit to perform procedures as much as possible with the standing patients and in those cases in which general anaesthesia is mandatory, improve balanced anaesthesia itself trying to reduce the risks associated.

In the first study two opioids, butorphanol and morphine, administer together with detomidine were evaluated in horses undergoing bone scintigraphy. The sedative protocol was evaluated with a "radiologist" point of view focusing on the immobility of the patients that it has been described to be most important in this diagnostic technique. Not only sedation score was evaluated but also the number of retakes necessary to achieve a high quality diagnostic image. Interestingly morphine resulted in achieving a better sedation quality, a reduction in number of retakes and in time. All these findings reflect a more stable patient with less movement. A result that should be highlighted is the reduction in time. Radiation dose to technologists working with human patients undergoing bone scintigraphy was measured and found to be only 0.5 μSv. The potential radiation exposure dose to nuclear medicine technologists and other personnel working with horses is higher than when scanning human patients. A radiation dose rate of 3

μSv/h has been documented at a distance of 2 m from the horse 2–3-h post injection. If comparing these doses of radiation, it is clear that reduction in time and consequently in radiation exposure is an important achievement that increases safety for personnel involved.

In the second study two different administration routes, CRI and subcutaneous injection, of dexmedetomidine as a part of balanced anaesthesia were evaluated in horses undergoing magnetic resonance. Cardiopulmonary parameters together with recovery quality were compared. Dexmedetomidine has been widely used in small animal anaesthesia while in equine there has been an increasing interest in the last years. In literature there are studies in horses of dexmedetomidine administration in standing horses and as CRI during general anaesthesia. Subcutaneous administration has been described in human paediatric patients while in veterinary medicine this route has not been investigated. Interestingly in our study subcutaneous administration of dexmedetomidine resulted in cardiopulmonary parameters within clinically acceptable levels and similar to CRI administration. Moreover, recovery scores resulted to be better in quality and also with minor attempts to stand with dexmedetomidine administered subcutaneously. These results are interesting not only because this route of administration did not result in any complication but moreover because its clinical effects are comparable and, in some cases better of CRI administration.

In the third work a new approach to ophthalmic locoregional anaesthesia has been evaluated. Peribulbar block, as safer alternative to retrobulbar block was performed on head cadavers. The locoregional blocks were performed using contrast medium, the likelihood of achieving anaesthesia was evaluated through computer tomography examination. Peribulbar block resulted to be a possible alternative to retrobulbar block even if evaluation in vivo to confirm our theory is needed. The research of new techniques for locoregional-anaesthesia could permit to improve the number of procedures that can be performed in standing avoiding general anaesthesia.

Conclusions

The present doctorate thesis includes studies of different nature, connected to each other by the aim of patient safety with particular attention to safety during diagnostic imaging investigations. Despite the diversity in objective of every single work, the global aim of this thesis was to improve and substitute, when possible, general anaesthesia with standing sedation and, to ameliorate balanced anaesthesia in those cases in which general anaesthesia in mandatory. In the two clinicals studies reported, these goals were reached. Standing sedation was evaluated from a "radiologist" point of view, not only evaluating the sedation quality but also the immobility of the patient fundamental to acquire good images and therefore a correct diagnosis. Dexmedetomidine subcutaneous administration maintained cardiopulmonary clinically acceptable parameters and, in some cases preferable if compared to CRI administration. Furthermore, recovery quality had a significantly higher quality and with a minor number of attempts with this new administration route. The locoregional technique investigated could be part of a balanced anaesthesia but also it could permit clinicians to perform ophthalmic surgeries with the standing horse.

In conclusion all clinicians that work with equine patients are well aware that we are still a long way from greatly reducing the mortality associated with equine anaesthesia. Improvements have been made, such as in the monitoring and supporting the cardiovascular system, so that anaesthesia itself is less likely to be fatal: however, we still lose horses after anaesthesia to a range of catastrophes that would not occur if the horses were not anaesthetized. Probably the most notable development is the increase interest in technological improvements to avoid when possible general anaesthesia and to improve anaesthesia itself when it is mandatory.