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EFFECTS OF XYLAZINE, ROMIFIDINE AND DETOMIDINE ON HAEMATOLOGY, SERUM BIOCHEMISTRY AND SPLENIC SIZE IN HORSES

by

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DECLARATION

I hereby declare that this dissertation, submitted for the degree MSc Companion Animal Clinical Studies, to the University of Pretoria, is my own work and has not been submitted to another university for a degree, and that the data in this dissertation are the results of my investigations.

Anne Kullmann

31 May 2011

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To my husband Carsten

and

Banshee, Nandi & Rigidor.

ACKNOWLEDGEMENTS

ABBREVIATIONS

%	Percent
α_2 agonist	Alpha-2 adrenoceptor agonist
ADH	Antidiuretic hormone
ANOVA	Analysis of variance
ANP	Atrial natriuretic peptide
BUN	Blood urea nitrogen
Cl^-	Chloride
CNS	Central nervous system
COP	Colloid osmotic pressure
Ca^{2+}	Ionized calcium
GIT	Gastro-intestinal tract
g/L	Grams per litre
Hb	Haemoglobin
ICS	Intercostal space
IM	Intramuscular
IV	Intravenous
K^+	Potassium
mg/kg	Milligrams per kilogram
ml	Millilitre
mm	Millimetre
mmHg	Millimetres of mercury
mmol/L	Millimoles per litre
mOsm/kg	Milliosmoles per kilogram
mOsm/L	Milliosmoles per litre

MPI	Minutes post-injection
Na$^+$	Sodium
NE	Norepinephrine
OTAU	Onderstepoot Teaching Animal Unit
OVAH	Onderstepoort Veterinary Academic Hospital
PCV	Packed cell volume
RBC	Red blood cell
SD	Standard deviation
SE	Standard error
TSP	Total serum protein
VAS	Visual analogue scale

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SUMMARY

EFFECTS OF XYLAZINE, ROMIFIDINE AND DETOMIDINE ON

HAEMATOLOGY, SERUM BIOCHEMISTRY AND SPLENIC SIZE IN HORSES

by

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Alpha 2 agonists are frequently used in equine medicine. This study focused primarily on α_2 agonist-induced changes in PCV and TSP. A secondary aim of this study was to investigate the effects of α_2 agonist on selected serum biochemical parameters and splenic size in order to identify potential causes for the changes seen in PCV and TSP.

Four healthy adult mares were treated in a blinded, randomized, cross-over design with a single dose of xylazine (0.5 mg/kg), romifidine (0.04 mg/kg) or detomidine (0.01 mg/kg) intravenously, or detomidine (0.02 mg/kg) intramuscularly. A 1-week washout period was allowed between treatments. Haematology, TSP, COP, plasma osmolality, glucose, BUN, serum lactate, electrolytes, venous blood pH,

ultrasonographic splenic size and degree of clinical sedation were evaluated at different time points post-injection and compared to baseline values.

All treatments induced similar clinical sedation in the mares. A significant change over time in PCV and TSP following each treatment was identified, with overall median (range) maximal reductions compared to baseline of 20.9% (12.9 - 27.3%) and 5.8% (3.0 - 10.3%), respectively. Additionally, changes over time were significant for RBC count, BUN, COP and Ca^{2+}, which decreased; and glucose, plasma osmolality, Na^+ and splenic size, which increased, when compared to baseline. There was no significant main effect of treatment on PCV, TSP or any other parameters measured except for glucose.

This study concluded that changes in PCV, TSP and other biochemical parameters induced by α_2 agonists should be taken into consideration when assessing critically ill horses that received these drugs. There was evidence of splenic RBC sequestration as well as fluid shifts; therefore, the results suggest a multifactorial cause for the changes in PCV and TSP.

Key words: *horse, alpha 2 adrenoceptor agonist, xylazine, romifidine, detomidine, packed cell volume, total serum protein, plasma osmolality, colloid osmotic pressure, splenic size*

CHAPTER 1 INTRODUCTION

Packed cell volume and TSP form an integral part of clinical assessment in equine medicine and are often referred to as indicators of hydration status.[1-3]

Critical patients, especially those suffering from colic frequently present dehydrated[3, 4] and often require transport to referral institutions. In addition, pain management is usually needed. Because of their predictable sedative and analgesic effects,[5-8] α_2 agonists, such as xylazine, romifidine and detomidine are routinely used for these purposes.[9, 10]

Previous reports indicate a decrease in PCV, following IV or IM α_2 agonist administration in several domestic species including bovines,[11-13] caprines,[14, 15] ovines[13, 16] and felines.[15] Decreases in PCV can also be expected following IV and IM administration of α_2 agonists in horses.[6, 17, 18] Few studies, however, evaluated or reported a reduction in TSP in horses.[19-21]

This suggests that administration of α_2 agonists may alter these parameters and hence, confound subsequent clinical assessment. Correct appraisal of hydration status in critically ill horses is imperative to establish proper and timely fluid therapy and is key to successful management of horses with gastrointestinal disease.[3, 22]

To the best of the author's knowledge, there are no published reports evaluating the effects of xylazine, romifidine and detomidine on PCV and TSP in healthy horses in conjunction with selected biochemical parameters and splenic size. The results of this study will add to the clinician's understanding of the physiological effects of α_2 agonist administration. In addition, it will help clinicians to assess horses that

received these drugs more appropriately, in order to make suitable treatment choices promoting a better clinical outcome.

CHAPTER 2 LITERATURE REVIEW

2.1. ALPHA 2 ADRENERENOCEPTOR AGONISTS

2.1.1. Mechanism of action

For over two decades α_2 agonists have been used by veterinarians to induce dose dependent sedation, analgesia and muscle relaxation.[23]

Alpha adrenoceptors are generally classified into α_1 and α_2 receptor subtypes, which upon central and peripheral activation produce varied physiological effects.[6, 7, 23] Briefly, α_1 adrenoceptors are found post-synaptically in the CNS, heart, smooth muscle and liver, producing increased awareness, positive inotropic effect, vasoconstriction as well as glycogenolysis and gluconeogenesis, respectively. On the other hand, α_2 adrenoceptors are found pre-synaptically in the CNS and post-synaptically in many organs, such as the GIT. Stimulation of central α_2 adrenoceptors causes hyperpolarisation of CNS neurons and a decrease in NE as well as dopamine release. This results in a reduced discharge rate of central and peripheral neurons and hence, cardiopulmonary depression, sedation, analgesia and muscle relaxation. In the GIT and the smooth muscle of the vasculature, α_2 adrenoceptor activation elicits reduced GIT tone with a decline in propulsive activity, and vasoconstriction, respectively. Stimulation of these receptors also promotes platelet aggregation.[6]

All α_2 agonists have some degree of α_1 adrenergic function and are hence, classified on the basis of their $\alpha_{2:}\alpha_1$ selectivity ratio. Of the three agonists investigated here,

romifidine (340:1) is the most selective for the α_2 adrenoceptors, followed by detomidine (260:1) and xylazine (160:1).[6, 7]

In horses, detomidine is more potent than romifidine and both are more potent than xylazine.[6] When considering the use of equipotent doses, detomidine produces the longest lasting sedative effect followed by romifidine.[24] Xylazine has the shortest duration of action.[25-28] The onset of sedation is usually within 5 minutes following IV injection of the three α_2 agonists investigated here; however, a delay of 10-20 minutes should be expected with IM injection of detomidine or romifidine.[26] Peak sedation is achieved 5, 10 and 15 minutes post-sedation with IV xylazine,[29, 30] IV romifidine[31] and IV detomidine,[28] respectively. The duration of sedation is dose-dependent and so are the side effects.[6]

2.1.2. Cardiopulmonary and other side effects

Alpha 2 agonists produce a number of well-known cardiopulmonary effects, including marked decreases in heart and respiratory rates as well as second degree atrioventricular blockade.[6, 7, 17, 26, 32] Furthermore, peripheral vascular resistance is elevated and that, combined with bradycardia, are the causes for a significant fall in cardiac output.[6, 7, 17, 32] Initial hypertension[25] (15 minutes with IV detomidine)[17] occurs, as a consequence of the drug-induced increase in peripheral resistance, which is followed by a mild[25] decrease in blood pressure.[6] Arterial blood pressure rarely falls below 20% of baseline values. Detomidine and romifidine, the more selective α_2 receptor agonists, cause a more prolonged primary increase in blood pressure compared to xylazine, which is much less marked following IM administration.[6] In general IV administration of all these drugs produces more rapid and pronounced

cardiovascular effects of shorter duration, presumably because of the higher plasma concentration of unbound drug, as opposed to a more prolonged course with IM administration.[17, 26]

Other important side effects are the result of α_2 agonist-induced changes in hormonal physiology. This includes hyperglycaemia[33-35] due to inhibition of insulin release from pancreatic islet cell.[6, 7, 32, 36] Another consequence is increased diuresis[36] with maximum urine output between 30-60 minutes post-administration.[6, 33] The latter is believed to be the result of inhibition of ADH release,[6, 7, 36] blocking of its activity on renal tubules,[6, 7, 32] decreased release of renin[7, 36] as well as increased release of ANP.[6, 21, 32]

2.1.3. Haematological effects in non-equine species

Various studies have been conducted on the haematological effects of α_2 agonists, in particular xylazine, in several species. Intramuscular injection of xylazine resulted in reduction of Hb concentration and PCV in caprines[14, 15] and ovines.[13, 16] Combinations of IM xylazine or detomidine with atropine also resulted in a decrease in PCV in goats.[37] Similar effects on PCV, Hb concentration and RBC count were observed during deep sedation following subcutaneous xylazine injection in felines[15] and 30 minutes after xylazine immobilisation of impalas.[38] In other wildlife species similar reductions in PCV associated with α_2 agonist sedation were observed with IM xylazine in red deer,[39] IM medetomidine in wild boars[40] and epidural xylazine administration in buffalo calves.[41] Epidural administration of xylazine in buffalo calves also resulted in a small but insignificant decrease in TSP,[41] whereas IM administration of medetomidine in wild boars decreased TSP slightly but

significantly.[40] Additionally, a decline in PCV has been identified in cattle subsequent to xylazine administration by several routes, namely, intravenously,[12] intramuscularly[11, 13] as well as epidurally.[42] In contrast to IV administration in pregnant cows,[12] caudal epidural injection of xylazine in cows induced a significant reduction in TSP.[42]

2.2. THE EQUINE PACKED CELL VOLUME

2.2.1. Effects of alpha 2 adrenoceptor agonists

Decreases in PCV have also been described subsequent to IV and IM α_2 agonist administration in horses.[6, 17, 18] Gasthuys et al. (1990) observed significant reductions in PCV as early as 5 and 15 minutes post-injection of IV detomidine and xylazine, respectively.[18] Wagner et al. (1991) however, detected a significant decrease in PCV at 30-60 minutes following IV or IM administration of a single dose of xylazine or detomidine.[17] In the latter study, a return to baseline PCV values after 120 minutes could not be established. Total serum protein was not monitored in either of these two studies.[17, 18] A similar effect on PCV was observed with IV xylazine in 28 day old foals, although much less pronounced.[19] In the latter study no significant change in TSP was identified.

Daunt et al. (1993) investigated the effects of detomidine at four different plasma concentrations in standing horses.[21] At the start of each infusion a drug bolus was administered to ensure the rapid achievement of adequate plasma concentrations. During subsequent constant rate infusions a significant decrease in PCV, with an associated small but significant reduction in TSP, was observed initially.

Interestingly, during later stages of the infusion process (i.e. at higher plasma levels of detomidine) the PCV normalised.

When xylazine was used in combination with guaifenesin and ketamine for maintenance of anaesthesia in horses, significant decreases in PCV, Hb concentration as well as RBC count were found compared to baseline values.[20] In this study a reduction in TSP was also observed; however, this change was not significant. Lastly, caudal epidural administration xylazine and detomidine in mares has also been shown to induce a significant decrease in PCV.[43]

In contrast, no significant change in PCV was identified following sedation in horses with a detomidine-atropine combination.[44, 45]

2.2.2. Effects of other sedatives

The phenomenon of PCV reduction is not exclusively found with α_2 agonists, but has also been identified with the use of phenothiazine tranquilisers. The influence of acepromazine maleate on the haematocrit of horses was investigated by Parrry and Anderson (1983).[46] In this study a significant decrease in mean PCV was observed following IM and IV administration, which was not related to the dose or route of administration and accompanied by a comparable small decrease in TSP.

A similar effect on PCV was identified by de Moor et al. (1978)[47] as well as Gasthuys et al. (1990)[18] following IV administration of promazine hydrochloride and a combination of propionypromazine and promethazine, respectively.

2.3. PROPOSED CAUSES OF THE PCV RESPONSE TO SEDATIVES

Several mechanisms have been postulated to explain the effect of α_2 agonists on PCV. These include erythrocyte lysis,[11] fluid shifts from the extravascular to the intravascular space in order to maintain cardiac output[17, 37] secondary to a decrease in blood pressure[20, 42] and RBC sequestration[12] in the spleen[17, 37, 47] or other blood reservoirs[48] as a result of decreased sympathetic tone following sedation.[17]

2.3.1. The role of the spleen

At rest the equine spleen is a great reservoir for red cells and is capable of holding one third of the circulating red cell volume, which can be released upon contraction following sympathetic stimulation (i.e. physical stress and excitement) and hence, markedly increase the PCV.[49] This is illustrated by studies measuring PCV during exercise in intact horses[50] compared to splenectomised ponies[51], where pre-exercise baseline PCV increased by 50% and 4% respectively. Torten and Schalm (1964) also concluded that the increase in PCV during excitement in horses is principally due to splenic contraction, rather than haemoconcentration as a result of fluid shift.[49] On the other hand, Persson (1967) showed that excitement alone can indeed cause a fluid shift with consequent reduction in plasma volume.[52]

The overall decreased sympathetic tone during sedation has been hypothesised to result in splenic RBC sequestration.[17] Although an increase in splenic size was found in dogs, no significant correlation was established between splenic size, determined by laparoscopic measurements, and the decrease in haematocrit following different

anaesthetic protocols including medetomidine and acepromazine.[48] However, splenic sequestration appears to be a widely accepted theory in the horse.

De Moore et al. (1978) investigated the role of the spleen on the decrease in PCV following administration of a single IV dose of promazine using only one pony.[47] Venous haematocrit and TSP were evaluated in this study. The experiment was then repeated eleven months after performing a complete splenectomy. In contrast to the PCV reduction seen in the intact pony, a gradual increase in haematocrit was identified from five hours until the end of observation period subsequent to splenectomy. The same results were obtained for the plasma protein concentration.[47] The significant decrease in TSP with promazine administration in this study was speculated to occur due to a water inflow from the interstitial fluid. The latter was believed to be the result of arterial blood pressure reduction, due to the vasodilatory effects of promazine, as well as a decrease in hydrostatic pressure in the microcirculation, due to the reduction in RBC.

This phenomenon was also explored by Parry and Anderson (1983) using intravenous Evans blue injections in horses.[46] Plasma concentrations of this compound were not altered following IV and IM acepromazine injection as would be expected if plasma volume expansion was responsible for the decline in PCV. It was therefore concluded that the PCV response was a result of splenic relaxation and the small associated change in TSP could be attributed to minor variations in plasma volume.

Turner and Hodgetts (1960) using radioisotope studies in sheep, demonstrated that the fall in PCV following chlorpromazine tranquilisation is due to the storage capacity

of the spleen and plasma dilution only plays a small contributing role in this species.[53]

2.3.2. Fluid dynamics

Essentially the fluid compartments in the body comprise the intracellular and the extracellular fluid components; the latter can be subdivided into the interstitial and the intravascular compartment, namely blood plasma.[54]

A continuous exchange of solutes takes place between the plasma and the interstitium, which is facilitated by the pores in the capillary membrane. These pores are highly permeable to all solutes except proteins and hence, the ionic compositions of plasma and interstitium are essentially similar.[54] Water and ionic solute exchange occur rapidly at capillary level and equilibrium is reached within 15 to 30 minutes.[4] Transcapillary fluid movement is dependent on Starling forces, with the hydrostatic and oncotic pressures acting from both compartments, namely plasma and interstitium.[55, 56]

On the other hand, the cellular membrane is highly permeable to water but largely impermeable to most ions and hence, the ionic compositions of the extracellular and intracellular fluids are substantially different. In contrast to transcapillary fluid movement the distribution of fluid between intra- and extracellular compartments is governed by the osmotic effect of the solutes acting across the cellular membrane. Water movement occurs from low to high solute concentration and is thus influenced by the osmolal concentration of the solution, which is the osmolality.[54, 57]

2.3.3. Colloid osmotic pressure

In contrast to smaller solutes, most proteins are unable to cross the capillary membrane due to their size and are hence, more abundant in plasma compared to the interstitium, contributing to its COP.[54] Although COP accounts for a mere 0.5% of the total plasma osmotic pressure, it plays an important role in transcapillary fluid dynamics by retaining appropriate intravascular volume.[58, 59]

The negative charge of plasma proteins attracts positively charged ions, such as Na^+, whose concentration is therefore slightly higher in plasma compared to the interstitium and contribute approximately one third to the colloid osmotic force of the plasma.[54, 55, 58] This phenomenon is known as the Gibbs Donnan effect. Conversely, the negative charges of proteins repel anions, which are more abundant in interstitial fluid.[54] As a result; COP is, at least to some extent, dependent on the ionic composition of the intravascular and interstitial fluid components.[55]

2.3.4. Plasma osmolality

Osmolality is the concentration of osmotically active solutes[4] including ions and proteins.[59] It provides an indication of plasma osmotic pressure.[60] Whereas osmolality expresses the total number of dissolved particles in a kilogram of water, osmolarity denotes the same number in a litre of solution.[59, 61] The terms are often used interchangeably when referring to the relatively dilute intra- and extracellular compartments of animals.[4, 54, 59, 61]

Normal adult horses have plasma osmolality values ranging from 280 to 310 mOsm/kg.[62] Sodium and chloride constitute about 90% of the ionic component of the

extracellular fluid compartment and are, therefore, the primary determinants of plasma osmolality.[54, 60, 63] Potassium, glucose, non-protein nitrogen and other trace elements contribute less than 10% to normal plasma osmolality,[60] with glucose accounting for approximately 2%.[54]

There are many formulas for the calculation of plasma osmolality.[60, 64] Brownlow and Hutchins (1982) concluded that, in horses, measured plasma osmolality was best predicted by the following formula:[60]

$$\text{Osmolality} = 1.86\ (Na^+ + K^+) + \text{glucose} + \text{BUN} + 9$$

It is important to note that this formula is only suitable if solute concentrations are given in mmol/L.[59]

Measured osmolality includes both effective and ineffective osmoles, whereas tonicity refers to the concept of effective osmolality of a solution.[57, 59, 65] Most cellular membranes are impermeable to Na^+ and since the osmotic effect of the solutes is largely dependent on its membrane permeability, Na^+ is considered a very effective osmole.[54, 57, 59] Since glucose is not readily diffusible across cellular membranes either, it also has a significant osmotic activity, even when it is only slightly increased in the blood.[57, 59] On the other hand, urea, commonly measured in the blood as BUN, is a very small molecule that essentially moves freely across cell membranes and contributes very little to the effective osmolality.[57, 59, 65]

Under steady state conditions where effective osmotic concentrations of intracellular and extracellular fluids are equal, water is in equilibrium across the cellular membrane.[61] In the very basic sense, modification of the solute content in either compartment affecting tonicity generates a transmembrane osmotic gradient, resulting in water movement until the equilibrium is re-established.[57, 59, 61] Causes for plasma hyperosmolality include hypernatremia, hyperglycaemia and severe

azotemia,[57, 65] whereas hyposmolality is almost always associated with hyponatremia.[57]

In order to restore fluid balance, elevations in plasma osmolality are detected by osmoreceptors in the hypothalamus, which govern ADH release.[63, 66-68] An increase in plasma osmolality of less than 1% leads to a marked increase in ADH.[63, 66, 67] Antidiuretic hormone regulates the permeability for water in the renal collecting ducts and hence, is very important for water balance in the body.[63, 67, 68] Once released, it causes a dramatic increase in renal water reabsorption,[63, 66-68] while Na^+ re-uptake is only slightly enhanced.[66, 67] Water is conserved more relative to Na^+, leading to solute dilution in the extracellular fluid and thus normalisation of plasma osmolality. Antidiuretic hormone also has a vasopressor effect leading to vascular smooth muscle contraction and an increase in blood pressure.[67] The reverse occurs in the event of hyposmolality: less ADH is released, little water reabsorption takes place and a large amount of dilute urine is formed.[67, 68]

Few reports have published results on serum osmolality measures following α_2 agonist administration. Thurmon et al. (1984) detected no significant differences in BUN, serum K^+, Na^+, and osmolality in healthy horses when comparing administrations of IV xylazine and saline solution, evaluated at fixed time periods over 180 MPI.[34] Trim and Hanson (1986) investigated the effects of a single dose of IV xylazine in ponies and also did not find a significant change in serum osmolality, K^+, Na^+, and Cl^- concentrations at 120 MPI compared to baseline values; however, no data was available between these times.[33] In addition serum concentrations of Na^+, K^+ and Ca^{2+} were also not significantly altered following sedation in horses with a combination of xylazine-atropine[69] or detomidine-atropine.[44] In contrast, in another

study, a significant increase in Ca^{2+} was detected, while serum Na^+ and K^+ did not vary significantly following sedation with a combination of detomidine-atropine.[45]

CHAPTER 3 OBJECTIVES AND HYPOTHESES

The main focus of this study was to evaluate the effects of α_2 agonist sedation on PCV and TSP in healthy horses. Furthermore, RBC count and degree of clinical sedation were assessed to verify drug-induced changes in PCV and equipotent sedative effect, respectively. Another aim was to identify possible causes for the observed changes. Two phenomena were investigated: splenic RBC sequestration and intravascular fluid shift. This was performed by measuring splenic ultrasonographic width as well as COP and plasma osmolality, respectively. Blood glucose, BUN, serum lactate and electrolytes were also evaluated, as they may contribute to plasma osmolality.[64] Lactate measurements were additionally used to identify whether tissue metabolism was compromised as a result of α_2 agonist administration. Venous blood pH was also monitored. The following specific primary and secondary objectives and corresponding hypotheses were formulated:

3.1. OBJECTIVES

3.1.1. Primary objectives

i.   To quantify the magnitude of change in PCV, TSP, lactate, COP, plasma osmolality and splenic size over time following IV administration of recommended equipotent dosages of three different α_2 agonists used clinically in horses, namely romifidine, xylazine and detomidine, as well as following IM administration of detomidine.

ii. To compare the magnitude of change in PCV, TSP, lactate, COP, plasma osmolality and splenic size over time for IV administration of romifidine, xylazine and detomidine.

iii. To compare the magnitude of change in PCV, TSP, lactate, COP, plasma osmolality and splenic size over time for IV administration and IM administration of detomidine.

3.1.2. Secondary objectives

i. To describe the trend and duration of the effects on PCV and TSP following administration of the studied drugs.

3.2. HYPOTHESES

3.2.1. Primary hypotheses

i. A significant decrease in PCV will be observed over time following each α_2 agonist administration. The maximum reduction in PCV is expected at 60 minutes MPI with a decrease of at least 15% from baseline values.

Splenic size will increase significantly over time to a maximum measurement at 60 MPI, at which an increase in ultrasonographic width of at least 30% from baseline values will be observed.

Plasma osmolality, lactate, TSP and COP will show no significant change over time for any of the drugs administered.

ii. The changes in PCV and splenic size over time will be similar following IV administration of romifidine, xylazine or detomidine.

Plasma osmolality, lactate, TSP and COP values will be similar following IV administration of romifidine, xylazine or detomidine.

iii. At 60 MPI, the reduction in PCV observed with IV detomidine will be significantly greater than with IM administration. The duration of the effect in hours will be significantly longer with IM injection of detomidine. The last time point studied will be set at 300 MPI.

At 60 MPI, the increase in splenic size observed with IV detomidine will be significantly greater than with IM administration.

At 60 MPI, plasma osmolality, TSP, lactate and COP observed with IV detomidine will not significantly differ from IM administration.

3.2.2. Secondary hypotheses

i. The PCV will decrease significantly over time following administration of all drugs to a nadir value at 60 MPI, after which it will return to baseline values. The last time point studied will be set at 300 MPI. No significant changes over time will be observed for TSP for any treatment group.

CHAPTER 4 MATERIALS AND METHODS

4.1. STUDY DESIGN

Four adult Nooitgedacht mares were selected from the Onderstepoort Teaching Animal Unit for this prospective, randomized, blinded, cross-over study. Mares of the same breed and similar age were chosen to lessen any possible gender, breed or age related variation. Before inclusion, the mares were determined to be clinically healthy based on a complete physical examination and routine haematology performed 48 hours prior to the commencement of the study. Clinical examination was repeated 24 hours prior to treatment administration. All mares were administered one of four treatments on four different days so each mare acted as its own control. A one week wash-out period was allowed between treatments as previously reported.[24, 31] Each horse received in random order, a single dose of xylazine[a] (0.5 mg/kg), romifidine[b] (0.04 mg/kg), detomidine[c] (0.01 mg/kg) intravenously, or detomidine[c] (0.02 mg/kg) intramuscularly. These IV doses were selected as they are considered to be equipotent[31] and the IV detomidine dose was doubled for IM use, as is generally recommended.[10, 32]

The study was conducted at the Equine Clinic of the OVAH, Faculty of Veterinary Science, University of Pretoria. The mares were stabled 24 hours prior to treatment for acclimatization. All experimental procedures and observations were performed in the stall to prevent stimulation of the mares. Food was withheld from the time of α_2 agonist administration until the end of the observation period to prevent oesophageal obstruction associated with drug induced changes in peristalsis.[70, 71] Mares had free access to water at all times. Ad libitum teff/lucerne hay was provided prior to and

following the sedation period. After completion of all recordings, the mares were monitored hourly for a total of 24 hours. Thereafter they were returned to the OTAU facilities for the duration of the washout period.

4.2. EXPERIMENTAL PROCEDURES

4.2.1. Blood sampling and drug administration

Intravenous sedative administration and blood collection were performed via an IV 14G polytetrafluoroethylene catheter[d] and a high flow extension set[e]. The catheter was placed in the morning of each treatment day in the left jugular vein aseptically as described previously[72] following surgical preparation of the site.[72, 73] The catheter was removed immediately following the observation period. The left lateral neck was used for IM detomidine[c] administration.

To prevent dilution of the blood samples with heparinised saline used to flush the catheter, 10 ml of blood were collected and discarded prior to sampling. Thereafter depending on the sampling point; 1 or 15 ml of blood were collected and transferred to microhaematocrit or serum, EDTA and heparin vacutainer tubes, respectively, for testing. Ten millilitres of heparinised saline were used to flush the catheter after drug administration and blood sampling to avoid clotting.

Packed cell volume, TSP, RBC count, COP, plasma osmolality, serum lactate, glucose, BUN, blood pH and electrolyte (Na^+, K^+, Cl^- and Ca^{2+}) concentrations were evaluated at the following times: -1 (before administration of any α_2 agonist), 30, 45, 60, 75 and 90 MPI. Additionally, PCV and TSP were measured at 15, 120 and hourly thereafter until 300 MPI.

Packed cell volume was determined using sodium-heparinised microhaematocrit tubes[f]. Four microhaematocrit tubes were prepared, in case breakage occurred and these were centrifuged at 11800 revolutions per minute (14000 g) for 5 minutes[74] using a microhaematocrit centrifuge[g]. Subsequently, two samples were randomly selected by the author and the PCV and TSP were determined by means of a haematocrit reader[h] and refractometer[i], respectively, and the average calculated. Plasma osmolality was quantified by freezing point osmometry[j] using a heparinised blood sample.[60] The sample was centrifuged[k] for 10 minutes at 2000 revolutions per minute (1028 g). The supernatant was then aspirated, transferred to microcentrifuge tubes and kept on ice until the analysis was performed.

Red blood cell count was measured by routine haematology[l] and COP by colloid[m] osmometry.[55, 75] Handheld monitoring systems were used for serum lactate[n] and glucose[o]. These monitoring systems remained constant during the duration of the study. Chloride and BUN were determined in serum[p], while Na^+, K^+, Ca^{2+} and venous blood pH were evaluated by venous blood gas analysis[q].

4.2.2. Splenic ultrasound

Splenic size was evaluated using a portable ultrasound[r] at -1, 30, 45, 60, 75 and 90 MPI. Ultrasonographic width of the spleen (in mm) was measured at five different locations: the level of the tuber coxae at the 17th ICS, the major trochanter of the femur at the 15th ICS, the tuber ischii at the 13th ICS, the point of the shoulder at the 11th ICS and the point of the elbow at the 9th ICS. To facilitate visualization as well as to ensure that ultrasonography was always performed in the same location during

the entire duration of the study, a small 4x1.5 cm area of hair was clipped in each of the above mentioned locations.

Prior to the study four investigators performed three splenic measurements each on all described locations of all four mares, in order to evaluate repeatability of ultrasonography for evaluation of splenic size. Specifically intra- and interobserver repeatability of the measurements were assessed. Once good agreement among observers was established further evaluations were carried out by only one individual to aid in the repeatability of the measurements. The ultrasonographer (MS) was blinded to the treatment received.

4.2.3. Sedation score

Clinical sedation was scored at -1, 30, 45, 60, 75 and 90 MPI by two blinded investigators; one of which was constant throughout the study (MS; observer 1), while the other varied weekly (observer 2). Sedation was evaluated using the VAS scale which has been employed in clinical studies elsewhere.[76] Additionally a categorical scoring system was created for this project to assess the mares' response to ultrasound (Appendix A). Briefly, the VAS scale consists of a 100 mm line with no markings, where a VAS score of 0 represents no sedation and 100 represents profound sedation, which was clinically observed as severe ataxia and head drop. For the response to ultrasound, a score of 1 was assigned to a lack of response and 4 to a marked response, such as moving away from the approaching ultrasonographer before or during contact with the ultrasound probe.

4.3. OBSERVATIONS

All observations are summarised in the sampling time line (Appendix B). The age of the mares, weekly body weights, PCV, TSP, glucose, serum lactate, RBC count, COP, plasma osmolality, BUN, Na^+, K^+, Cl^-, Ca^{2+}, venous blood pH, ultrasonographic splenic size and sedation scores were collected and recorded on a standardised data recording sheet (Appendix C).

4.4. STATISTICAL ANALYSIS

Descriptive statistics were calculated including mean and standard deviation for continuous variables satisfying the normality assumption and median and range for non-normally distributed variables. The distributional form of the outcome variables was assessed by calculating descriptive statistics, plotting of histograms and performing the Anderson-Darling test for normality. Continuous data that violated the assumption of normality were rank-transformed prior to statistical analysis.

Repeated measures ANOVA was used to test for differences in outcome variables among treatment groups over time using simple contrasts with baseline values set as the reference category. Post-hoc tests were employed for outcome variables significantly different overall, with Bonferroni correction for multiple comparisons.

Repeatability of ultrasonographic measurements was assessed by calculating the coefficient of variation among and within subjects. The proportion of variability in splenic size within observers was estimated by calculating the coefficient of variation for each observer. A variance components analysis was performed to assess the

amount of variability in measured splenic size attributed to subject, ICS and observer.

Lastly Pearson product-moment correlation coefficients were computed to assess the relationship between selected variables. Measurements were standardised by the following formula: standardised value = (measured value - mean) / SD, and described using scatter plots.

Analyses were performed using commercially available software[s,t] and results were interpreted at the 5% level of significance.

4.5. ETHICAL CONSIDERATIONS

Materials used in this experiment posed no undue health risk to the researchers or the mares involved in the study.

Each horse received a single intramuscular injection in the left lateral neck over the entire course of the study. All intravenous drug administration and blood collections were performed via an IV catheter. Intramuscular injections as well as intravenous catheterisation are routinely performed and are quick procedures causing minimal pain and discomfort. Lignocaine[u] was injected subcutaneously at the catheter site to prior to IV catheterization to provide local anaesthesia and hence, decrease the discomfort associated with this procedure. Ultrasonography is a non-invasive diagnostic procedure.

Approval from the University of Pretoria Animal Use and Care Committee was obtained prior to initiation of the project (Protocol V053-10).

CHAPTER 5 RESULTS

The mean (± SD) age and weight of the mares was 11.3 years (±1.6 years) and 406.7 kg (± 13.3 kg), respectively.

Haematology and clinical examination prior to the study revealed no abnormalities in any of the mares and the clinical parameters remained within normal limits during subsequent pre-treatment clinical examinations.

Repeated measures ANOVA for haematology and serum biochemistry are summarised in appendix D1.

PCV: There was a significant change in PCV over time (p<0.001). Packed cell volume decreased subsequent to drug administration with all treatments to a nadir, following which it slowly recovered. The maximal reductions following each treatment and the time to maximal reductions were not significantly different among treatments (p=0.415 and p=0.125, respectively). The overall median (range) maximal reduction in PCV compared to baseline was 20.9% (12.9 - 27.3%). The overall median (range) time to reach the nadir was 75 MPI (15 - 120 MPI). The mean PCV ± SE for all treatments is summarised in figure 5.1.

There was no significant effect of treatment on PCV for the three IV treatments (p=0.229) or for the comparison between IV and IM detomidine administration routes (p=0.198). Even though, there was no significant main effect of treatment, the treatment by time interaction was significant (p<0.001). This appears to be due to the shorter duration of the xylazine effect, which is illustrated in figures 5.1 and 5.7.

Figure 5.1: Mean (± SE) PCV over time following administration of IV xylazine, IV romifidine, IV detomidine and IM detomidine.
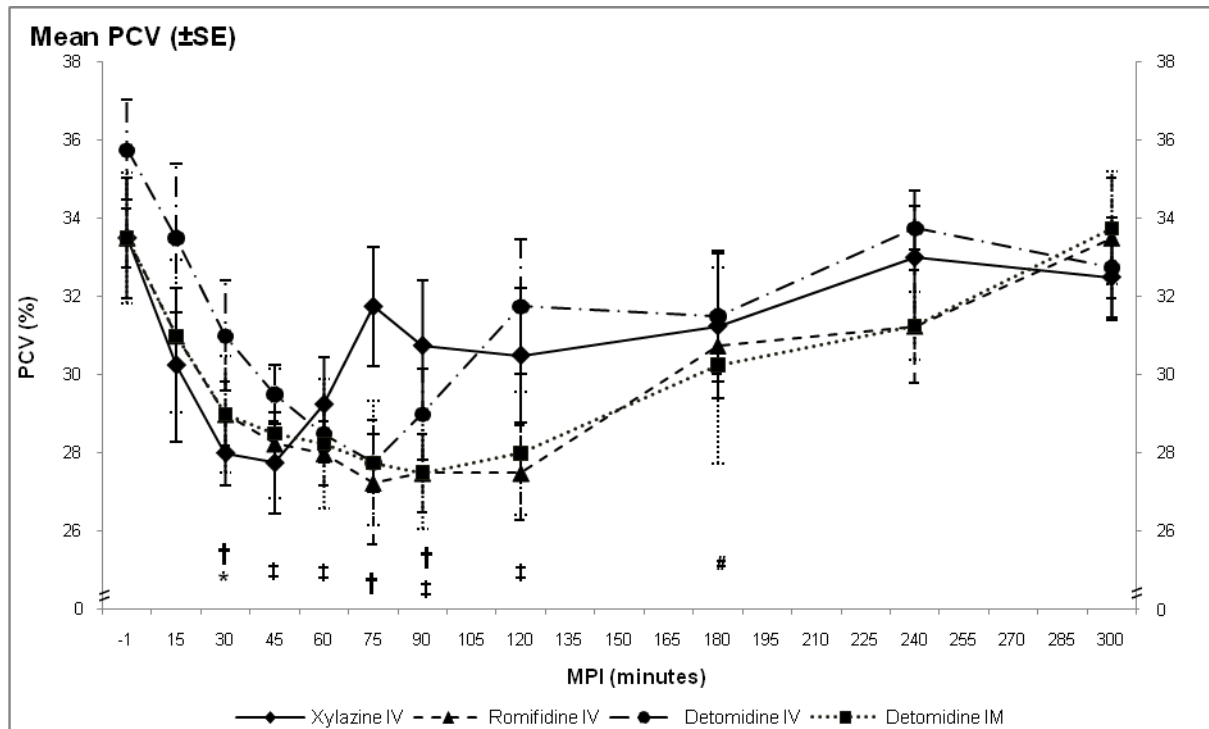
Data statistically different from baseline ($p<0.05$) are indicated by * for IV xylazine, # for IV romifidine, † for IV detomidine and ‡ for IM detomidine.

The PCV responses over time for the individual mares following each treatment are shown in figures 5.2 - 5.5.

Xylazine IV (Figure 5.2): The maximum decrease in PCV occurred at 15 MPI (Mare 3) and at 45 MPI (Mares 1, 2 and 4). At the nadir, the median (range) maximal reduction in PCV compared to baseline was 21.1% (12.9 - 21.9%). During the observation period, two of the mares' PCV returned to baseline values. This occurred at 90 MPI (Mare 3) and 120 MPI (Mare 1).

Figure 5.2: PCV response following IV xylazine administration.
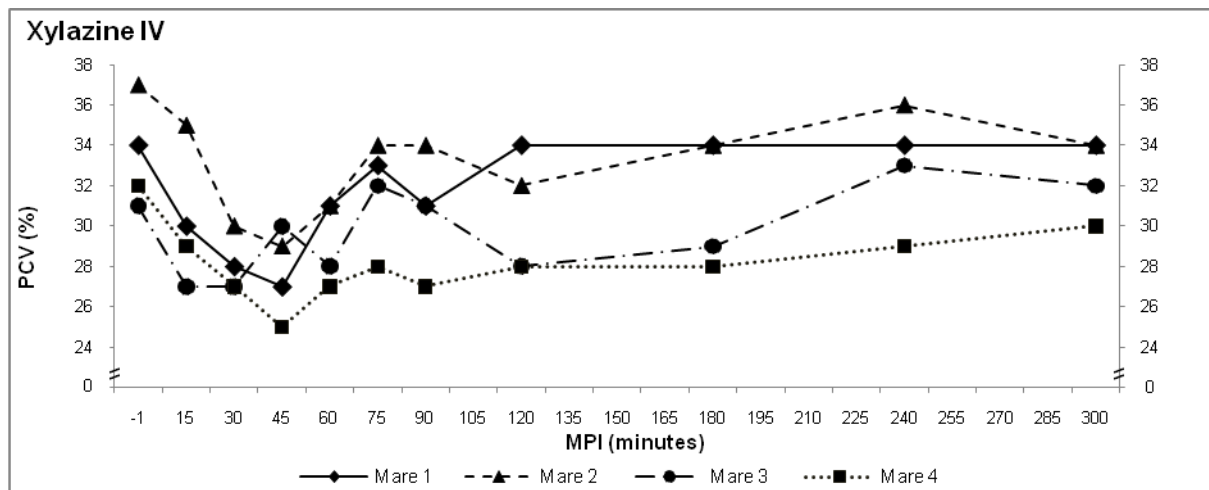
Romifidine IV (Figure 5.3): The maximum decrease in PCV of each mare occurred at different time points, namely 45 MPI (Mare 2), 75 MPI (Mare 1), 90 MPI (Mare 4) and 120 MPI (Mare 3). At the nadir, the median (range) maximal reduction in PCV compared to baseline was 23.2% (15.6 - 27.3%). During the observation period, the PCV of three mares returned to or exceeded baseline values. This occurred at 240 MPI (Mare 3) and 300 MPI (Mares 1 and 2).

Figure 5.3: PCV response following IV romifidine administration.
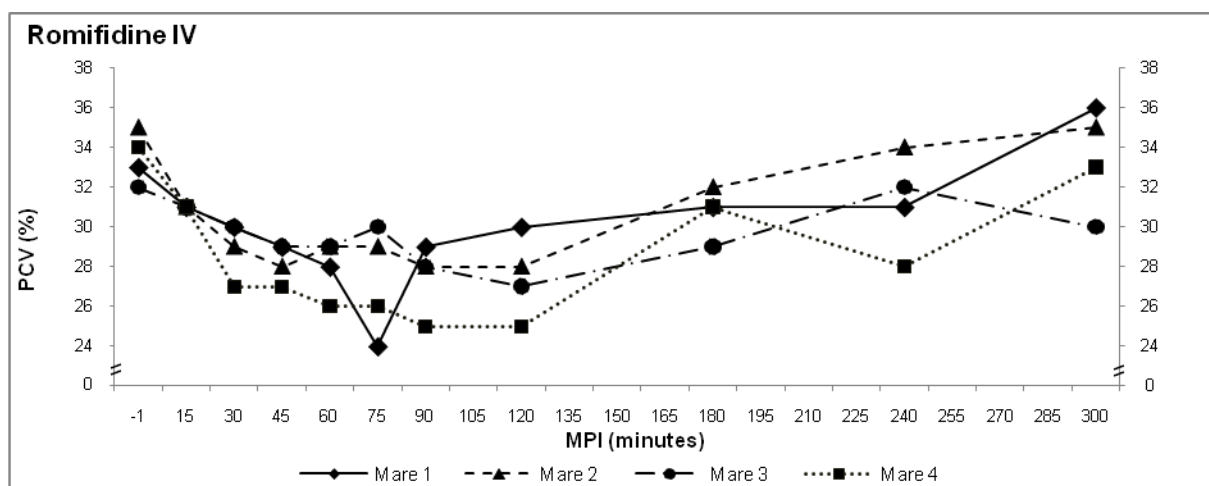
Detomidine IV (Figure 5.4): The maximum decrease in PCV occurred at 45 MPI (Mare 4) and at 75 MPI (Mares 1 - 3). At the nadir, the median (range) maximal

reduction in PCV compared to baseline was 22.8% (17.1 - 26.3%). During the observation period, the PCV of only one mare returned to baseline value (240 MPI; Mare 3).

Figure 5.4: PCV response following IV detomidine administration.
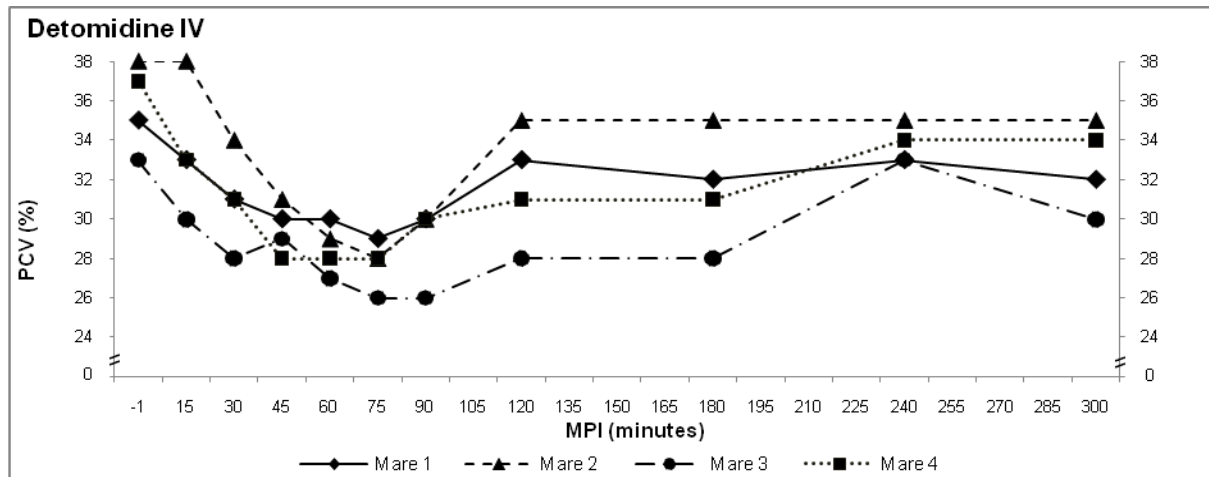
Detomidine IM (Figure 5.5): The maximum decrease in PCV occurred at 45 MPI (Mare 3), 75 MPI (Mares 1 and 4) and 90 MPI (Mare 2). At the nadir, the median (range) maximal reduction in PCV compared to baseline was 17.4% (16.2 - 23.5%). During the observation period, two of the mares' PCV returned to or exceeded baseline values. This occurred at 180 MPI (Mare 3) and 300 MPI (Mare 2).

Figure 5.5: PCV response following IM detomidine administration.
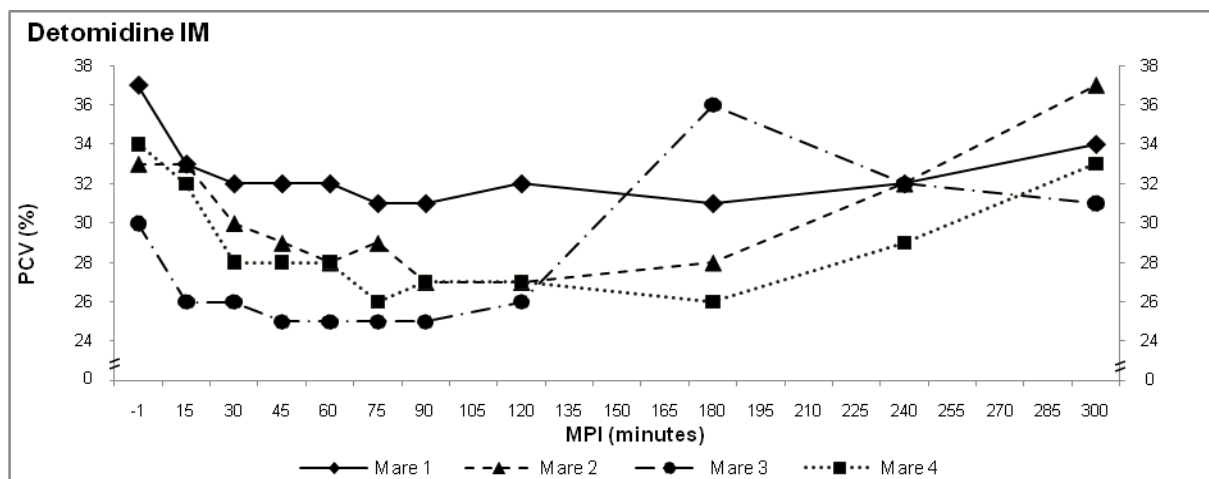
RBC count: There was a significant change in RBC count over time ($p<0.001$). Red blood cell count decreased subsequent to drug administration with all treatments. The overall median (range) maximal reduction in RBC count compared to baseline was 17.7% (9.2 - 24.5%). The overall median (range) time to reach the nadir was 60 MPI (30 - 90 MPI). The mean RBC count ± SE for all treatments is summarised in figure 5.6. The change in RBC count over time paralleled that of PCV (Figure 5.7).

There was no significant effect of treatment on RBC count for the three IV treatments ($p=0.823$) or for the comparison between IV and IM detomidine administration routes ($p=0.862$).

Figure 5.6: Mean (± SE) RBC count over time following administration of IV xylazine, IV romifidine, IV detomidine and IM detomidine.
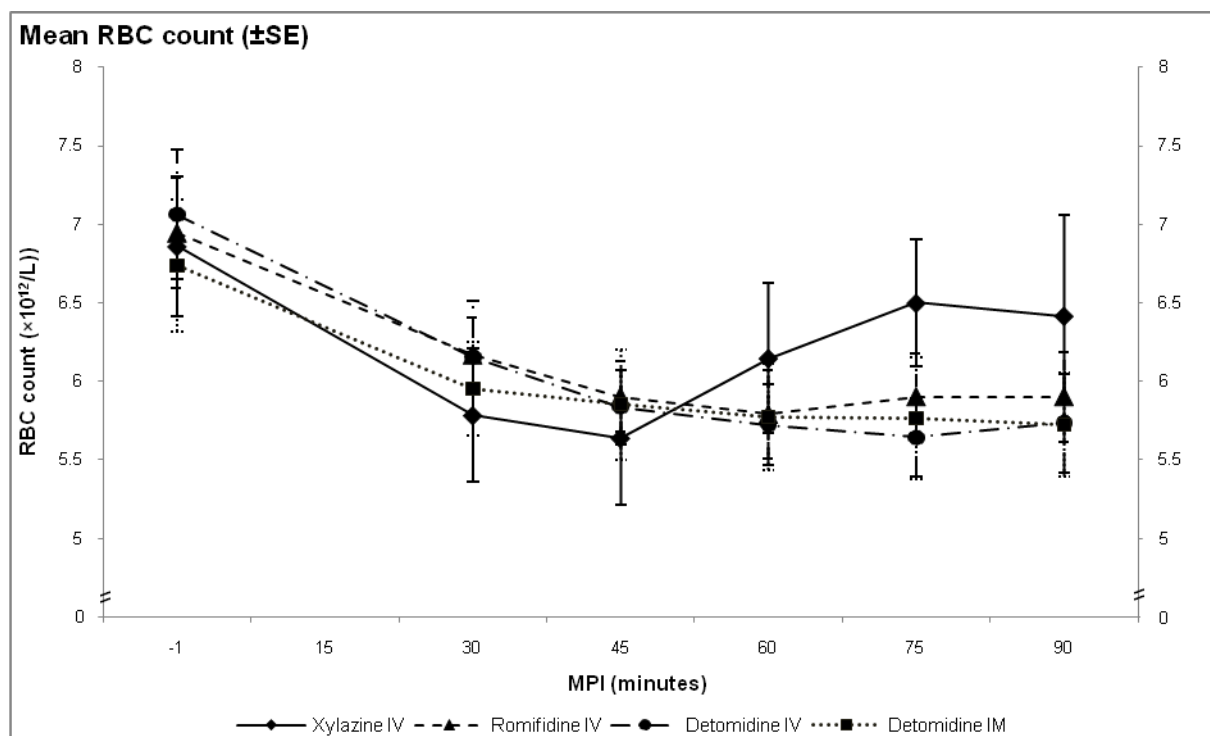
Figure 5.7: Mean RBC count and PCV over time following administration of IV xylazine, IV romifidine, IV detomidine and IM detomidine.
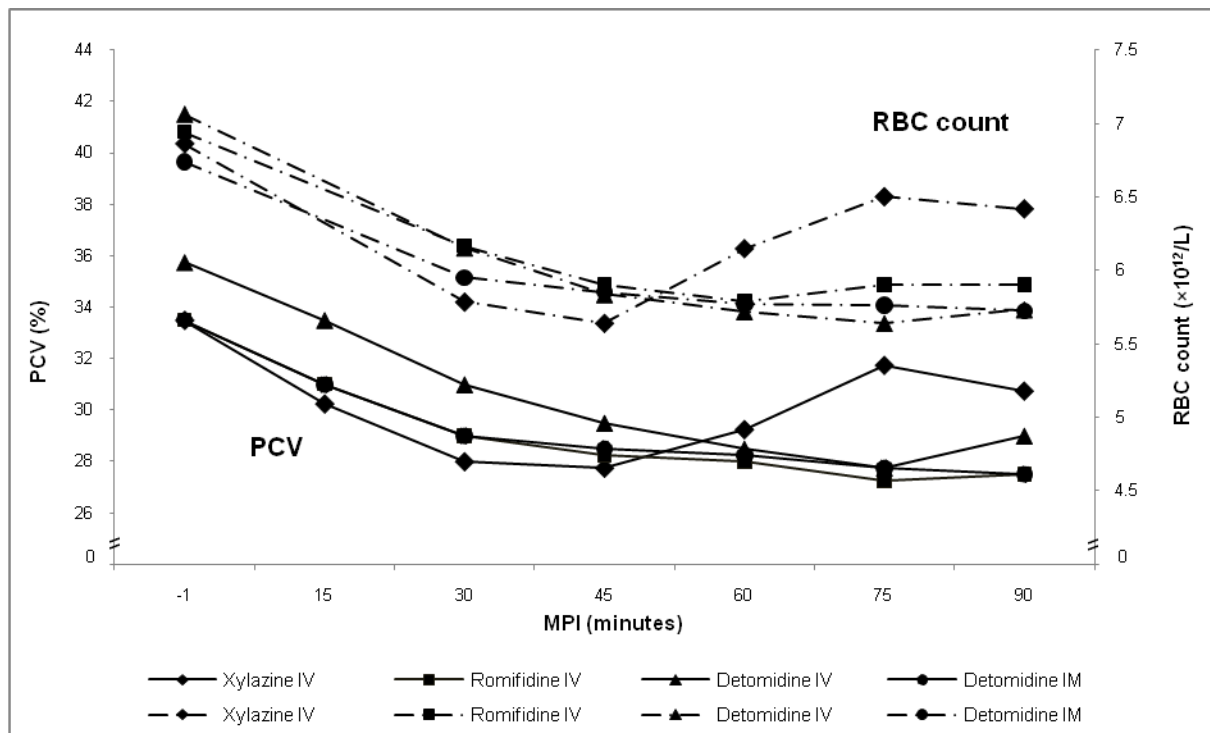
TSP: There was a significant change in TSP over time ($p<0.001$). Total serum protein decreased subsequent to drug administration with all treatments to a nadir, following which it slowly recovered. The maximal reductions following each treatment was not significantly different among treatments ($p=0.660$). The overall median (range) maximal reduction in TSP was 5.8% (3.0 - 10.3%) compared to baseline. The time to maximal reductions was significantly different among treatments ($p=0.043$). The overall median (range) time to reach the nadir was 52.5 MPI (15 - 120 MPI). The mean TSP ± SE for all treatments is summarised in figure 5.8.

There was no significant effect of treatment on TSP for the three IV treatments ($p=0.536$) or for the comparison between IV and IM detomidine administration routes ($p=0.798$). Treatment by time interaction for TSP was also not significant ($p=0.915$).

Figure 5.8: Mean (± SE) TSP over time following administration of IV xylazine, IV romifidine, IV detomidine and IM detomidine.
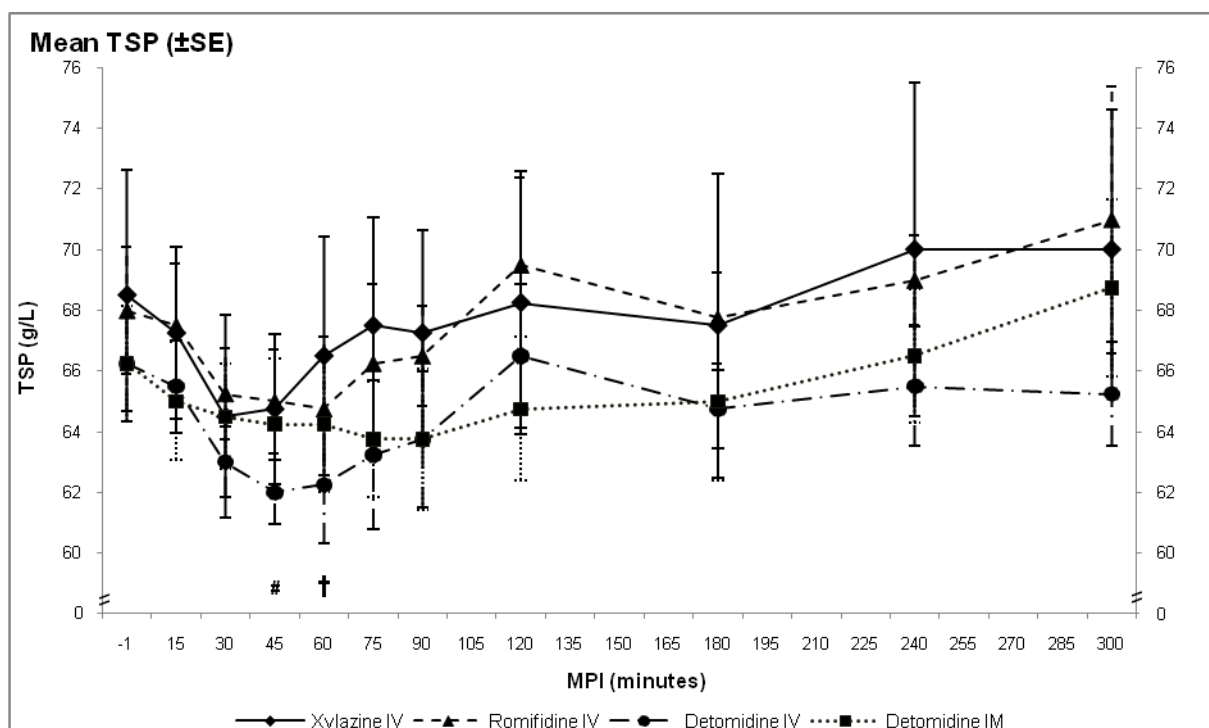
Data statistically different from baseline are indicated by [#] for IV romifidine ($p=0.05$) and [†] for IV detomidine ($p<0.05$).

The TSP responses over time for the individual mares following each treatment are shown in figures 5.9 – 5.12.

Xylazine IV (Figure 5.9): The maximum decrease in TSP occurred at 30 MPI (Mares 2 - 4) and at 45 MPI (Mare 1). At the nadir, the median (range) maximal reduction in TSP compared to baseline was 5.3% (4.7 - 10.3%). During the observation period, the TSP of all four mares returned to baseline values. This occurred at 60 MPI (Mare 2), at 75 MPI (Mare 4) at 90 MPI (Mare 3) and 120 MPI (Mare 1).

Figure 5.9: TSP response following IV xylazine administration.
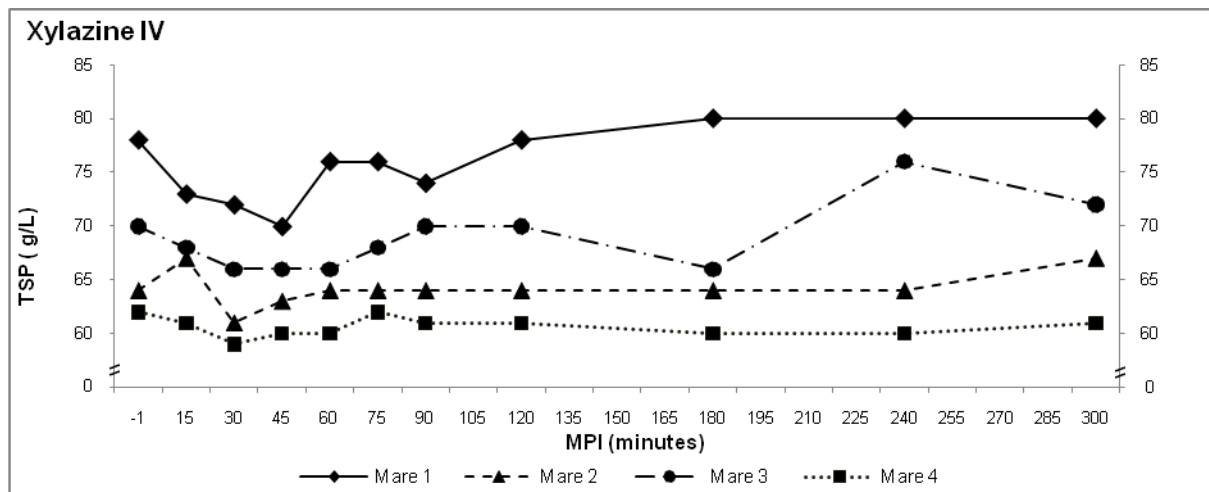
Romifidine IV (Figure 5.10): The maximum decrease in TSP occurred at 30 MPI (Mare 1) and at 60 MPI (Mares 2 - 4). At the nadir, the median (range) maximal reduction in TSP compared to baseline was 5.6% (4.7 - 6.1%). During the observation period, the TSP of all four mares returned to or exceeded baseline values. This occurred at 75 MPI (Mare 2), 90 MPI (Mare 4), 120 MPI (Mare 3) and 300 MPI (Mare 1).

Figure 5.10: TSP response following IV romifidine administration.
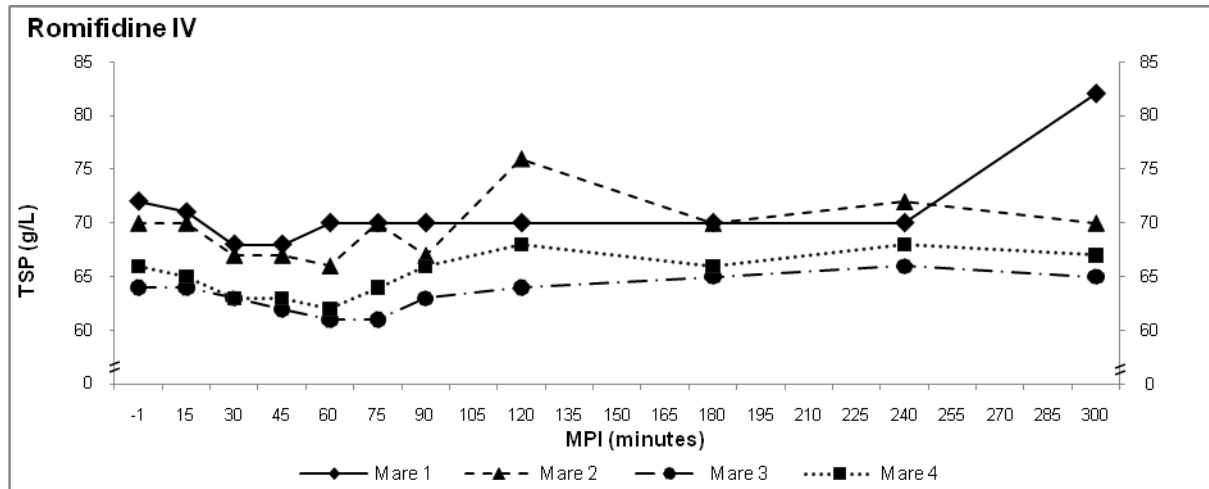
Detomidine IV (Figure 5.11): The maximum decrease in TSP occurred at 30 MPI (Mare 1), at 45 MPI (Mare 4) and at 60 MPI (Mares 2 and 3). At the nadir, the median (range) maximal reduction in TSP compared to baseline was 7.0% (5.9 - 8.7%). During the observation period, the TSP of three mares returned to or exceeded baseline values. This occurred at 75 MPI (Mare 4) and at 120 MPI (Mares 1 and 3).

Figure 5.11: TSP response following IV detomidine administration.
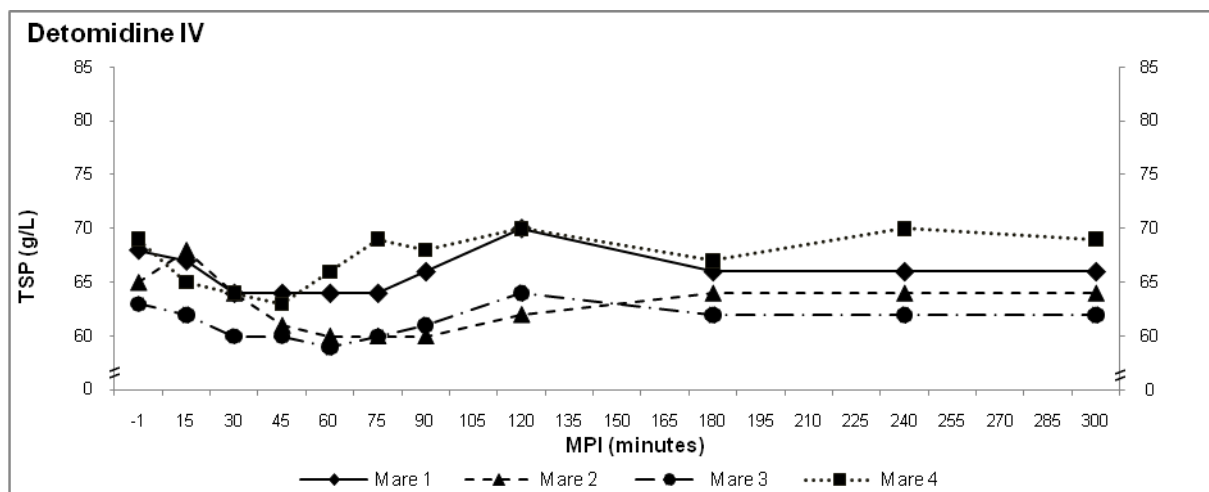
Detomidine IM (Figure 5.12): The maximum decrease in TSP of each mare occurred at different time points, namely 15 MPI (Mare 3), 60 MPI (Mare 1), 90 MPI (Mare 4) and 120 MPI (Mare 2). At the nadir, the median (range) maximal reduction in TSP compared to baseline was 5.5% (3.0 - 8.6%). During the observation period, the TSP of three mares returned to or exceeded baseline values. This occurred at 90 MPI (Mare 3), 240 MPI (Mare 4) and 300 MPI (Mare 2).

Figure 5.12: TSP response following IM detomidine administration.
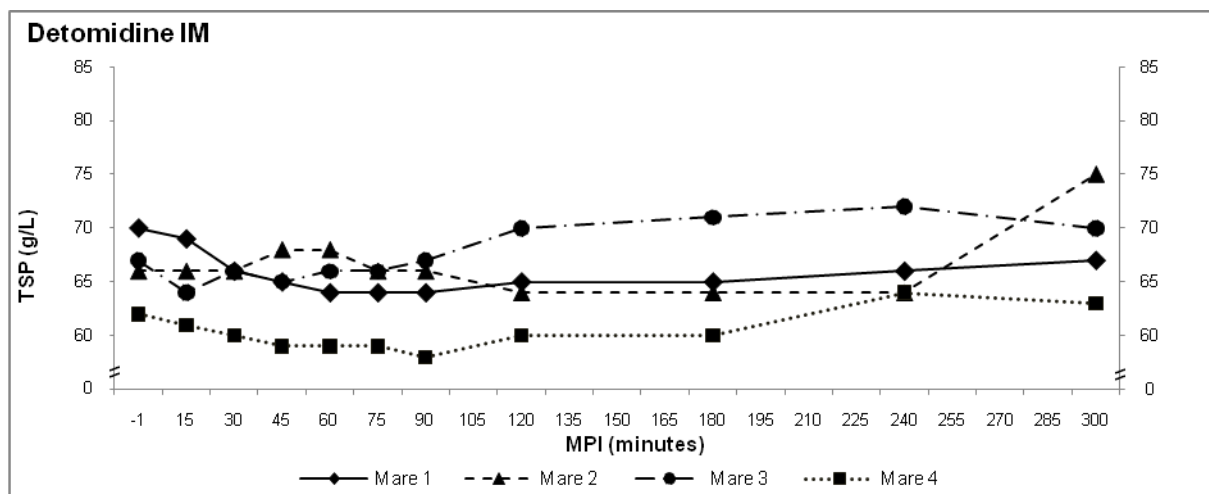
COP: There was a significant change in COP over time (p=0.001). Colloid osmotic pressure decreased subsequent to drug administration with all treatments. During the observation period a recovery of COP towards baseline values was observed for the three IV treatments, but not for IM detomidine administration. The overall median (range) maximal reduction in COP compared to baseline was 9.3% (0 - 16.2%). The overall median (range) time to reach the nadir was 45 MPI (-1 - 90 MPI). The mean COP ± SE for all treatments is summarised in figure 5.13.

There was no significant effect of treatment on COP for the three IV treatments (p=0.221) or for the comparison between IV and IM detomidine administration routes (p=0.618).

Figure 5.13: Mean (± SE) COP over time following administration of IV xylazine, IV romifidine, IV detomidine and IM detomidine.
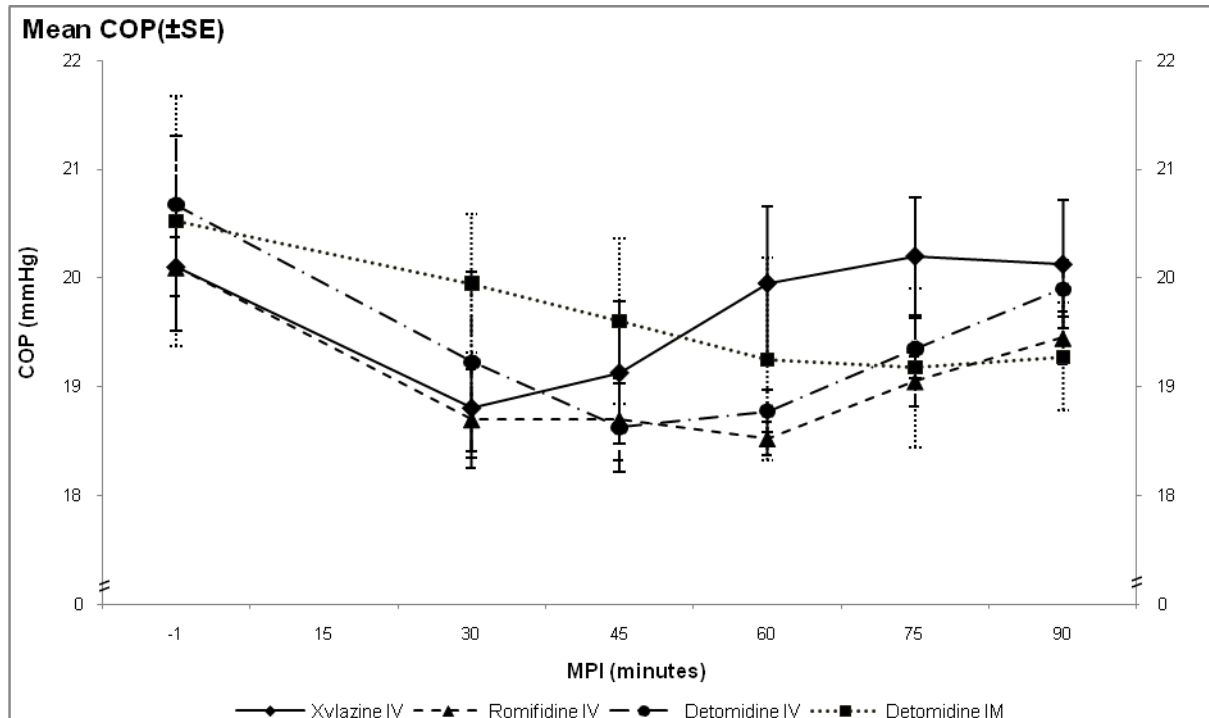
A Pearson product-moment correlation coefficient was computed to assess the relationship between TSP (g/L) and COP (mm Hg). Overall, there was a positive correlation between TSP and COP (n=96, r=0.462, p<0.001). Total serum protein and COP measurements were standardised by the following formula:

Standardised value = (measured value - mean) / SD

These values were displayed graphically using a scatter plot (Figure 5.14).

Figure 5.14: Correlation between COP and TSP.

Blood glucose: There was a significant change in blood glucose over time (p<0.001). Blood glucose increased subsequent to drug administration with all treatments. The overall median (range) maximal increase in blood glucose compared to baseline was 28.8% (4.9 - 54.6%). The overall median (range) time point of these peak measurements was 37.5 MPI (30 - 90 MPI). The mean glucose concentration ± SE for all treatments is summarised in figure 5.15.

There was a significant effect of treatment on blood glucose for the three IV treatments (p=0.011) and the treatment by time interaction was also significant (p=0.022). Mean blood glucose over time was lower following IV xylazine compared to IV detomidine treatment (4.32 versus 4.90 mmol/L; p<0.05 including Bonferroni correction; Appendix D1). The route of detomidine administration did not have a significant effect on glucose concentration (p=0.881).

Figure 5.15: Mean (± SE) blood glucose over time following administration of IV xylazine, IV romifidine, IV detomidine and IM detomidine.
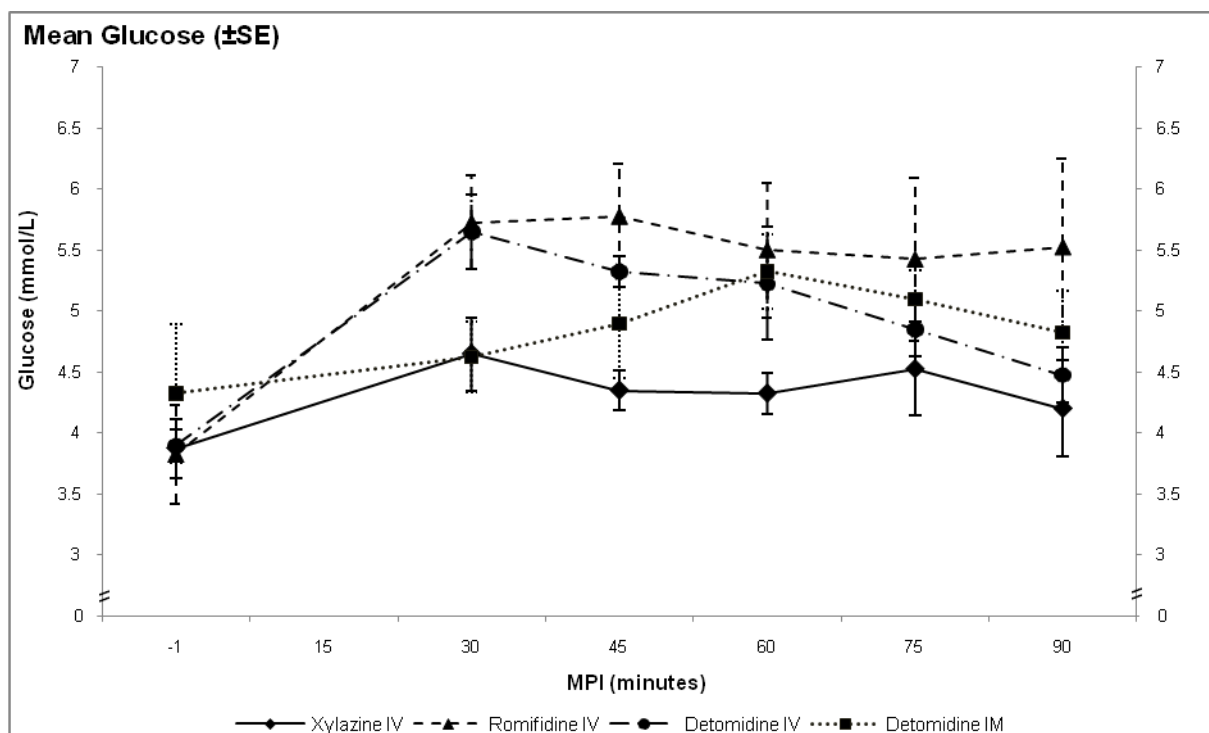
BUN: There was a significant change in BUN over time (p=0.005). Blood urea nitrogen decreased subsequent to drug administration with all treatments. The overall median (range) maximal reduction in BUN compared to baseline was 6.4% (0 - 11.0%). The overall median (range) time to reach the nadir was 75 MPI (-1 - 90 MPI). The mean BUN ± SE for all treatments is summarised in figure 5.16.

There was no significant effect of treatment on BUN for the three IV treatments (p=0.827) or for the comparison between IV and IM detomidine administration routes (p=0.994).

Figure 5.16: Mean (± SE) BUN over time following administration of IV xylazine, IV romifidine, IV detomidine and IM detomidine.
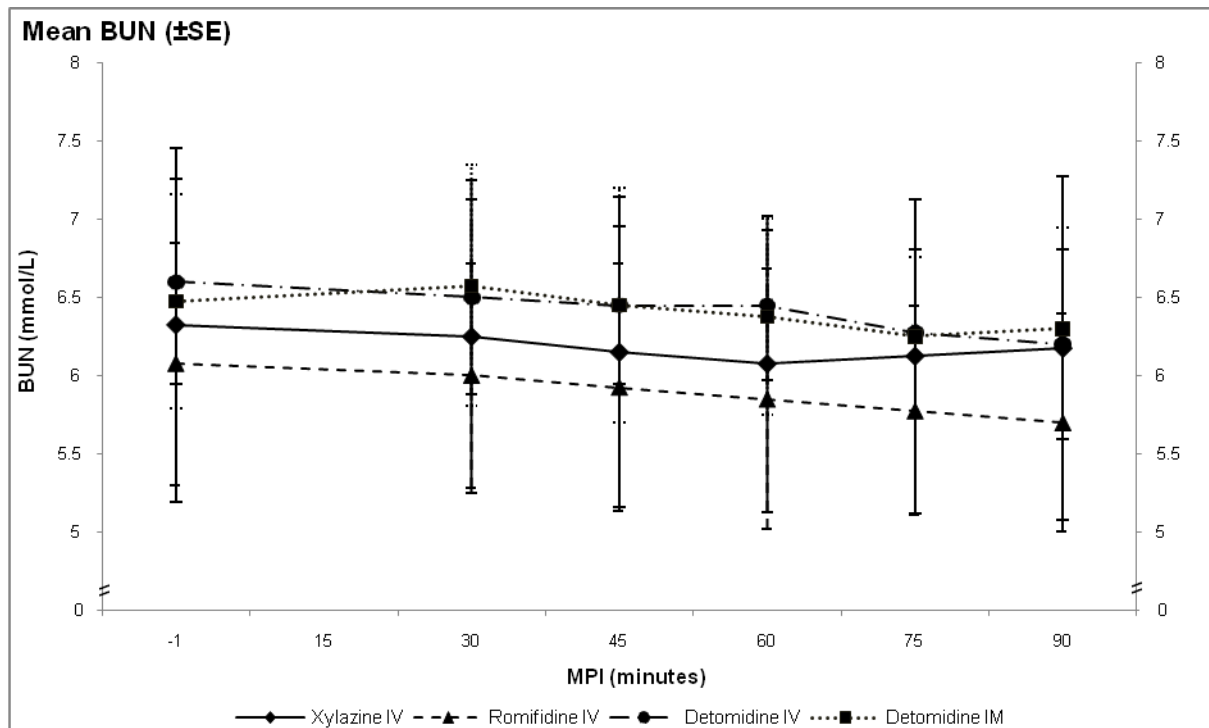
Na⁺: Na^+: There was a significant change in Na^+ concentration over time (p=0.025). Serum Na^+ increased slightly subsequent to drug administration with all treatments. The overall median (range) maximal increase in Na^+ concentration compared to baseline was 0.7% (0 - 2.2%). The overall median (range) time point of these peak measurements was 60 MPI (-1 - 90 MPI). The mean Na^+ concentrations ± SE for all treatments are summarised in figure 5.17.

There was no significant effect of treatment on Na^+ for the three IV treatments (p=0.598) or for the comparison between IV and IM detomidine administration routes (p=0.211).

Figure 5.17: Mean (± SE) Na^+ over time following administration of IV xylazine, IV romifidine, IV detomidine and IM detomidine.
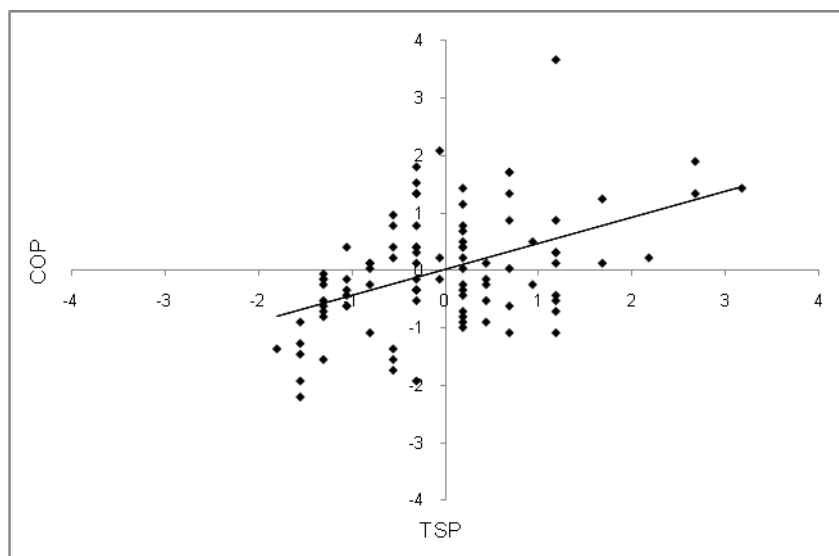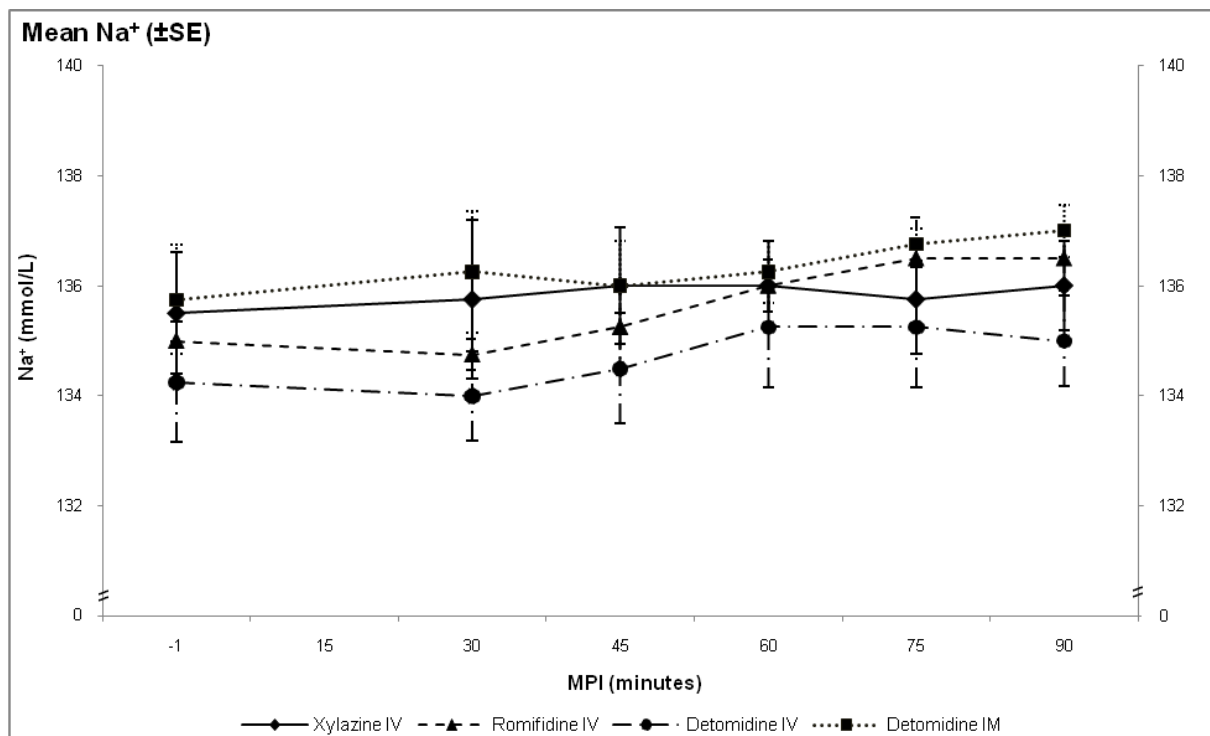
Ca^{2+}: There was a significant change in Ca^{2+} concentration over time ($p<0.001$). Serum Ca^{2+} decreased subsequent to drug administration with all treatments until 30 to 60 MPI, following which it slowly recovered. The overall median (range) maximal reduction in Ca^{2+} compared to baseline was 5.9% (2.0 - 12.7%). The overall median (range) time to reach the nadir was 60 MPI (30 - 75 MPI). The mean Ca^{2+} concentrations ± SE for all treatments are summarised in figure 5.18.

There was no significant effect of treatment on Ca^{2+} for the three IV treatments ($p=0.366$) or for the comparison between IV and IM detomidine administration routes ($p=0.381$).

Figure 5.18: Mean (± SE) Ca^{2+} over time following administration of IV xylazine, IV romifidine, IV detomidine and IM detomidine.

K^+: There was no significant change in K^+ concentration over time (p=0.202). There was no significant effect of treatment on K^+ for the three IV treatments (p=0.860) or for the comparison between IV and IM detomidine administration routes (p=0.158).

Cl^-: There was no significant change in Cl^- concentration over time (p=0.162). There was no significant effect of treatment on Cl^- for the three IV treatments (p=1.0) or for the comparison between IV and IM detomidine administration routes (p=0.381).

Lactate: There was no significant change in serum lactate over time (p=0.839). There was no significant effect of treatment on lactate for the three IV treatments (p=0.201) or for the comparison between IV and IM detomidine administration routes (p=0.086).

Blood pH: There was no significant change in venous blood pH over time (p=0.330). There was no significant effect of treatment on venous blood pH for the three IV treatments (p=0.273) or for the comparison between IV and IM detomidine administration routes (p=0.605).

Plasma osmolality: There was a significant change in plasma osmolality over time (p=0.028). Osmolality increased subsequent to drug administration with all treatments. The overall median (range) maximal increase in plasma osmolality compared to baseline was 1.1% (0.3 - 5.4%). The overall median (range) time point of these peak measurements was 52.5 MPI (30 - 90 MPI). The mean plasma osmolality ± SE for all treatments is summarised in figure 5.19.

There was no significant effect of treatment on plasma osmolality for the three IV treatments (p=0.879) or for the comparison between IV and IM detomidine administration routes (p=0.241). The relationship between the change over time in plasma osmolality and PCV is shown in figure 5.20.

Figure 5.19: Mean (± SE) plasma osmolality over time following administration of IV xylazine, IV romifidine, IV detomidine and IM detomidine.
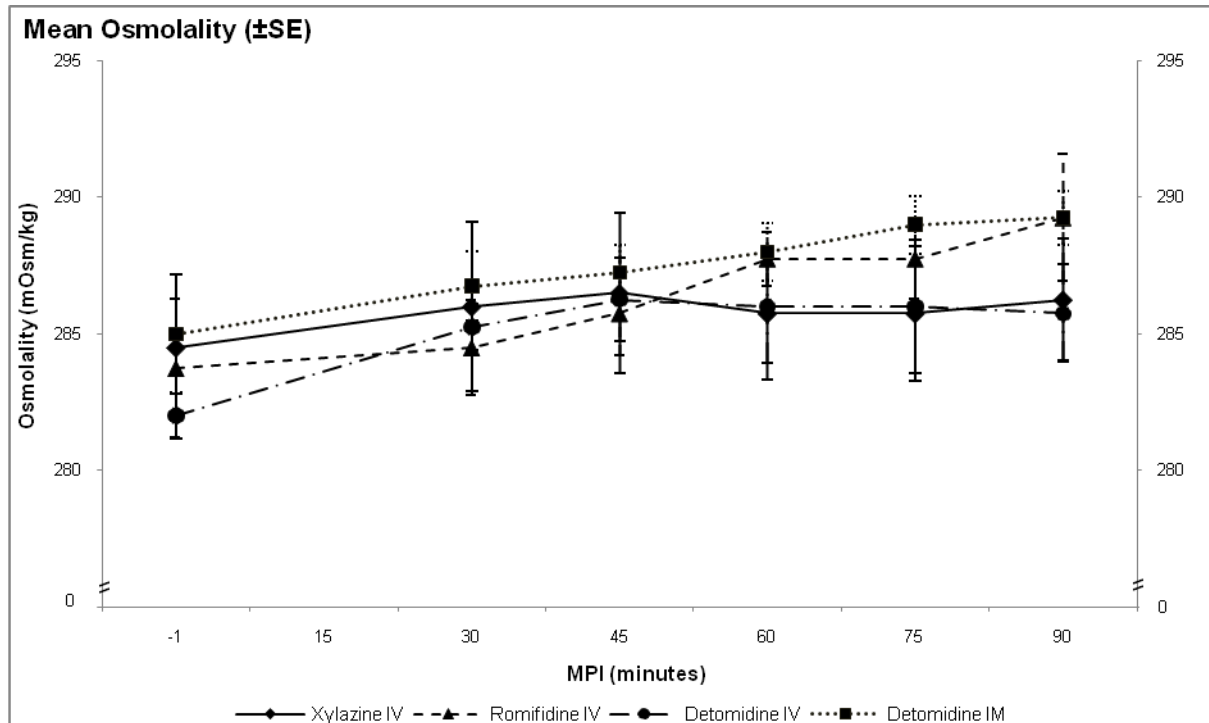
Figure 5.20: Mean plasma osmolality and PCV over time following administration of IV xylazine, IV romifidine, IV detomidine and IM detomidine.
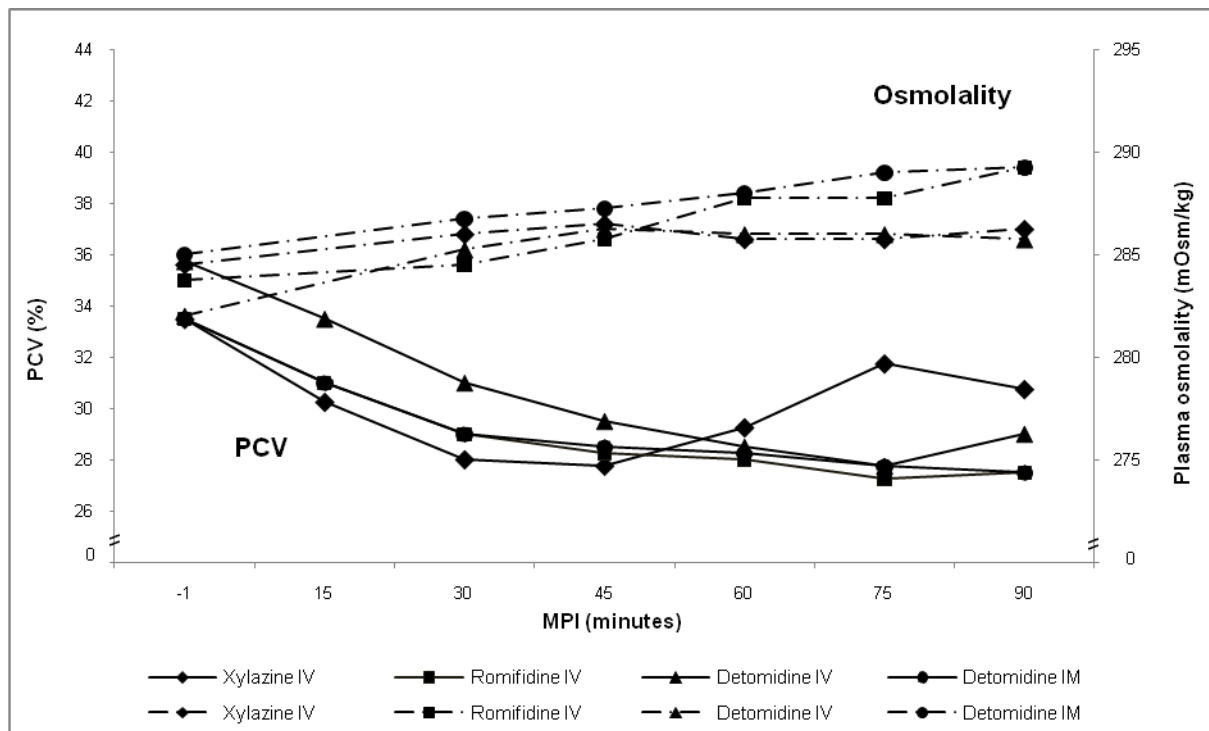
Ultrasonographic splenic size: The intraobserver coefficients of variation (range) for observers 1 - 4 were 8.3% (1.4 - 15.3%), 8.5% (0.6 - 23.3%), 6.5% (0.0 - 18.2%) and 6.5% (0.6 - 22.5%), respectively. The overall mean intraobserver coefficient of variation (range) was 7.5% (0 - 23.3%). Based on the variance components analysis, the amount of variability in measured splenic size that could be attributed to ICS, subject and ultrasonographer was 46.8%, 24.2% and 1.0%, respectively. Twenty-eight percent of the variation was unexplained by these variables.

Repeated measures ANOVA for all splenic measurements and respective p values are summarised in appendix D2.

There was a significant change in ultrasonographic splenic size over time in three out of five anatomical locations, namely ICS 9 (p=0.045), ICS 11 (p=0.003) and ICS 13 (p=0.032). There was no significant effect of treatment on splenic size at any location for the three IV treatments or for the comparison between IV and IM detomidine administration routes.

The coefficients of variation in measured splenic size in the three anatomical locations, where a significant change over time was detected, were 7.5% for ICS 9, 10.5% for ICS 11 and 7.7% for ICS 13. The repeatability of ultrasonographic measurements was best in ICS 9 and therefore all further analyses were performed using only this ICS.

ICS 9: There was a significant change in ultrasonographic splenic size over time (p=0.045). Splenic size at ICS 9 increased subsequent to drug administration in all but one case (IM detomidine; Mare 1). The overall median (range) maximal increase in splenic size at ICS 9 compared to baseline was 14.4% (0.0 - 52.9%). The median

(range) time point of these peak measurements was 45 MPI (-1 - 90 MPI). The mean splenic sizes at ICS 9 ± SE for all treatments are summarised in figure 5.21.

There was no significant effect of treatment on splenic size at ICS 9 for the three IV treatments (p=0.689) or for the comparison b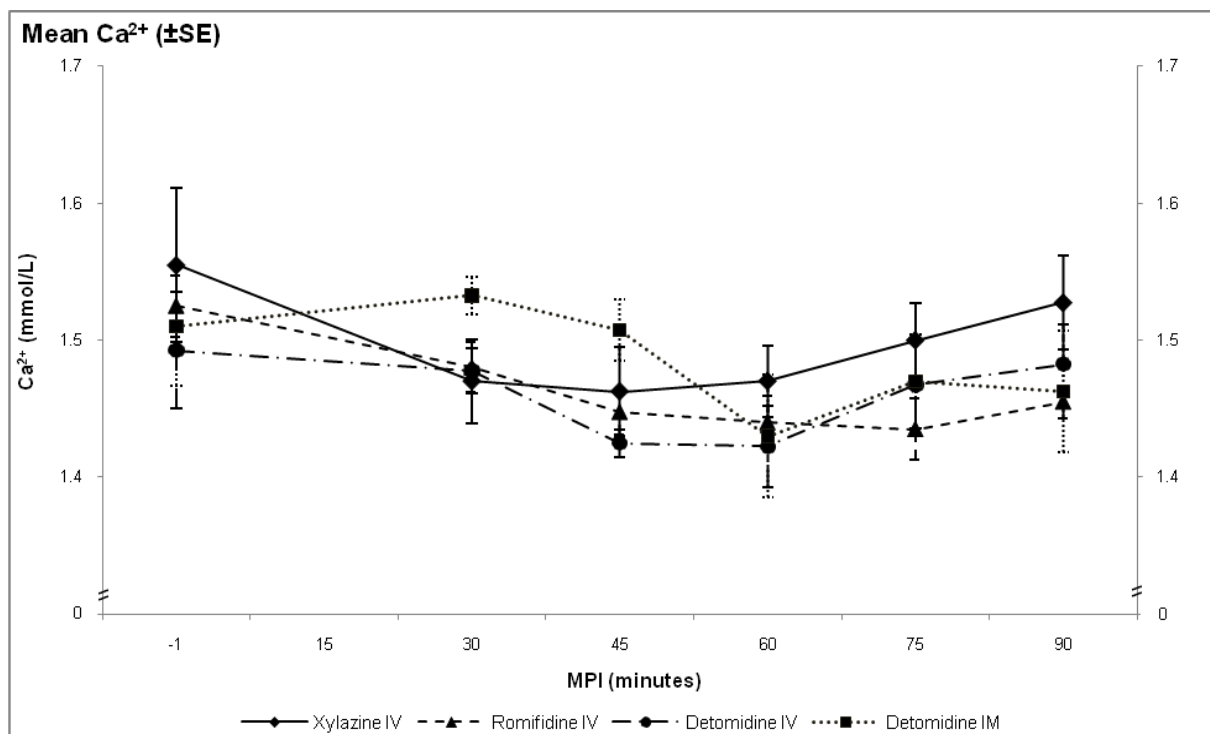etween IV and IM detomidine administration routes (p=0.902). The relationship between the change over time in splenic size at ICS 9 and PCV is shown in figure 5.22.

Figure 5.21: Mean (± SE) splenic size at ICS 9 over time following administration of IV xylazine, IV romifidine, IV detomidine and IM detomidine.
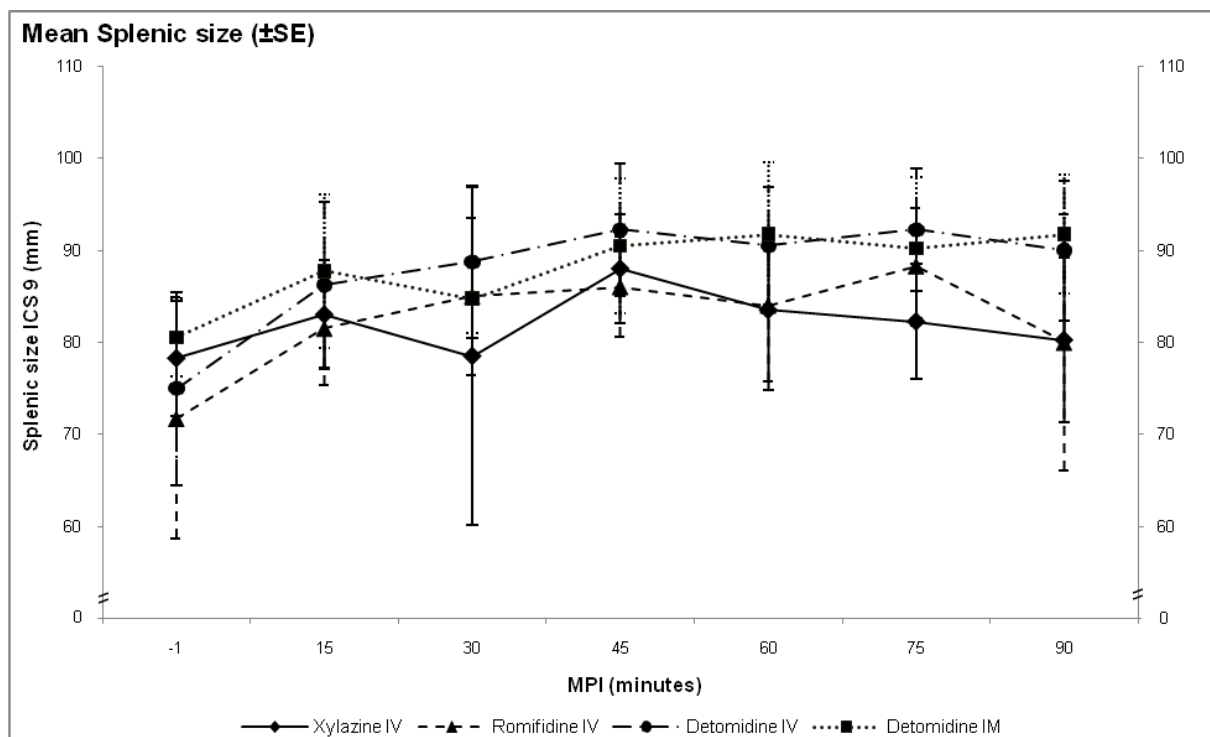
Figure 5.22: Mean splenic size at ICS 9 and PCV over time following administration of IV xylazine, IV romifidine, IV detomidine and IM detomidine.
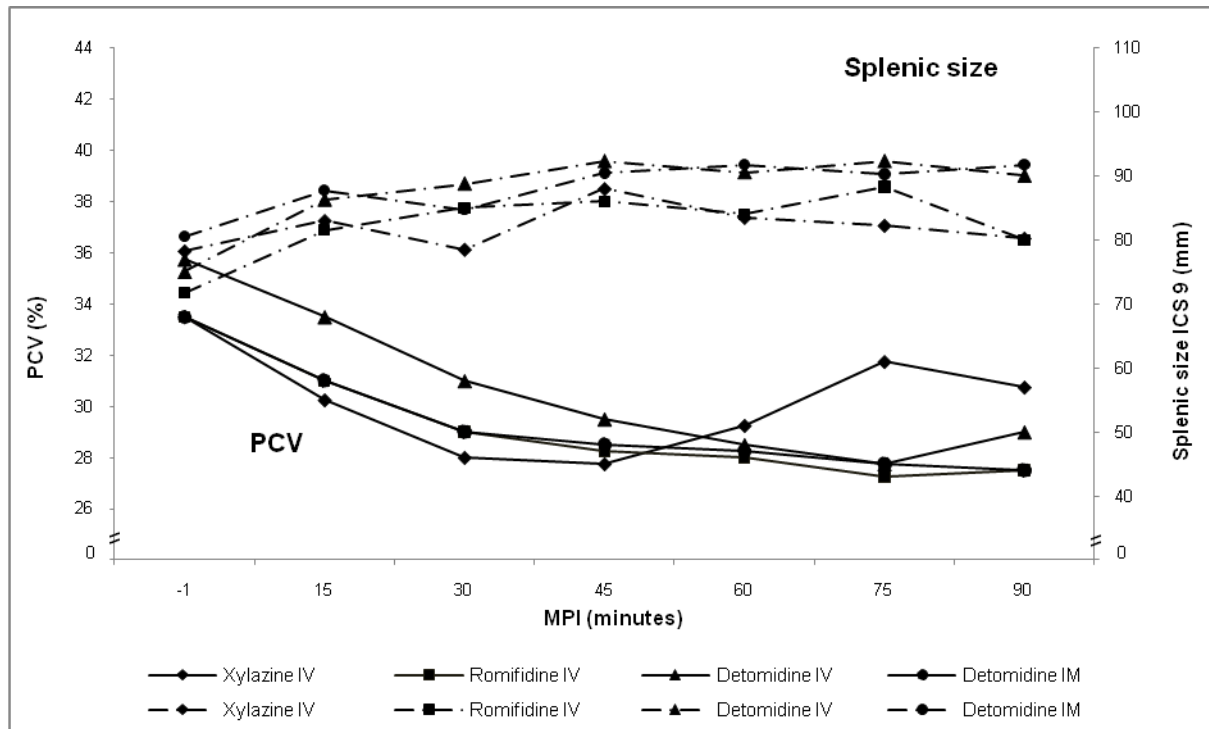
A Pearson product-moment correlation coefficient was computed to assess the relationship between PCV (%) and ultrasonographic splenic size at ICS 9 (mm). Overall, there was a negative correlation between PCV and splenic size at ICS 9 (n=112, r=-0.358, p<0.001). Splenic size and PCV measurements were standardised by the following formula:

Standardised value = (measured value - mean) / SD

These values were displayed graphically using a scatter plot (Figure 5.23).

Figure 5.23: Correlation between PCV and splenic size at ICS 9.
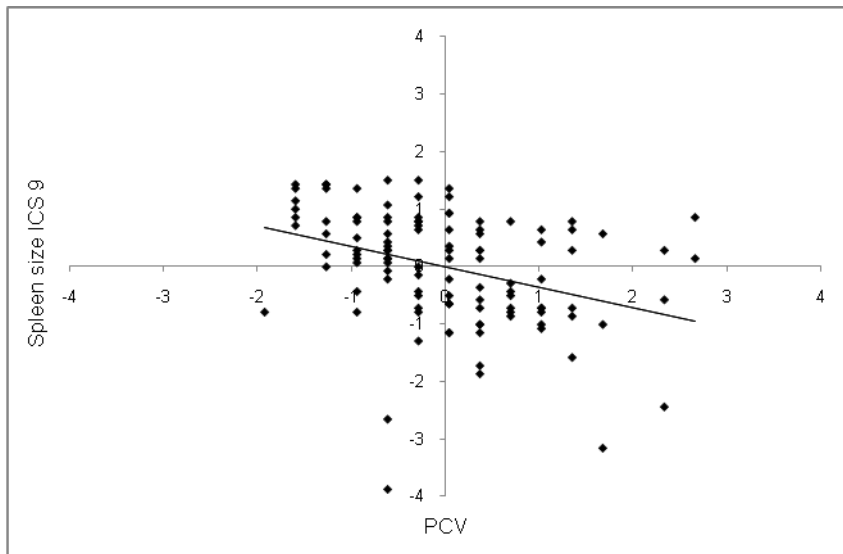
The changes over time in splenic size at ICS 9 for the individual mares following each treatment are shown in figures 5.24 – 5.27.

Xylazine IV (Figure 5.24): The maximum increase in splenic size occurred at 30 MPI (Mare 3) and at 45 MPI (Mares 1, 2 and 4). At peak measurements, the median (range) increase in splenic size compared to baseline was 14.8% (7.3 - 17.5%).

Figure 5.24: Splenic size at ICS 9 following IV xylazine administration.
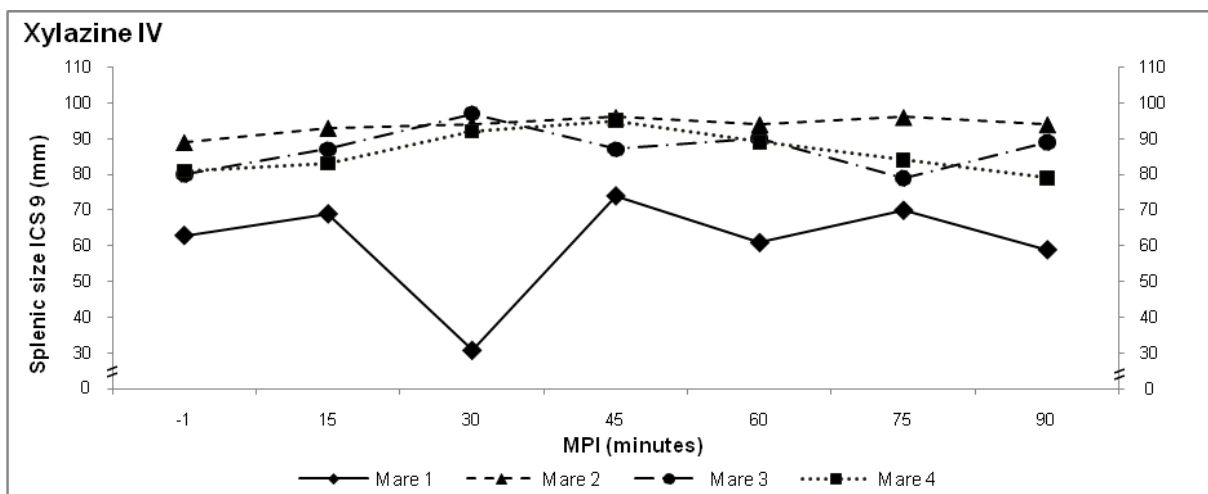
Romifidine IV (Figure 5.25): The maximum increase in splenic size of each mare occurred at different time points, namely 30 MPI (Mare 3), 45 MPI (Mare 2), 60 MPI (Mare 1) and 90 MPI (Mare 4). At peak measurements, the median (range) increase in splenic size compared to baseline was 15.6% (7.7 - 52.9%).

Figure 5.25: Splenic size at ICS 9 following IV romifidine administration.
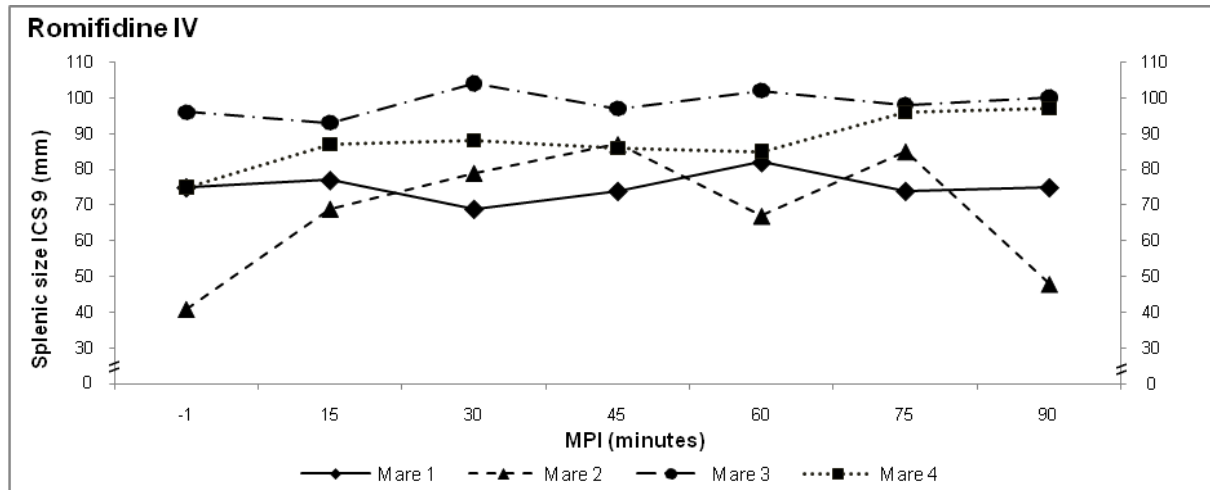
Detomidine IV (Figure 5.26): The maximum increase in splenic size of each mare occurred at different time points, namely 30 MPI (Mare 3), 45 MPI (Mare 4), 60 MPI (Mare 1) and 90 MPI (Mare 2). At peak measurements, the median (range) increase in splenic size compared to baseline was 12.7% (9.0 - 44.0%).

Figure 5.26: Splenic size at ICS 9 following IV detomidine administration.
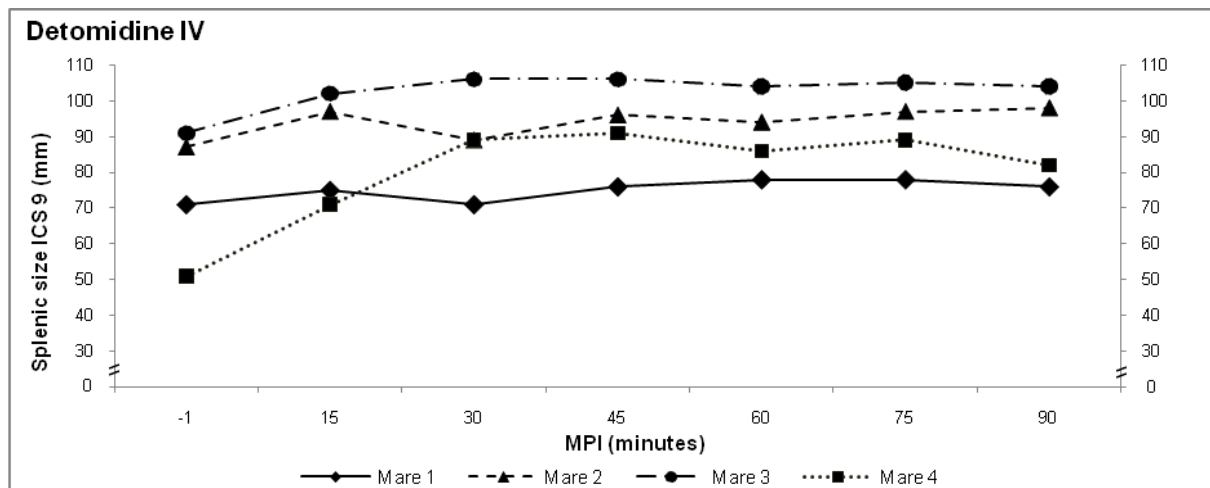
Detomidine IM (Figure 5.27): The maximum increase in splenic size of three mares occurred at different time points, namely 15 MPI (Mare 3), 60 MPI (Mare 2) and 90 MPI (Mare 4). The splenic size of mare 1 did not increase during the observation period. At peak measurements, the median (range) increase in splenic size compared to baseline was 14.4% (0 - 24.7%).

Figure 5.27: Splenic size at ICS 9 following IM detomidine administration.
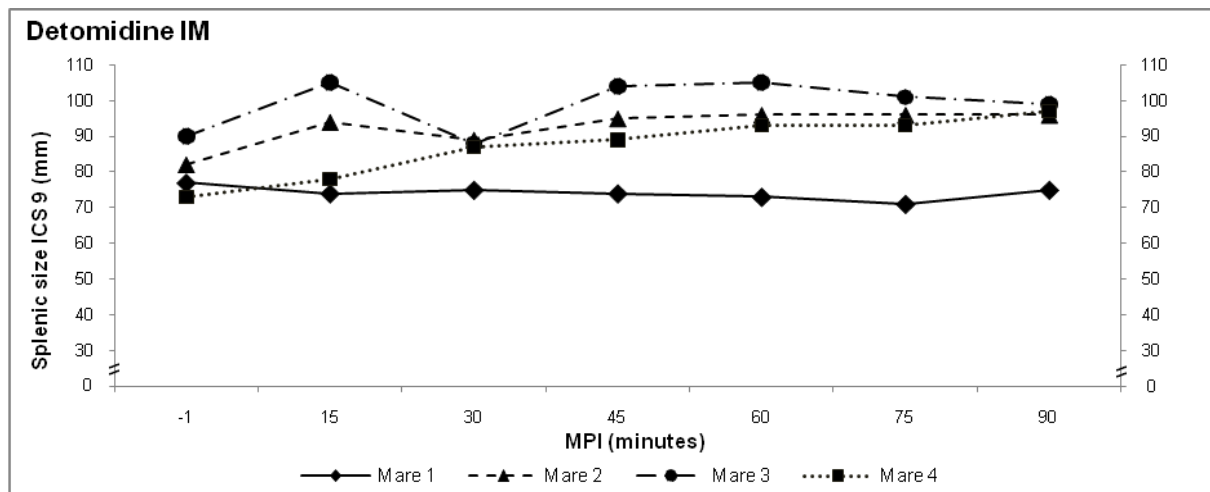
Sedation score - VAS: There was a significant change in VAS sedation scores for both observers over time (p<0.001). Repeated measures ANOVA for all sedation scores are summarised in appendix D3.

VAS observer 1: There was no significant effect of treatment on VAS 1 for the three IV treatments (p=0.352), and neither was there a treatment by time interaction (p=0.148). Detomidine administration route also did not have an effect on VAS 1 (p=0.603).

There was an increase subsequent to drug administration with all treatments to a peak at 15 MPI, following which VAS 1 declined. At 15 MPI the overall mean (± SD) VAS 1 score was 65.4 (± 17.4). The mean VAS 1 scores ± SE for all treatments are summarised in figure 5.28.

Figure 5.28: Mean (± SE) VAS observer 1 over time following administration of IV xylazine, IV romifidine, IV detomidine and IM detomidine.
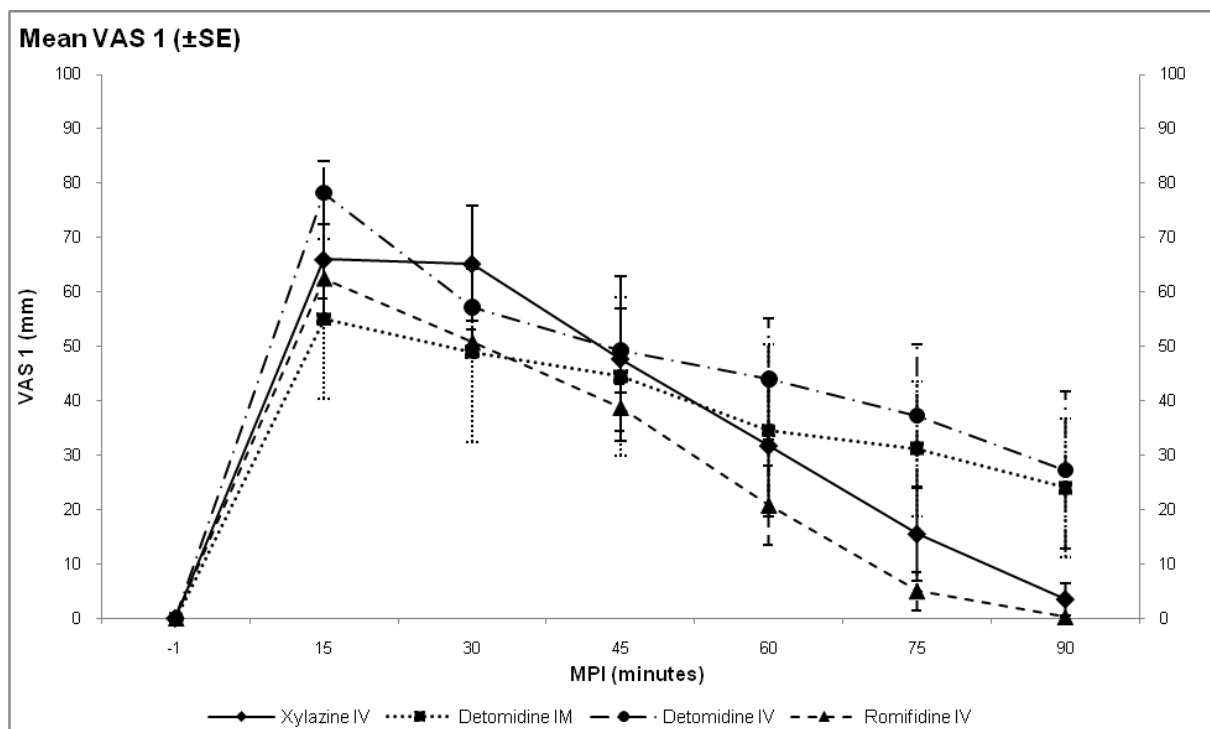
VAS observer 2: There was no significant effect of treatment on VAS 2 for the three IV treatments (p=0.051), and neither was there a treatment by time interaction (p=0.585). Following IV detomidine treatment, VAS 2 was higher compared to IV romifidine treatment (55.5 versus 32.5 mm; p<0.05 including Bonferroni correction; Appendix D3). The route of detomidine administration did not have a significant effect on VAS 2 (p=0.414).

There was an increase subsequent to drug administration with the three IV treatments to a peak at 15 MPI, following which VAS 2 declined. Following IM detomidine administration; however, a peak VAS 2 score was only observed at 45 MPI. At peak measurements, the overall mean (± SD) VAS 2 score was 66.3 (± 20.5). The mean VAS 2 scores ± SE for all treatments are summarised in figure 5.29.

Figure 5.29: Mean (± SE) VAS observer 2 over time following administration of IV xylazine, IV romifidine, IV detomidine and IM detomidine.
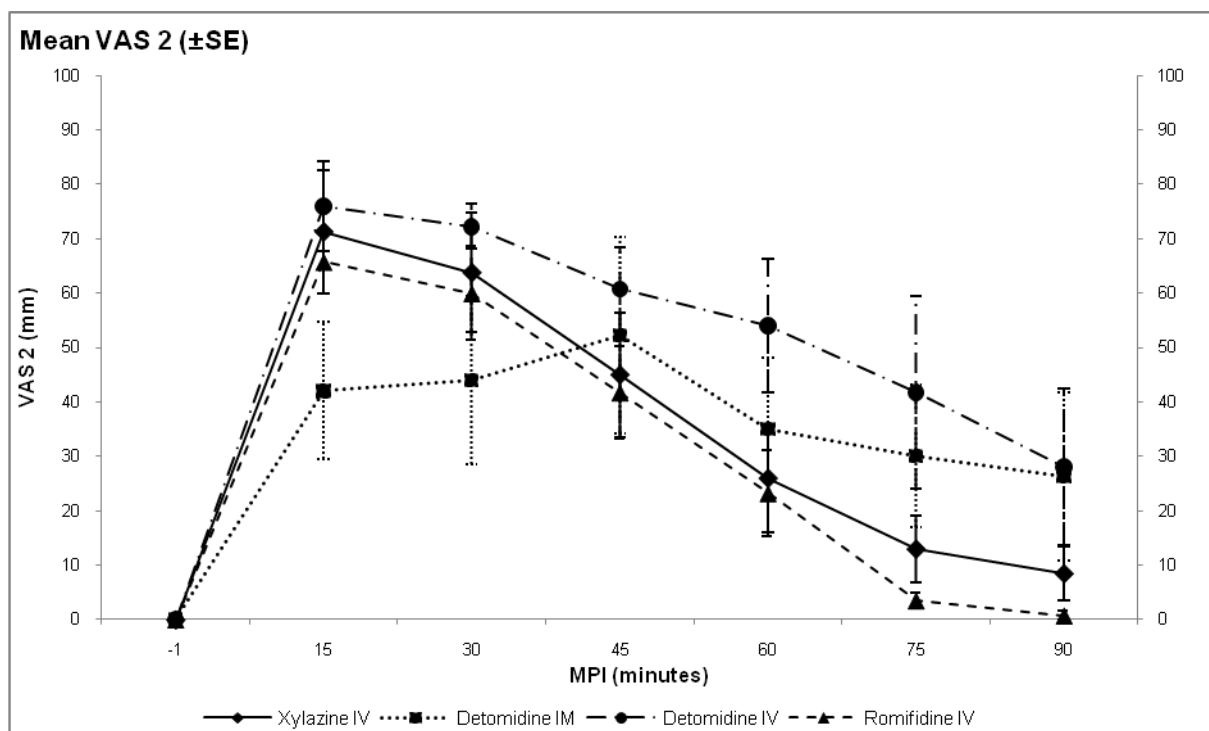
***Sedation score - Response to Ultrasound:*** There was no significant change in this sedation score over time for observer 1 (p= 0.119) or for observer 2 (p=0.135). There was no significant effect of treatment on response to ultrasound for the three IV treatments (observer 1 p=0.804; observer 2 p=0.355). The route of detomidine administration did not have a significant effect on response to ultrasound (observer 1 p=0.182; observer 2 p=0.718).

CHAPTER 6 DISCUSSION

The dosages of α_2 agonists used in the present study were based on previous reports.[10, 31, 32] Equipotency was confirmed by the assessment of sedation using the VAS sedation scores, which showed no significant differences in any of the treatments. No change over time was observed for the measured response to ultrasound, as it is a non invasive technique that produces minimal or no discomfort in horses.

As hypothesised, a significant reduction in PCV was observed over time following α_2 agonist administration, which was paralleled by the change in RBC count. All treatments induced a similar decrease in PCV, which followed a comparable pattern over time. However, the duration of this decrease was shorter for xylazine, as expected due to its reported shorter duration of action.[25, 26] The time to maximal reduction in PCV was not significantly different among drugs, which was unexpected, as xylazine has been reported to have a shorter onset of peak sedation.[29, 30] Despite the lack of statistical significance, the maximum reduction in PCV induced by xylazine occurred earlier than the other drugs (i.e. 45 min versus 75 min). The small sample size in this study probably prevented a statistical significance, but this difference is clinically significant. The hypothesised decrease in PCV of at least 15% from baseline values was observed in 93.8% of PCV measurements following α_2 agonist administration. Previous studies also reported reductions in PCV subsequent to IM or IV xylazine sedation in several domestic species, such as caprine,[14, 15] ovine,[13, 16] bovine,[11-13] and feline,[15] as well as wildlife species such as red deer,[39] impala[38] and wild boar.[40] In horses this phenomenon was identified following IM or IV

xylazine or detomidine administration.[17, 18] Despite the different dosages used in the latter studies, the reductions in mean PCV observed (23 - 29%) were in line with decreases reported here. Based on these results, the clinician should be cautious when assessing critical patients with a history of prior administration of α_2 agonists. Packed cell volume may decrease by up to 27% after α_2 agonist administration and these changes must be taken into account when using PCV to evaluate the patient's hydration status. Furthermore, this effect may outlast clinical sedation, as it was the case for romifidine administration, where the PCV remained significantly below baseline values even at three hours post-sedation. In addition cautious use of these drugs is advised in patients that already have a low PCV, such as patients suffering from anaemia or experiencing acute haemorrhage. Administration of α_2 agonist will further decrease the PCV in these patients and this, together with the drug-induced decrease in cardiac output,[6, 7, 17, 32] may lead to cardiovascular compromise and inadequate tissue oxygenation.

Total serum protein concentration decreased significantly over time following α_2 agonist administration, with no differences among drugs. The overall maximal reductions in TSP were much lower than those observed for PCV (10.3% and 27.3%, respectively). Nonetheless, TSP should also be evaluated with caution in patients that received α_2 agonists. Few studies evaluated TSP responses subsequent to α_2 agonist administration. No significant change in TSP was identified with IM xylazine in goats[14] and IV xylazine administration in 28 day old foals[19] and pregnant cows.[12] On the other hand, epidural injection of xylazine in cows[42] as well as buffalo calves[41] did show a small but non-significant reduction in TSP. Intramuscular administration of medetomidine in wild boars decreased TSP slightly but significantly.[40] In horses, a significant reduction in TSP was seen with IV

detomidine after a bolus administration followed by an infusion.[21] When xylazine was used in combination with guaifenesin and ketamine for maintenance of anaesthesia in horses, a decrease in TSP was also observed; however, this change was not significant.[20]

Blood glucose increased after α_2 agonist administration. The trend towards hyperglycaemia identified in this study compares to earlier reports of IV xylazine,[33, 34] IV detomidine[35] and IV detomidine-atropine combination[44] on blood glucose in horses. Hyperglycaemia in response to α_2 agonist administration alone or in combination with atropine has also been identified in other species including feline[77] and caprine.[14, 37] This effect is believed to be due to α_2 agonist-induced inhibition of insulin release from pancreatic islet cells.[6, 7, 32, 36, 77]

A significant decrease in BUN was identified in the present study following α_2 agonist administration, with no differences among treatments. Thurmon et al. (1984), conversely, detected no significant differences in BUN of healthy horses when comparing administrations of IV xylazine and saline solution, evaluated at fixed time points over 180 minutes.[34] No change was either observed with IM xylazine in goats.[14] Despite the significant decrease in BUN in the present study, all measurements remained within normal reference ranges for adult horses (Appendix E), and therefore these changes were considered clinically insignificant. It should be also noted that there was a high baseline BUN variation among mares.

No significant changes over time were observed for K^+ and Cl^- for either treatment, as previously reported in horses.[33, 34]

Additionally, no significant change in serum lactate was observed. Lactate has been previously monitored in response to α_2 agonist administration post-exercise in

horses. A significant decrease in lactate was reported shortly after exercise subsequent to xylazine administration, while no changes were observed following treatment with detomidine or a combination of xylazine and acepromazine.[78]

There was no significant change in venous blood pH following α_2 agonist administration.   Previous studies report various results on pH responses. While the arterial pH in cattle was significantly increased with IM xylazine administration,[11] no significant change was observed in horses with IV or IM xylazine or detomidine administration.[17, 29] Additionally, no changes in venous or arterial pH were observed in mares with caudal epidural administration of xylazine or detomidine.[43]

Ionized calcium concentrations decreased significantly following α_2 agonist administration. Despite moderate urinary calcium excretion following sedation with xylazine and atropine in healthy mares, significant changes in total and ionized calcium concentrations were not observed by Gasthuys et al (1986).[69] Additionally, no significant change in total and Ca^{2+} concentrations was observed using a detomidine-atropine combination.[44] One study, however, reported a significant increase in Ca^{2+} along with a small but significant increase in urinary excretion following detomidine-atropine sedation.[45] The decrease in Ca^{2+} in the present study is not considered clinically significant, as the measurements remained within normal reference ranges for adult horses (Appendix E2). Multiple factors such as changes in TSP concentration, protein binding capacity and affinity, serum pH[79] as well as complexed calcium interact to determine the Ca^{2+} concentration, independent of total calcium.[80] Blood pH can be excluded as a causative mechanism, as no significant changes were observed. In cases of hypoalbuminaemia in horses, total hypocalcemia results, while Ca^{2+} usually remains within normal reference ranges,[79] as observed here. Although albumin was not measured in this study, there was a

small decrease in TSP, which could account for a small change in total calcium but not in Ca^{2+}. Total serum concentrations and urinary excretion of Ca^{2+} were not measured in this study, and therefore, the physiological reason for the observed decrease of Ca^{2+} remains unknown.

A significant change in serum Na^+ in response to α_2 agonist administration was identified. There was a gradual tendency of Na^+ to increase during the observation period. The Na^+ concentrations, however, were within normal reference ranges established for adult horses (Appendix E1). Former published results on the effects of IV xylazine,[33, 34] xylzaine-atropine[69] and detomidine-atropine combination[44, 45] indicate no significant changes in serum Na^+ despite significant diuresis in conjunction with increased urinary clearance of Na^+. Diuresis has also been reported in horses following IV administration of detomidine.[35] Alpha 2 agonist-induced diuresis is believed to be the result of inhibition of ADH release,[6, 7, 36] blocking of its activity on the renal tubules,[6, 7, 32] decreased release of renin[7, 36] as well as increased release of ANP.[6, 21, 38] Urine osmolality as well as specific gravity was significantly lower after IV xylazine administration,[33, 34] indicating production of a rather dilute and hypotonic urine. As hypotonic fluid loss has been associated with hypernatraemia,[81] the small increase in Na^+ observed in this study is speculated to occur due to a relatively higher renal loss of water compared to Na^+.

A significant reduction in COP was observed over time following α_2 agonist administration, with no differences among drugs. To the best of the author's knowledge, the effects of α_2 agonists on COP have not been previously reported. In this study TSP and COP were positively correlated, which is expected as COP is determined mainly by the plasma proteins, especially albumin.[55, 75] The relationship between COP and TSP has been described as nonlinear.[55] Other factors contribute

to COP apart from the plasma proteins, in particular the Gibbs Donnan effect[55, 56, 58, 75] and blood pH.[82] As no significant change in pH was observed and the serum Na^+ actually increased, the decrease in COP in this study can only be explained by the decrease in TSP.

Two main mechanisms have been postulated in the literature to explain the effect of α_2 agonists on PCV namely fluid shift from the extravascular into the intravascular space[12, 17, 20, 37, 42] and RBC sequestration in the spleen.[12, 17, 37] The present study attempted to evaluate these two mechanisms by measuring plasma osmolality and ultrasonographic splenic size, respectively.

A significant change in plasma osmolality, with a tendency to increase over time, was observed following α_2 agonist administration. The results of this study are in contrast to previously published reports in horses, in which no significant changes in plasma osmolality were found following IV xylazine administration.[33, 34] Sodium and chloride are the primary determinants of plasma osmolality[54, 63] (Osmolality = 1.86 $(Na^+ + K^+)$ + glucose + BUN + 9).[60] Potassium, glucose, non-protein nitrogen and other trace elements contribute less than 10% to normal plasma osmolality,[60] whereas glucose alone accounts for approximately 2%.[54] The increases in glucose and Na^+ may explain the small increase in plasma osmolality observed in this study. Plasma osmolality has been defined as the gold standard for identifying dehydration in adult working horses, subject to the limitation that it may fall within reference range even if dehydration is present.[83] Plasma osmolality increases with true hypertonic dehydration.[60] Since plasma osmolality also increased post-sedation, interpretation of measurements in clinical practice may be confounded by previous α_2 agonist administration. However, plasma osmolality in this study remained within reference ranges reported for normal adult horses (Appendix E2) and hence, measurements

above these reference values may still be used as an indicator of dehydration in the clinical situation, despite prior administration of α_2 agonists.

An intravascular fluid shift in response to the change in plasma osmolality could have occurred in this study. While some authors considered the change in PCV entirely due to the inflow of fluid from the interstitial fluid,[20] most authors attribute the change in PCV to RBC sequestration and the smaller change in TSP to consequent changes in plasma volume.[46, 47] The increase in osmolality and the decrease in TSP in this study substantiate the hypothesis of plasma volume expansion and haemodilution[40] contributing, at least partially, to the decline in PCV. Additionally, α_2 agonist-induced hypotension may also play a role. Although this decrease in blood pressure has been described as mild[25], rarely falling below 20% of baseline measurements,[6] it could be sufficient to stimulate a fluid shift. This was speculated by Wagner et al (1991)[17] and De Moor et al (1978)[47] to explain the post-sedation decrease in PCV and TSP, respectively. Blood pressure was not measured in the present study and any possible correlation with the decrease in PCV remains speculative.

On the other hand, the observed decrease in COP, an important osmotic force in retaining appropriate intravascular volume,[56, 58, 59] would suggest fluid movement in the opposite direction; from the intra- to the extravascular space. However, the initial change in COP was probably a consequence, and not the cause, of the decrease in TSP, secondary to the initial fluid shift. It is likely that the decrease in COP contributed, at least partially, to the subsequent restitution of the fluid balance.

As hypothesised, a significant increase in ultrasonographic splenic size was observed over time following α_2 agonist administration in three out of five anatomical locations chosen (ICS 9, 11 and 13), which was similar for all treatments. However, the time to obtain the maximum splenic size measurements was different among

treatments. Ultrasonographic splenic size had a tendency to increase over time as PCV decreased, which indicates the likelihood of splenic RBC sequestration, as previously reported.[12, 17, 47] The horse's spleen is a great reservoir for red cells and is capable of holding one third of the circulating red cell volume,[49] which upon splenic contraction following stress or exercise is released, as illustrated by studies using intact and splenectomised horses.[49-51] By the same argument the decreased sympathetic tone following sedation with phenothiazines and α_2 agonists could lead to splenic relaxation and RBC sequestration.[17, 46] This was shown following promazine administration in ponies, where a decrease in haematocrit was observed in intact but not in splenectomised ponies.[47] Additionally, there may be other RBC pools besides the spleen, as has been suggested in horses by Torten and Schalm (1964)[49] and in dogs by Wilson et al (2004).[48]

The results of the present study, therefore, suggest interplay of splenic sequestration as well as intravascular fluid shifts as possible mechanisms for the observed decrease in PCV following α_2 agonist administration.

Some of the limitations of this study include the small sample size used, which limited the power of the study, as well as the short sampling period. Since the maximal decrease in PCV was hypothesised to occur at 60 MPI, the observation period of 90 MPI was considered sufficient to try to elucidate the mechanism for this reduction. However, for some variables such as plasma osmolality and Na^+, a return to baseline values was not achieved in that time frame and hence, a longer observation period may have been beneficial.

In addition, the study lacks a control group where no treatment was administered, which would have been ideal. However, due to financial constraints this group was not included. Any possible changes in the study outcomes due to the mares

becoming accustomed to the experimental situation or due to diurnal variation would have affected all treatments equally as they were randomized.

Furthermore, ultrasonographic evaluation of splenic size has not been previously validated in the horse and hence it may not be the ideal method to measure splenic volume. Nonetheless, there are no other non-invasive methods described in the literature to evaluate splenic volume in the horse to the author's knowledge. Generally there was a high baseline variation in splenic size among mares. Although a small area of hair was clipped to ensure that ultrasonography was always performed in the same location, slight tilting of the transducer could lead to substantial errors in measurements. Nevertheless, ultrasonographic evaluation of splenic size was shown to have a high repeatability, with an interobserver variance of 1% and a mean intraobserver coefficient of variation of 7.5%. Coefficients of variation between 20 – 30% are assumed to be acceptable for the validation of serological tests.[84]

The volume of blood sampled is not believed to have affected the results of this study. A total of approximately 205 ml of blood was sampled during each treatment day. This represents 0.50 ml/kg BW or 0.70% of the mare's total blood volume assuming the mean weight of the mare is 407 kg and the total blood volume of an adult horse is 72 ml/kg.[85] Half of this volume was replaced by heparinised saline. It is highly unlikely that, the small overall loss in blood volume caused a significant fluid shift. Furthermore, changes in TSP and PCV due to fluid shifts after significant acute blood loss are only clinically appreciated 6 hours post-bleeding.[86] While the investigation into the mechanism to explain the PCV response was concluded at 90 MPI, even the 5 hour sample should not have been affected by a fluid shift large enough to influence the PCV and TSP results.

CHAPTER 7 CONCLUSIONS

In conclusion, this study showed that α_2 agonist administration in horses induced significant and clinically relevant changes in PCV, which may outlast sedation. Total serum protein concentration also decreased, but to a lower extent than PCV. Therefore, these parameters cannot be solely relied upon when assessing hydration status in horses that have recently received these drugs.

Significant changes over time were also identified in other biochemical parameters namely, COP, plasma osmolality, blood glucose, Na^+, BUN and Ca^{2+}. Clinically significant hyperglycaemia was observed following α_2 agonist administration. Although COP, plasma osmolality, Na^+, BUN and Ca^{2+} measurements reported here were within normal reference ranges for horses, these changes should be taken into consideration when assessing critically ill horses that have received α_2 agonists.

Based on the results of this study, it appears that the changes in PCV and TSP are a result of an interaction between fluid shifts and splenic sequestration. Further research is required to determine the exact underlying physiology of these changes.

FOOTNOTES

[a]Rompun 2% Injection, Bayer (PTY) Ltd., Animal Health Division, Isando, RSA

[b]Sedivet ® 1%, Boehringer Ingelheim Pharmaceuticals (Pty) Ltd., Randburg, RSA

[c]Domosedan ®, Pfizer Laboratories (PTY) Ltd., Sandton, RSA

[d]14G Jelco I.V. catheter, Smiths International Ltd, Rossendale, Lancashire, UK

[e]High Flow T extension set (12gax17cm), Mila Iintenational Inc., Erlanger, KY, USA

[f]Microhematocrit tubes, Marienfeld Laboratory Glassware, Lauda-Königshofen, Germany

[g]Jouan Hema-C Microhematocrit Centrifuge, Hawksley and Sons, Ltd., Sussex, United Kingdom

[h]Hematocrit reader, Hawksley and Sons, Ltd., Sussex, United Kingdom

[i]Clinical refractometer, Kyron Laboratories (PTY) Ltd. , Benrose, RSA

[j]Roebling Automatic Micro-osmometer, Herrmann Roebling Messtechnik, Berlin, Germany

[k]Tabletop Centrifuge PLC-05, Gemmy Industrial Corp., Taipei, Taiwan

[l]ADVIA ® 2120 Hematology System, Siemens Healthcare Diagnostics, Deerfield, IL, USA

[m]Colloid Osmometer 4420, Wescor Inc., Logan, UT, USA

[n]Accutrend ®Plus, Roche Diagnostics, Mannheim, Germany

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APPENDICES

APPENDIX A: Criteria used to assign a sedation score based on the response to ultrasound.

Score	Response to Ultrasound
1	No response
2	Response slow and hesitant
	Horse looks at person performing ultrasound
	No movement of the horse
3	Response slow and hesitant
	Horse looks at person performing ultrasound
	Horse moves away from ultrasonographer
4	Response marked and rapid
	Horse responds to approach of ultrasound before or during contact of the probe

APPENDIX B: Sampling time line.

Sedation scores
US spleen
PCV
TSP
RBC
Osm
Na$^+$, K$^+$, Cl$^-$, Ca^{2+}
Lactate
Glu
BUN
pH

Sedation scores
US spleen
PCV
TSP

Sedation scores
US spleen
PCV
TSP
RBC
Osm
Na$^+$, K$^+$, Cl$^-$, Ca^{2+}
Lactate
Glu
BUN
pH

PCV
TSP

-1   0   15   30   45   60   75   90   120   180   240   300

Time 0 = injection of α$_2$ agonist

End point: 300 MPI

77
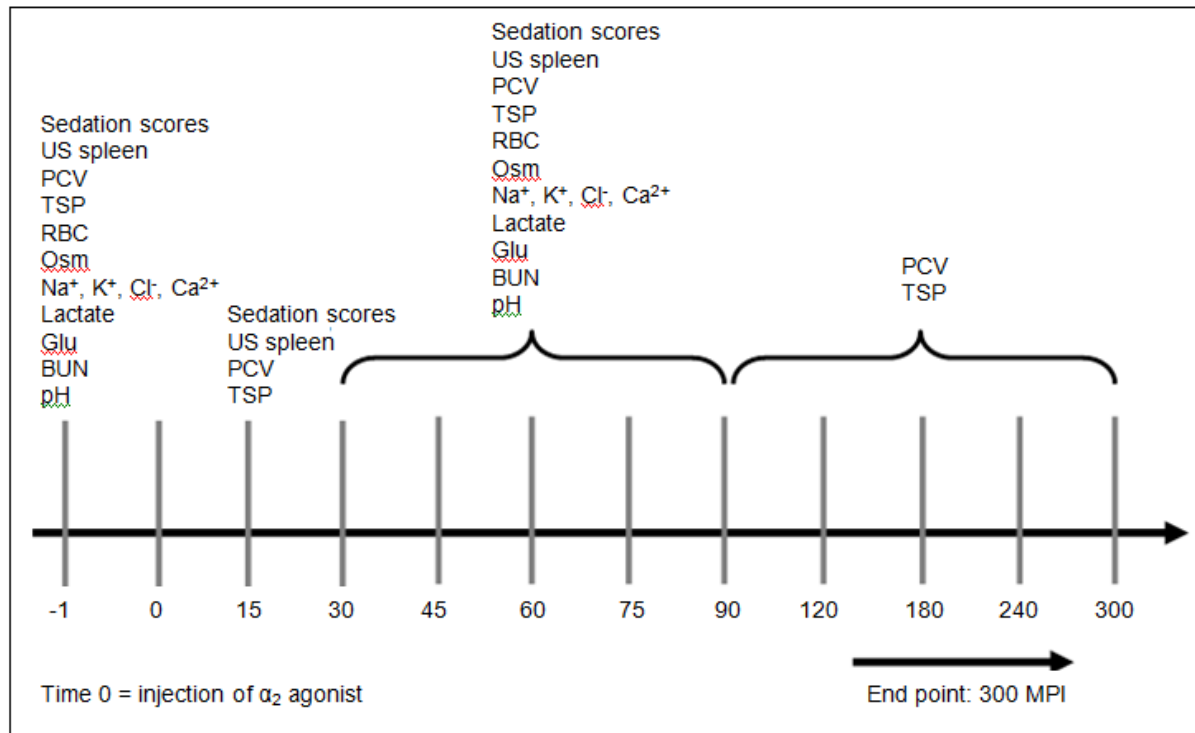
APPENDIX C: Standardised data recording sheet.

Horse		Injection time	
Week		Weight	
Date		Age	

Variables

MPI	Time	VAS 1	VAS 2	Resp to US 1	Resp to US 2	PCV (%)	TSP (g/L)	Lactate (mmol/L)	Glucose (mmol/L)	RBC (×10^{12}/L)	Osmolality (mOsm/kg)	COP (mmHg)	BUN (mmol/L)	Na$^+$ (mmol/L)	Cl$^-$ (mmol/L)	K$^+$ (mmol/L)	Ca^{2+} (mmol/L)	Venous blood pH
-1																		
15																		
30																		
45																		
60																		
75																		
90																		
120																		
180																		
240																		
300																		

Ultrasonographic splenic width in mm

MPI	Time	Tuber coxae 17th ICS	Major trochanter of femur 15th ICS	Tuber ischii 13th ICS	Point of shoulder 11th ICS	Point of elbow 9th ICS
-1						
15						
30						
45						
60						
75						
90						

APPENDIX D: Repeated measures ANOVA with (1) Haematology and serum biochemistry, (2) Splenic size and (3) Sedation scores.

(1)   Haematology and serum biochemistry

Parameter	Treatment groups				P values		
	Xylazine IV	**Romifidine IV**	**Detomidine IV**	**Detomidine IM**	**IV Treatment***	**Detomidine†**	**Time‡**
	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)			
PCV (%)	30.6[a] (2.9)	29.8[a] (2.8)	31.3[a] (3.1)	29.9 (3.3)	0.229	0.198	<0.001
TSP (g/L)	66.9[a] (5.8)	67.3[a] (4.1)	64.4[a] (3.1)	65.2 (3.7)	0.536	0.798	<0.001
RBC (×10^{12}/L)	6.23[a] (0.85)	6.10[a] (0.59)	6.03[a] (0.69)	5.97 (0.65)	0.823	0.862	<0.001
COP (mmHg)	19.7[a] (1.1)	19.1[a] (0.7)	19.4[a] (1.0)	19.6 (1.3)	0.221	0.618	0.001
Osm (mOsm/kg)	286[a] (4.1)	287[a] (3.5)	285[a] (3.0)	288 (2.5)	0.879	0.241	0.028
Lactate (mmol/L)	0.90[a] (0.46)	1.24[a] (0.80)	0.93[a] (0.48)	1.27 (0.77)	0.201	0.086	0.839
Glucose (mmol/L)	4.32[a] (0.51)	5.30[a,b] (1.08)	4.90[b] (0.72)	4.85 (0.67)	0.011	0.881	<0.001
BUN (mmol/L)	6.18[a] (1.58)	5.89[a] (1.16)	6.41[a] (0.89)	6.40 (1.03)	0.827	0.994	0.005
Na (mmol/L)	135.8[a] (1.6)	135.7[a] (1.1)	134.7[a] (1.6)	136.3 (1.2)	0.598	0.211	0.025
K (mmol/L)	3.51[a] (0.40)	3.48[a] (0.53)	3.42[a] (0.31)	3.60 (0.29)	0.860	0.158	0.202
Cl (mmol/L)	101.2[a] (0.6)	101.2[a] (4.1)	101.2[a] (1.1)	103.2 (4.2)	1.0	0.381	0.162
iCa (mmol/L)	1.50[a] (0.07)	1.46[a] (0.04)	1.46[a] (0.05)	1.49 (0.07)	0.366	0.381	<0.001
Blood pH	7.437 (0.02)	7.446 (0.02)	7.427 (0.02)	7.429 (0.02)	0.273	0.605	0.330

* Based on repeated measures ANOVA comparing the within subject treatment effect. Means without superscripts in common are significantly different (P <0.05) based on Bonferroni adjustment for multiple pairwise comparisons

† Based on repeated measures ANOVA comparing IV versus IM administration of Detomidine

‡ Based on repeated measures ANOVA

(2) Splenic size

Intercostal space	Treatment groups				P values		
	Xylazine IV	Romifidine IV	Detomidine IV	Detomidine IM	IV Treatment*	Detomidine†	Time‡
	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)			
9 (mm)	82.0^a (15.0)	82.4^a (15.2)	87.9^a (13.6)	88.2 (11.0)	0.689	0.902	0.045
11 (mm)	54.2^a (7.6)	62.8^a (8.3)	63.4^a (10.1)	63.9 (17.7)	0.332	0.938	0.003
13 (mm)	63.1^a (11.3)	70.0^a (13.9)	59.2^a (17.4)	67.2 (14.8)	0.611	0.488	0.032
15 (mm)	75.9^a (19.2)	77.3^a (22.6)	81.1^a (18.3)	89.1 (12.4)	0.860	0.123	0.212
17 (mm)	45.1^a (11.7)	45.0^a (11.7)	44.3^a (10.6)	45.4 (12.3)	0.782	0.610	0.531

* Based on repeated measures ANOVA comparing the within subject treatment effect. Means without superscripts in common are significantly different ($P < 0.05$) based on Bonferroni adjustment for multiple pairwise comparisons

† Based on repeated measures ANOVA comparing IV versus IM administration of Detomidine

‡ Based on repeated measures ANOVA

(3)   Sedation scores

Parameter	Xylazine IV	Romifidine IV	Detomidine IV	Detomidine IM	IV Treatment*	Detomidine†	Time‡
	Treatment groups				**P values**		
	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)			
VAS 1	38.3[a] (29.6)	29.7[a] (24.3)	48.9[a] (23.0)	39.7 (24.8)	0.352	0.603	<0.001
VAS 2	37.9[a,b] (28.5)	32.5[a] (27.8)	55.5[b] (24.7)	40.6 (26.7)	0.051	0.414	<0.001
Response US 1	1.3[a] (0.7)	1.3[a] (0.6)	1.2[a] (0.5)	1.1 (0.4)	0.804	0.182	0.119
Response US 2	1.3[a] (0.7)	1.3[a] (0.6)	1.1[a] (0.3)	1.1 (0.4)	0.355	0.718	0.135

* Based on repeated measures ANOVA comparing the within subject treatment effect. Means without superscripts in common are significantly different (P <0.05) based on

Bonferroni adjustment for multiple pairwise comparisons

† Based on repeated measures ANOVA comparing IV versus IM administration of Detomidine

‡ Based on repeated measures ANOVA

APPENDIX E: Reference ranges for horses with (1) Selected haematology and serum biochemistry (Clinical Pathology Laboratory, OVAH)[v] and (2) Ionized calcium,[87] plasma osmolality,[62] COP[88] and venous blood pH.[89]

(1)   Selected haematology and serum biochemistry (Clinical Pathology Laboratory, OVAH).[v]

Variable	Units	Reference range
PCV	%	24 - 44
RCC count	$\times 10^{12}$/L	5.5 - 9.5
TSP	g/L	66 - 83
Na^+	mmol/L	132 - 146
K^+	mmol/L	2.6 - 5.1
Cl^-	mmol/L	99 - 109
BUN	mmol/L	5.4 - 10.7
Glucose	mmol/L	3.1 - 5

(2)   Ionized calcium,[87] plasma osmolality,[62] COP[88] and venous blood pH.[89]

Variable	Units	Reference range
Ca^{2+}	mmol/L	1.26 - 1.9*
Plasma osmolality	mOsm/kg	280 - 310
COP	mmHg	15 – 22
Venous blood pH		7.38 – 7.54*

* Mean ± 2 SD