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Effects of various anaesthetics in chemical capture on different species of ungulates and carnivores

PhD Thesis

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DECLARATION

I hereby declare that I have done this thesis entitled "**Effects of various anaesthetics in chemical capture on different species of ungulates and carnivores"** independently. All texts in this thesis are original, and I have provided a complete reference to the resources that are used in this document according to the citation rules of the Faculty of Tropical Agrisciences. All figures and photographs are used with authorisation and with appropriate citation.

In Prague, 18th of May 2020 ..……..

Abid Mehmood

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LIST OF AUTHOR PAPERS

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Date..... Signature.....…

ABSTRACT

Chemical capture of animals has advanced as an essential tool in wildlife management over the past few years. However, the need to assess the implications such as cardiorespiratory depression and success (without any severe complication) of a given combination of anaesthetics persists for various species. This thesis was designed to assess the effects of various combinations of anaesthetics (ketamine-medetomidine, KM; ketamine-xylazine, KX: thiafentanil-ketamine-medetomidine, TKM; thiafentanil-ketamine-xylazine, TKX; and ketamine-xylazine-medetomidine, TXM) on respiration rate, heart rate, rectal temperature, blood oxygen saturation, non-invasive blood pressure, quality and duration of induction, anaesthesia and recovery in Arabian striped hyaena (*Hyaena hyaena sultana*), Northeast African cheetah (*Acinonyx jubatus soemmeringii*), Patterson's eland (*Taurotragus oryx pattersonianus*), Barbary sheep (*Ammotragus lervia*), and Beisa oryx (*Oryx beisa beisa*). The data were collected during different routine management operations requiring chemical capture. The animals were immobilised by projecting a dart through a dart gun. The vital signs were monitored through a pulse oximeter. The arterial blood gas samples were analysed through ISTAT point of care hand-held analyser, and the haematological and biochemistry samples were analysed through Abbott CELL-DYN® 3700 Haematology Analyser or Abbott Architect c4000 clinical chemistry analyser; or through an external laboratory. The data were analysed through the Shapiro–Wilk test (for normality), general linear model, and Wilcoxon matched-pairs test. KM combination was more suitable for the immobilisation of Arabian striped hyaena, providing a better quality of induction, anaesthesia and recovery compared to KX. Similar results were achieved with TKX and TKM Patterson's eland, and with KX and KXM for Barbary sheep and Beisa oryx. However, KX resulted in hypoxaemia in Barbary sheep, whereas no hypoxaemia was observed with KXM. There was a significant difference ($P<0.05$) in arterial oxygen saturation, the partial pressure of O_2, the arterial partial pressure of CO_2, bicarbonates, and pH before and after oxygen supplementation in Arabian striped hyaena and Northeast African cheetah suggesting that the oxygen may adequately treat hypoxemia during field immobilisation with ketamine-medetomidine. Further studies are suggested to assess the implications of KX and KXM combinations in Barbary sheep and Beisa oryx. The outcomes of the current study provide valuable information on the physiological responses, critical

complications associated with various combinations on the studied species, and field compensation of these complications to the practitioners working with semi-captive species of carnivores and ungulates.

Key Words: Acid-base balance; anaesthesia; arterial blood gas; α-2 adrenergic agonist; carnivores; chemical immobilisation; hypoxaemia; island; opioids; oximetry; oxygen semi-captive; supplementation; ungulates; wildlife management

Table of Contents

List of Figures

List of Tables

1. INTRODUCTION

Capturing wild and semi-wild animals for various management interventions, research, translocations or for treatments is inevitable. However, most of the physical capture techniques such as trapping, netting, snaring or herding the animals into a boma can cause life-threatening levels of stress to the animals (Brivio et al. 2015). In wild animals, these interventions can cause a partial or complete change in the behavioural response to the environment. These may include reduced foraging, avoiding areas of habitat where the capture procedure occurred, lack of activity, loss of social status, exclusion from the territory or social group, exhibition of stereotypic behaviours and fear. All of these can lead to an undesired or unexpected result (Proulx et al. 2012; Brivio et al. 2015).

For many years, the use of chemical restraint of wild and dangerous animals in wildlife research and management is a successful intervention (Kreeger 1996). Live capture of wild animals by chemical immobilisation was introduced in the 1950s (Rausch & Ritcey 1961; Bush 1992; McKenzie et al. 1993). After the completion of the Kariba Dam across the Zambezi River in Zimbabwe and rescue of many strangled animals, there was a tremendous improvement in the chemical capture and immobilisation techniques and equipment (Nielsen 1999). Immobilising drugs are selected to allow safe handling of wildlife in an efficient, predictable, and minimally stressful manner. Furthermore, anaesthetic protocols that allow quick induction, short processing time, and rapid reversal are necessary for researchers performing minimally invasive procedures, e.g. morphometric measurements or attachment of radio collars and sample collection (Rockhill et al. 2011). Chemical immobilisation and anaesthesia are vital constituents in conservation, diagnostic and surgical procedures in wild animal species (Wack 2003).

Although chemical immobilisation is a vital management and research intervention, there are some adverse effects associated with it. These negative impacts, especially on wild or semi-wild animals that are not used to handling or restraint and immobilisation, can turn a welfare intervention into a life-threatening situation for these animals. Conversely, it is not the only extent of successful chemical immobilisation that an animal survives the procedure. Some animals show absolutely no signs of capture and

immobilisation stress right after the procedure but do develop complications in the following hours (Arnemo et al. 2006).

The monitoring of physiological parameters and blood variables of the animals during immobilisation is unavoidable to assess the stress and its implications on the animal (Brivio et al. 2015). Also, the expertise of the staff performing the procedure, the selection of drugs and doses and the duration of the procedure have significant effects on the stress level experienced by the animal. The release of stress countering hormones such as cortisol can also provide a better picture of post immobilisation stress levels and behavioural response of the animals (Brivio et al. 2015).

Another essential tool to assess the effect of anaesthetic drugs and response of animals is the analysis of arterial blood gas variables. It is a valuable intervention in assessing the animals' blood gas status and acid-base balance. The abnormal values of blood gas and acid-base variables can suggest what complications are arising and to what extent. If these complications are not dealt with timely, they can cause serious detrimental effects on the animals and may lead to a life-threatening situation (Sood et al. 2010). One of the effects of drug agonists on the central nervous system is the depression of the respiratory centre. It can lead to hypoxaemia that can further give rise to severe complications such as hypoxia and organ failure (Hennessey & Japp 2016).

There is enormous scope for study in the field of chemical immobilisation and its effects on the animals' physiology during and after the procedures due to the continuous development of new drugs and combinations, and sometimes constraints of budget and availability of the drugs. The current thesis discusses research work conducted with various combinations of anaesthetics on different animals. It unveils some of the effects of these drugs and combinations on the physiological parameters of these animals in the conservation breeding protected wildlife reserve. Moreover, it also discusses the changes in the blood gas and acid-base homeostasis of the anaesthetised animals.

2. REVIEW OF LITERATURE

2.1. Species account

2.1.1. Carnivores

Carnivores are essential indicators of functioning ecosystems. They influence broader aspects of the system through predation by diverting what they do not need for their energetic requirements to scavengers, detritivores, and microorganisms. However, large-carnivore populations are declining globally, and all are subject to a multitude of pressures, including habitat degradation, conflict with agriculture, hunting, disease, and commercial trade (Sillero-Zubiri & Laurenson 2001). For instance, most of the species of family Felidae are either classified as threatened or endangered, except for the domestic cat (*Felis catus*) (Marker et al. 2002).

Carnivores play critical ecological roles and may be nominated as keystone species. The capability of carnivores to regulate prey numbers diverges due to different factors and often is useful in the short tenure. The ancillary effects of carnivores on the community structure and diversity can be momentous. The role of carnivores can be regarded as essential as that of umbrella species (i.e. whose habitat necessities incorporate the habitats of several other species) (Noss et al. 1996).

2.1.1.1. Arabian striped hyaena

Hyaenas are scavengers (Prater 1971; Kruuk 1975, 1976; Mills & Hofer 1998). They seek their food by scent. Overall, the animal is built neither for the attack nor the swift pursuit of prey. Its structure fits its particular mode of life, which is to feed on prey killed by other animals (Prater 1971). The general appearance of hyena suggests its relation with the canids. However, the structure of the skull, the teeth and other points of anatomy relate them to the felids of the order Carnivora. Due to these considerations, hyenas are placed in a separate family Hyaenidae (Prater 1971). Family Hyaenidae has four species in three genera, the spotted hyena (*Crocuta crocuta*), brown hyena (*Hyaena brunnea*), striped hyena (*Hyaena hyaena*) and aardwolf (*Proteles cristata*) (Macdonald 1984).

The hyenas are distributed in Africa except for Sahara and Congo basin, Turkey, and the Middle East to Arabia, south-west of former Union of Soviet Socialist Republics and India (Prater 1971; Kruuk 1976; Macdonald 1984). They prefer chiefly dry, open grassland and brush, open plains, deserts, rocky scrub-covered hills, nullahs and open forest. They usually avoid the interior of dense forests and live more commonly in the drier area (Prater 1971). The length of the hyaenas from head to tail ranges between 1.2 – 1.5 meters. The average weight of an adult hyaena is between 25 to 55 kg. The fur is brown, grey or sandy coloured depending on specific species. The female hyaena has a unique feature to possess an enlarged clitoris which is also known as pseudopenis. The females use these protruding genitalia for their sexual activities, and it also provides them with control in mate selection.

The Arabian striped hyaena is a critical scavenger found in tropical forest and grassland ecosystems. The striped hyena is a classic scavenger, existing around human settlements and feeding by dried bones, carcasses and sometimes by fruits, insects and reptiles (Kruk 1976; Hofer 1998). It is listed as 'Near-threatened' according to the IUCN Red List of Threatened Species (AbiSaid & Dloniak 2015). Its distribution ranges from Yemen, Saudi Arabia, Oman, and to the United Arab Emirates. The last authentic sighting in the wild within the UAE was in 1996 (Hellyer & Aspinall 2005). Its role in clearing off carrion in tropical ecosystems and in recycling mineral compounds from dead organic matter enhances its biological importance. The striped hyena is a medium-sized carnivore in appearance resembling that of dog. They have a pointed muzzle, broad head and pointed ears. Their hind parts slope down towards the tail. They possess vertically positioned black stripes; a distinguishing feature and thus reason for its common name. The colouration of the fur is greyish pale or beige, but it may vary according to the region (Rosevear 1974). The distinguishing feature of Arabian subspecies is that they have an accentuated black dorsal mane and the mid-dorsal hairs are approximately 20 cm long (Mills & Hofer 1998).

The striped hyena has been considered exclusively nocturnal and solitary (Prater 1971; Kruuk 1976; Macdonald 1984). Also, during the night, they spend considerable time resting. The animal appeared more active in the first part of the night, followed by a period of rest and became again active in the last part of the night. The striped hyena

spends a significant part of the activity searching for food. Their range is relatively broad in Serengeti for instance; one radio collard female had a range of 44 $km^{2,}$ and a male had a range of 72 km^2 (both were 1 - 2 years old). Striped hyena covered a mean distance of 19 km per night with the shortest distance per night of 7 km; the most extended 27 km (Kruuk 1976).

Figure 1: Arabian striped hyaena (Photo credit: Abid Mehmood)

2.1.1.2. Northeast African cheetah

The cheetah is remarkably different in both their behaviour and anatomy as compared to other felids (Ewer 1986; O'Brien et al. 1983). They are also known as the fastest terrestrial mammals. However, the maximum speed is attained for short distances only (James 1968). To reach such high speeds, the cheetahs have several physical adaptations such as small head, binocular vision, enlarged nostrils, short muzzle, narrow-body and legs with specific muscles (Ewer 1986; Hilderbrand 1959; Hildebrand 1961). Moreover, the cheetah has semi-retractable, short and blunt claws, making it different from other cats. This adaptation helps them to have extra traction (Ewer 1986).

The population of the cheetah has declined exponentially and has resulted to relegate their conservation status further to vulnerable (Durant et al. 2015). According to recent information, less than 7000 wild cheetahs remain in the world. Majority of its remaining wild population is only on the African continent, and that too comprises only 22 % of their historical range (Durant et al. 2015). It is challenging to study the population of the

cheetahs in the wild due to many contributing factors such as their cryptic nature, use of various habitat types, scattered populations, lower densities, lower survival, lower breeding rates, the influence of other predators, and humans (Sunquist & Sunquist 2002; Marker et al. 2003; Funston et al. 2010; Wachter et al. 2011; Thorn et al. 2012; Boast et al. 2013). All these factors hinder the adequate data collection and assessment of species population decline (Mills & Mills 2014; Boast et al. 2013).

The North African cheetah is distributed in Ethiopia and South Sudan, and an Australian scientist Leopold Fitzinger named this subspecies as *Cynailurus soemmeringii* in 1855. It is comparatively larger subspecies and resembles East African cheetah. Its belly is mostly white, and the fur is darker as compared to eastern subspecies. Cheetah is categorised as 'Vulnerable' according to the IUCN Red List of Threatened Species (Durant et al. 2015).

Figure 2: North African cheetah (Photo credit: Abid Mehmood)

2.1.2. Ungulates

Ungulates can be broadly defined as those animals that possess hooves. Depending on the number of toes, ungulates can be further subdivided into two orders of odd and even-toed animals, namely Perissodactyla and Artiodactyla. The odd-toed animals include horses, rhinoceroses, asses, zebras and tapirs. They also are characterised by digestion of cellulose in their intestine rather than in chambered stomach like in even-toed animals. The order Artiodactyla with chambered stomach includes animals such as deer, giraffe, sheep, cattle and camels (Ursing & Arnason 1998).

2.1.2.1. Patterson's Eland

Eland belongs to a group of animals within the family Bovidae known as antelopes and is amongst the largest of the antelopes. Although, the morphological appearance of both sexes is somewhat similar, with tawny coat colour having between ten to sixteen white stripes. The tail is long with a black-haired tuft at the end. Males develop tuft of hair on their heads; their necks and shoulders become muscular and thick. Moreover, the males can weigh up to two times to that of females (Buijs et al. 2016).

The common eland is classified as 'Least Concerned' according to the IUCN Red List of Threatened Species (IUCN SSC Antelope Specialist Group 2016). Common eland further has three subspecies, namely Cape eland (*Taurotragus oryx oryx*), East African eland (*Taurotragus oryx pattersonianus*) and Livingstone eland (*Taurotragus oryx livingstonii*) (Groves & Grubb 2011). The Patterson's eland is an opportunistic feeder and adaptable herbivore and can have up to 12 stripes. They avoid thick forests as well as pure deserts and can be found in grasslands with low tree cover. Water consumption and dependence in eland is not studied efficiently, but inferences from their presence in the area with no water suggest that they can meet this requirement with the food they consume (Pappas 2002).

Figure 3: Female Patterson's eland (Photo credit: Abid Mehmood)

2.1.2.2. Barbary sheep

The Barbary sheep is a goat-like species of subfamily Caprinae found in the rocky mountains of Northern Africa. For a long time, Barbary sheep was under dispute for its classification until it reached an agreement of *Ammotragus lervia* having further six subspecies mainly based on their distribution. It has been introduced to Europe, the United States and other parts of the world. Barbary sheep are ranked as 'Vulnerable' in the IUCN Red List of Threatened Species (Cassinello et al. 2008).

Barbary sheep depicts common characteristics from both goat and sheep. The genus name *Ammotragus* means sand goat due to its sandy colouration and a general appearance and a massive goat. They have a unique feature where their mane profoundly extends from their neck down to their legs. Their horns resemble those of mouflon and are curved backwards. They show resemblance to sheep in their behaviour and prefer a range of habitats from grasslands, forests and rocky slopes (Cassinello 1998).

Figure 4: Barbary sheep (Photo credit: Abid Mehmood)

2.1.2.3. Beisa oryx

The Beisa oryx is one of the oryx species native to Africa from Sudan, Uganda, Kenya, Somalia to Tanzania. It further has two subspecies *viz. O. b. beisa* the Beisa oryx and *O. b. callotis* the fringe-eared oryx. Beisa oryx is 'Near Threatened' under the IUCN Red List of Threatened Species (IUCN SSC Antelope Specialist Group 2018).

Beisa oryx usually occurs in arid areas and grasslands. Due to anthropological factors, they have severely declined from Uganda and Somalia. However, their population is still intact to an extent in other areas of its distribution where human disturbance is rather low (IUCN SSC Antelope Specialist Group 2018). Beisa oryx is a grazer and feeds on a variety of grasses. They tend to take more browse during dry seasons. Beisa oryx can accommodate its water requirement from succulent tubers and bulbs, roots and melons, but drinks frequently if the water is available (IUCN SSC Antelope Specialist Group 2018).

Figure 5: Beisa oryx – female (Photo credit: Abid Mehmood)

2.2. What is the Art of Chemical Immobilisation

Anaesthesia of wildlife is an integral part of wildlife management, and veterinary medicine; as the wild nature and extensive procedures on these animals with minimal stress do require the use of remote application of anaesthetics (Thurmon & Short 2007; Hernandez 2014). The word anaesthesia is of Greek origin and refers to a complete or partial loss of senses (Hernandez 2014). Anaesthesia is widely used in wildlife examinations, as they are much safer than other orthodox methods of animal restraint and handling both for the animals and humans (Thurmon & Short 2007). There is a difference between general anaesthesia, local anaesthesia, regional anaesthesia, tranquillisation and sedation. The word analgesia also originates from Greek, where it is used to describe the absence of pain (Hernandez 2014). Whereas tranquillisation is inducing relaxation and

reduction of stress, but the animal is fully aware of its surroundings. The term sedation is the induction of drowsiness where the animal is not aware of its surroundings. Local anaesthesia or analgesia is a loss of pain sensation in a specific area of the body (but the animal is still apparently aware). In contrast, regional analgesia is a loss in a larger area. General anaesthesia induces depression of the central nervous system (Hernandez 2014; Thurmon & Short 2007).

Various mechanisms and systems aid in the drug delivery depending on the species under the procedure and the distance from which the medicine needs to be injected. The different types of drug delivery systems based on their application and efficacy in various scenarios include handheld syringes and needles, pole-syringes or jab stick, darts, blowpipes, and dart guns/pistols (Kock & Burroughs 2012).

The handheld syringes with appropriate needle gauges and syringe sizes are very important in all of the immobilisation missions. The typical uses include filling the medicine in the darts, taking blood samples, injection of medicine or anaesthetic drugs, and administration of reversal drugs (West et al. 2007). Pole-syringes or jab-sticks are other convenient tools to inject medicine from small but safe distances. The syringes are modified so as their plungers fit into a long pole. These are very useful in crush cages, night quarters, live-traps, and top up a partially sedated animal from a safe distance. Darts are a modified type of syringes with needles. They are used to inject medicine through a projectile that can be either a blowpipe or a dart-gun.

A dart consists of a syringe or chamber for the drug, a plunger, an air chamber to pressurise the plunger, needle, a rubber sleeve or collar to block medicine spillage before impact and a tailpiece to assist in trajectory (Kock & Burroughs 2012). Blowpipes are a handy tool in cases where the distance is about eight meters or less and where there are chances that the pressure of the gun can harm the animal or the species is very small or in night quarters or boma. It is a hollow pipe with the appropriate internal diameter to accommodate the dart; one end may have a mouthpiece from where the operator will blow the dart aiming at the animal. Dart guns or pistols are powered projectiles to deliver the darts at a much higher distance than blowpipes. They are usually powered by a CO_2 cylinder and are very useful in darting ferocious and wild animals from a safe distance (Kock & Burroughs 2012).

Anaesthetic drugs can be defined on the broad spectrum as drugs that cause anaesthesia or in other words, reversible loss of sensations in the animals. These drugs mimic the characteristics of neurotransmitters and alter the standard transmission of the nerve impulse. The broad categories of anaesthetic drugs include; opioids, cyclohexylamines, neuromuscular blockers, α – 2 agonists, and butyrophenones (West et al. 2007).

Opioids are the drugs derived from morphine. The opioids have three major classes of opioid receptors *viz.* μ, κ, δ (mu, kappa, and delta, respectively). All of these three opioid receptors are G – protein-coupled receptors that act on the GABAergic neurotransmission of animals (West et al. 2007). Examples of opioids include etorphine and thiafentanil oxalate. Etorphine (also known as M99) has a molecular formula of $C_{25}H_{33}NO_4$ and is a non-selective full agonist of the μ-, δ-, and κ- opioid receptors. It is reversible with an antagonist named diprenorphine with a molecular formula of $C_{26}H_{35}NO_4$. Thiafentanil oxalate (also known as A3080) with a molecular formula of $C_{24}H_{30}N_2O_2S$, induces similar analgesia as morphine and produces immediate immobilisation. It is reversible with an antagonist known as naltrexone hydrochloride. (Kock & Burroughs 2012).

Cyclohexylamines are also another category of anaesthetic drugs used to immobilise animals. This group of anaesthetics causes a state of dissociative anaesthesia with distinctive hypertonicity of the muscles. There is a big drawback with cyclohexylamines that they do not have any reversal or antagonist. The body of animals metabolises them over time. This group of drugs is usually mixed with opioids or other sedatives to reduce their side effects and to increase their efficacy (Kock & Burroughs 2012). Ketamine hydrochloride is a widely used cyclohexylamine with a molecular formula of $C_{13}H_{16}Cl_2NO$. It produces anaesthesia and induces a trance-like state while providing pain relief, sedation, and memory loss to the animal. There is no antagonist for ketamine hydrochloride, it usually metabolises into less potent units, and it is excreted in the urine (West et al. 2007).

Neuromuscular blockers cause a paralytic effect on the muscles of the animals by blocking the nerve transmission in the motor neurons ending in the muscles. During normal functioning, the nerve transmission releases acetylcholine that causes the muscles

to contract. Although these drugs cause the paralysis of the muscular system of the animals, yet they are entirely aware of their surroundings and can feel pain. Therefore, a standalone use of these drugs for any treatment or surgery is not recommended. The examples of neuromuscular blockers include gallamine that can be reversed by neostigmine and succinylcholine chloride that has no antidote (West et al. 2007; Kock & Burroughs 2012).

The α-2 agonists are the drugs that bind to the α-2 receptors at synaptic levels. They belong to the imidazole group, and the animals are sedated with efficient muscle relaxation and analgesia. Their effects can be quickly reversed with α-2 antagonists (Kock & Burroughs 2012). Their examples include medetomidine hydrochloride ($C_{13}H_{16}N_2$) with an antagonist, atipamezole hydrochloride ($C_{14}H_{16}N_2$). Medetomidine is known to be ten times more potent than xylazine. Xylazine hydrochloride ($C_{12}H_{16}N_2S$) can be reversed with Yohimbine. Xylazine is usually used in combination with ketamine (West et al. 2007).

Tranquillisers change the adrenergic neurotransmission and are used to suppress the anxiety and agitation in animals. They are used to calm the animals. In these drugs, an overdose may cause adverse side effects as opposed to sedatives, where a higher dosage may bring the drug effects quicker and smoother. The examples may include acetylpromazine maleate, azaperone, and haloperidol (Kock & Burroughs 2012).

An immobilising drug can be administered from various routes. These can be intramuscular, intravenous or subcutaneous. The drugs are then absorbed from the site of introduction into the bloodstream from where it is distributed to the sites of action. After this stage, the drug is metabolised and excreted out of the body of the animals (Kock & Burroughs 2012).

After the injection of the drug into the body of an animal, the drug starts to absorb in a unidirectional manner where it moves from the site of administration to the bloodstream. The speed of absorption is directly proportional to the route of administration, i.e. intravenous administration will have the fastest absorption as the drug is directly administered into the bloodstream, intramuscular administration such as most of the darts will take further time to absorb from the muscle into the bloodstream. Moreover, the speed

with which a drug is absorbed into the bloodstream also determines the time required by a drug to produce induction (Kock & Burroughs 2012).

After absorption of the drug into the bloodstream, it is distributed to the site of action. It is a multifunctional process where the drug is distributed to the tissues and organs. Anaesthetics directly affect the brain and spinal cord. The drugs move along with concentration gradient, i.e. from a higher concentration to a lower concentration. The most critical physiological barriers that affect the distribution of the drugs are blood-brain barriers and placenta-blood barrier from where only those drugs can pass through which are lipids soluble, unionised, and not bound to plasma protein and all the anaesthetics have these characteristics (Kock & Burroughs 2012).

During later stages of absorption, the drug is modified to facilitate excretion out of the body. It usually occurs in the liver. Inside the liver, the lipid-soluble drugs are converted into water-soluble entities. Once the drugs enter into the liver, their state of activity is changed, and if the liver is overactive, the efficacy of the drugs is reduced and vice versa. Moreover, the efficacy of the drug will also reduce if it is administered orally, where it enters the liver before proceeding to the site of action. As opposed to intramuscular or intravenous administration, where it will go to the site of action first and then end up in the liver (Kock & Burroughs 2012). After the breakdown of the drugs inside the liver into water-soluble parts, it is excreted through kidneys mostly. The excretion of the drugs is the one-way process usually; however, sometimes the drugs are re-absorbed and may re-sedate the animal (Kock & Burroughs 2012).

When the anaesthetic drugs are distributed to the site of action, they exert their activity by directly binding to the receptor sites. These receptor sites are the complex macromolecules which are incorporated into the cell membranes. The shape of each receptor site is essential in the determination of the type of drugs that will bind to them precisely in the same manner as different locks have different keys. Broadly, we can categorise the drugs into two types that can bind to these receptor sites. These are agonists and antagonists. Agonists are the drugs that have an intrinsic activity once they bond to the receptor site and result in the reduced activity of the central nervous system. On the other hand, antagonists are the drugs that do not show any intrinsic activity once they bond to the receptor site and result in blocking the receptor site for the agonists. Attaching

of each of these two drug types is a reversible process, and the number of receptors occupied by each type is determined by the concentration of these drugs (Kock & Burroughs 2012).

The most prominent side effects of opioids include respiratory depression (that can be fatal if the animal is not administered correctly), prolonged excitement in case of under-dosage or improper administration of the drug, muscular hyper-tonicity, hyperthermia, hypertension and immobility of the gut. Amongst side effects above, respiratory depression and hyperthermia can be significant causes leading the mortality of the animals under the effect of opioids (Kock & Burroughs 2012).

If cyclohexylamines are used in less quantity compared to opioids in a combination, it might result in extended excitement phase during the induction stage that can lead to exhaustion of animals. Moreover, muscular hyper-tonicity, convulsions and hyperthermia may also be observed when cyclohexylamines are administered. If ketamine is used with xylazine or medetomidine, it can result in vomiting. Another side effect is that cyclohexylamines cause the eyes to be left entirely opened; that may damage the eyes from direct sunlight or dirt (Kock & Burroughs 2012).

The anaesthetic drugs mimic the neurotransmitters and attach to the receptor sites, thus interfering with the standard neural transmission. As a result, the centre for posture, balance and movement control known as cerebellum cannot receive the nerve impulses and signals. Eventually, the animal cannot maintain its posture. The spinal cord functions in association with the primitive brain and cerebellum to control essential mechanisms such as respiration, movement, blood pressure regulation and balance of the body. Therefore, all these critical mechanisms are disturbed to an extent depending on the nature of the drug as well as the dosage used. Medulla oblongata and hypothalamus that control the complex body functions such as feeding, urinary bladder control, heat control and cardiac rhythm are also suppressed. In addition to the above, the sympathetic nervous system that controls stress in fight or flight situations and also controls blood pressure and thermoregulation is also compromised (Kock & Burroughs 2012).

Due to the depression of the activity of cerebellum, the respiration of the animals is compromised. The respiratory depression can lead to a condition called hypoxia, which

is defined as the deficiency of oxygen reaching the tissues. The blood starts appearing dark blue and the increased blood carbon concentrations may lead to respiratory acidosis. The respiratory acidosis may be followed by an increased plasma potassium level that may lead to heart failure. The α-2 agonists result in ventilation-perfusion mismatching, i.e. they alter the relationship between blood and airflow. The energy in the body is stored as ATP that is used for muscle movement. When the energy is required, glycogen breaks down into glucose by the process known as glycogenolysis. The glucose obtained after this process further breaks down into pyruvate and after the Krebs citric cycle into the hydrogen ions. These hydrogen ions are oxidised to produce energy. After the suppression of respiratory centre and lack of oxygen in the body, the process of oxidisation is suppressed, and lactic acid formation takes places, resulting in the damages to the muscle tissues (Kock & Burroughs 2012).

Some of the α-2 agonists are reputed seriously to affect the circulatory system of the animals. They can cause a reduction in the functionality of myocardium and vasodilation of the blood vessels leading to anaphylactic shock. Due to the myopathy of the myocardium, heart failure can result. It is due to the acidosis and increased potassium ion that disturb the cardiac rhythm and leading to cardiac shock (Kock & Burroughs 2012).

Anaesthetic drugs can have severe effects on the digestive system of the animals, especially ruminants. Ruminants usually produce large volumes of gases because of the fermentation process. During fermentation of the feed, carbon dioxide and methane gases are produced and are then collected in the dorsal hind sac of the rumen. The animals get rid of them by eructating through cardia as the reticulum relaxes. While in an immobilised state, the opening of cardia collapses due to the position of the animal and central nervous system loses the coordination of eructation of these gases out of the body. The consequences of this discoordination result in bloating. Ruminants also produce alkaline saliva to neutralise the acids produced during the process of fermentation. Moreover, the anaesthetics are also known to relax the sphincter muscle of the ruminal cardia and may result in aspiration as well (West et al. 2007).

As the anaesthetic drugs directly affect the central nervous system, they also affect the brain centre that controls body temperature or maintains the thermoregulatory system of the animal's body. Neurons in the central nervous system (CNS) measure the body

temperature and accordingly stimulate responses to whether to lose or conserve the heat. These responses are mediated by the sympathetic nervous system. When the anaesthetics suppress the activity of CNS, thermoregulation is not monitored. It can cause an abrupt increase in the body temperature leading to mortality (Kock & Burroughs 2012).

2.2.1. Chemical immobilisation in carnivores

Van Jaarsveld (1988) explored chemical anaesthesia of spotted hyaena (*Crocuta crocuta*) using zoletil. He suggested a dosage of 4 mg/kg for wild adults and a reduced dosage of 2 mg/kg for captive adult individuals. They also tested this dosage on various age groups and in females with different stages of pregnancy. They found it suitable and safe without any adverse effect on the animals or foetus.

Van Jaarsveld & Skinner (1992), studied the adrenocortical responses to an induced stressor (such as immobilisation) in spotted hyaena using zoletil. There was an insignificant difference between sexes and cortisol concentrations levels. However, females expressed a more substantial percentage increase in cortisol concentrations due to dominance.

A study on chemical immobilisation of wild foxes including *pseudalopex griseus and pseudalopex culpaeus* in Chile was conducted. Three different drug combinations *viz.* ketamine with medetomidine (KM), ketamine with xylazine (KX) and tiletamine – zolazepam (Z) were used. It was found that all of these three combinations were successful in inducing quick and smooth anaesthesia. However, KM combination produced better results with higher SpO_2; stabilised heart and respiration rates and other physiological factors. Whereas, animals darted with Z had much-elevated pulse and respiration rates compared to the other two combinations. Therefore, in foxes KM combination was recommended for anaesthesia (Acosta-Jamett et al. 2010).

The chemical immobilisation of captive Cougars (*Puma concolor*) with a combination of tiletamine-zolazepam, ketamine and xylazine at rates of 2 mg/kg, 1.6 mg/kg, 0.4 mg/kg respectively, showed no significant difference ($p>0.05$) in the vital parameters throughout immobilisation. Although, there was no significant difference due to anaesthetic and cardiorespiratory effects on the animals. The animals showed minor signs of respiratory

depression, and it was recommended to use respiratory support with this combination (Lescano et al. 2014).

Belsare & Athreya (2010) studied a combination of xylazine and ketamine for the emergency immobilisation of wild leopards (*Panthera pardus fusca*). They immobilised 55 wild leopards between 2003 until 2008 using a dosage of 1.4±0.3 mg/kg of xylazine and 5 ± 2 mg/kg of ketamine. The study results proved no adverse effects of the anaesthetics on the animals even after a month of post-procedure monitoring.

Luengos Vidal et al. (2016) worked on the field capture, chemical immobilisation, and morphometric measurements of a little-studied South American carnivore, the lesser grison (*Galictis cuja*). They captured the animals through live trapping and also radio marked some individuals. For immobilisation, they used ketamine and xylazine or tiletamine and zolazepam through a handheld syringe. During chemical immobilisation, the animals showed a high frequency of thermoregulation issues. Therefore, they suggested monitoring the body temperature of sedated animals regularly.

Jacquier et al. (2006) conducted the reversible immobilisation of free-ranging African lions (*Panthera leo*) with a combination of medetomidine-tiletamine-zolazepam and reversal with atipamezole. They used a dosage of 0.07±0.01 mg/kg medetomidine and 1.8±0.5 mg/kg tiletamine-zolazepam. The success of chemical capture was assessed with smooth inductions with an average of 14.1±6 minutes, analgesia and muscle relaxation. Only one lion had experienced bradypnea i.e. low respiratory rate and was treated. All other physiological parameters including heart rate, respiration rate and body temperature, were stable in other animals. The anaesthesia was reversed using atipamezole. The dosage was 0.3±0.1 mg/kg (intramuscularly) for reversal of anaesthesia. There were no mortalities even up to eighteen months of post-capture monitoring.

Fernando et al. (2013) performed chemical immobilisation of Bornean leopard cats (*Prionailurus bengalensis borneoensis*). They used tiletamine and zolazepam (also known as Zoletil©) and sedated nine animals. The mean dose was 6.92 ± 1.06 mg/kg. Although, some animals required top-up with either Zoletil© with a mean dose of 2.6±0.33 mg/kg or ketamine with a mean dose of 3.5± 0.05mg/kg. However, there was a

difference between the duration of anaesthesia of these two top-up doses *viz*. ketamine had anaesthesia time of 43.5 ± 2.1, and Zoletil© had 89.5 ± 6.36 minutes.

de Villiers et al. (1997) studied social dynamics and cortisol response to the stress related to immobilisation in African wild dogs (*Lycaon pictus*). They used a combination of fentanyl with a dosage range of 2-2.5 mg per animal and xylazine with a dosage range of 15-25 mg per animal. They did not find a significant relationship between immobilisation stress and the secretion of cortisol.

Fyumagwa et al. (2012), investigated anaesthesia quality and cost in free-ranging lions (*Panthera leo*) with ketamine - medetomidine or ketamine – detomidine and concluded that ketamine – detomidine combination was more effective to anaesthetise lions. It also saved the cost up to five times to that of medetomidine. They immobilised 32 lions in total and 16 animals with each combination. The comparison showed a minor difference in the quality of anaesthesia between the two combinations.

2.2.2. Chemical immobilisation in ungulates

Opioids are one of the frequently used and reliable immobilisation drugs for anaesthesia of wild and ferocious animals. The most popular are thiafentanil, fentanyl, etorphine and carfentanil (Nielsen 1999) despite their potential toxicity to humans (Haigh & Haigh 1980). Consequently, veterinarians and wildlife biologists require a special permit for their acquisition and use; opioids are, therefore, not readily available in many countries. As an alternative, xylazine as well as medetomidine, pure or in combination with ketamine, are commonly used for the immobilisation of deer in Europe (Jalanka & Roeken 1990; Janovsky et al. 2000). However, the use of α-2 adrenoceptor agonists in even relatively high doses is often unsatisfactory, particularly in free-ranging deer or in deer kept in large paddocks (Pond & O'Gara 1996; Haigh & Hudson 1993). Red deer (*Cervus elaphus hippelaphus*) was first chemically immobilised with nicotine salicylate and gallamine, although they had a low success rate. Later, copious narcotic drugs were tested around the world to anaesthetise deer. However, many of the tested drugs did not succeed (Janovsky et al. 2000). Janovsky et al. (2000) studied the standard dosage of zoletil and rompun in red deer. They concluded that the 1:1 combination of zoletil and

xylazine was an appreciated alternative for opioids for the immobilisation of an adult red deer.

Smuts (1973) successfully immobilisation gemsbok, eland and kudu using etorphine / xylazine or fentanyl / xylazine combinations. In most of the cases, he did not find any difference in immobilisation on kudu between etorphine-xylazine or fentanyl-xylazine combinations. In both combinations, the animals showed rapid recumbency. Some behavioural observations described in the study included slow swaying gait, head dropping backwards, standing in the same posture, walking in tight circles and sternal recumbency with chin resting on the ground. Some gemsbok took 7-19 hours to recover from the effects of anaesthetics completely. During this time they exhibited paralysis of the tongue, deep sleep, salivation with eyes closed. One of the gemsbok in their study showed that particles of grass in the lungs resulted in aspiration.

Smuts (1973) recorded respiration rate and found shallow irregular respirations in overdosed animals, whereas, all other animals exhibited regular breathing rates. Rectal temperatures varied between 37.6 °C to 42.2 °C. Instances, where the rectal temperature was observed on higher values, were correlated to muscular stress animals went through during immobilisation procedures. In gemsbok, it was observed that xylazine had a side effect when overdoses. The overdosed animals took longer to get off the effects of sedatives. These animals after a short while of administration of antidote, ran normally and then were found lying on their sides, which resulted in bloating.

Pye et al. (2001) anaesthetised Eastern giant eland (*Taurotragus derbianus*) at the White Oak Conservation Centre. Giant eland is a problematic species to anaesthetise. They are huge and prone to hyperthermia, capture myopathy, tympani, and fatal aspiration pneumonia resulting from regurgitation. Two anaesthetic drug combinations were compared to improve current anaesthesia techniques. The combination of A3080-medetomidine-ketamine (reversal with naltrexone and atipamezole) was compared to a combination of carfentanil-xylazine (reversal with naltrexone and yohimbine). The carfentanil combination was preferred to the A3080 combination due to its less respiratory depression. Regurgitation could occur using either combination, so endotracheal intubation was highly recommended. Antoninova et al. (2006) anaesthetised Western giant eland (*Taurotragus derbianus derbianus*) using etorphine (3-7 mg) and

xylazine (50-150 mg) combination to transport the animals from Bandia Reserve to the Fathala Reserve in Senegal. All the immobilisations were successful and no post immobilisation complication was recorded with these combinations.

Ancrenaz (1994) used atipamezole to reverse xylazine tranquilization in captive Arabian oryx (*Oryx leucoryx*). Twenty-seven hand-reared male Arabian oryx, with a mean (±SD) weight of 86.9 (±16.9) kg, were darted in the muscle with xylazine at a mean (±SD) dosage rate of 0.5 (±0.07) mg/kg. This dosage was sufficient to induce recumbency in 24 animals in a mean (±SD) time of 9.4 (±5.6) min. Three animals never became recumbent at this dosage but were mildly sedated and still could be handled. Atipamezole was used as antagonist agent in a mean (±SD) time of 32.1 (±9.6) min after injection of xylazine. Two-thirds of the total amount of atipamezole were given intravenously while one third was injected subcutaneously at a mean (± SD) total dosage of 0.087 (±0.014) mg/kg. The mean (±SD) reversal time (time to stand up after the injection of atipamezole) was 87.1 (±43.2) sec for the 24 recumbent oryx. A resedation period (lowering of the ears and the head, unsteady gait and sometimes recumbency), lasting up to two hours, occurred between two and five hours after the injection of atipamezole in 21 animals.

Auer et al. (2010) studied the total intravenous anaesthesia with midazolam, ketamine, and xylazine or detomidine, following induction with tiletamine, zolazepam and xylazine in red deer that were required to undergo surgery. They immobilised deer with 1.79±0.29 mg/kg xylazine and 1.79±0.29 mg/kg tiletamine/zolazepam. They reported smooth induction with mentioned drug dosages. Fournier et al. (1995), studied the effect of zoletil in 46 free-ranging and 17 hands reared *Sus scrofa*. He recorded the time taken by different stages of anaesthesia using various ranges of the zoletil to calculate the mean successful dosage for *Sus scrofa* as 6.8-9.2 mg/kg for anaesthesia ranging from 15-65 min.

Arnemo et al. (2003) used a wide-ranging of drugs to capture free-ranging moose (*Alces alces*). Their results showed that potent opioids are considered the drugs of choice for the capture of free-ranging moose. Doses of carfentanil at 0.01 mg/kg or etorphine at 7.5 mg/adult was used. Combining an opioid with a sedatives such as xylazine could increase aspiration of rumen contents, the risk of bloat, regurgitation. The best non-opioid alternative was medetomidine at 40-50 mg/adult combined with ketamine at 600 mg/adult. Carfentanil, etorphine, and medetomidine-ketamine have wide safety margins

in moose, and the risk of severe anaesthetic side effects in healthy animals is minimal. Chemical immobilisation from a helicopter in winter is considered the best capture method for moose. Due to animal welfare considerations and a low therapeutic index, neuromuscular blocking agents were not used in moose. A mortality rate greater than 2% during immobilisation and a one-month post-capture period are not acceptable for routine moose captures.

Janicki et al. (2006) tested the combination of tiletamine-zolazepam - xylazine hydrochloride for reversible chemical immobilisation of fifteen live-trapped wild red deer (*Cervus elaphus*) in Baranja district of Croatia. Their results showed that the combination of 2.0 ± 0.29 mg/kg of tiletamine-zolazepam and 2.76 (±0.85) mg/kg of xylazine hydrochloride was safe and proficient for chemical immobilisation of Red deer calves. The study also revealed that the mean combination of 1.9 mg/kg of tiletamine-zolazepam and 2.24 mg/kg of xylazine hydrochloride is efficient for adult animals. In calves, the average induction period was 5.88 (±2.17) min and 5.1 (±2.6) min for adults. Atipamezole was used as a reversal for the recovery of the animal. The mean dosage of atipamezole used for the recovery of calves was 0.14 mg/kg for calves and 0.10 mg/kg for adults. The average recovery duration for the calves was 12.25 min and 13.2 min for adults.

Bergvall et al. (2015) conducted a study on 50 free-ranging fallow deer (*Dama dama*) (29 males and 21 females) to assess the effect of the needle length, sex, and body condition on chemical immobilisation induction time. The distance between the darting sites and where they recovered the immobilised animal was measured. The time interim between the two events was also noted. The needles of 2.0 × 30 or 2.0 × 40 mm with side ports were used during the study. An important findings of their study was the 10 mm long needle shorten the recovery time significantly (>20 min) until the point when an animal is under observation. The mean recovery time of animal reduced from 51 to 29 min, and the separation reduced from 519 m from the darting area to 294 m. They recommend that a needle length of 40 mm was ideal for immobilisation of wild fallow deer, particularly for animals in over-average-to-fat body condition.

Chemical immobilisation is a dynamic tool in conservation with rapidly increasing immobilisation drugs and advancements in the field of animal anaesthesia. With availability of measuring equipment, now chemical immobilisation is not considered just

the knock-down of animals and then reversal. Considering the effects of anaesthetics on the central nervous system and physiology of the animals, it is necessary to evaluate these impacts and changes in detail, e.g. at blood gas level, acid-base status, blood chemistry, and changes in stress hormones (West et al. 2007).

3. AIMS OF THE THESIS

In general, the thesis aimed to determine the effects of different anaesthetic combinations on various ungulate and carnivore species and to investigate the physiological and cardiorespiratory response of these animals in semi-captive conditions of conservation breeding reserve, Sir Bani Yas Island (UAE). The specific objectives of the thesis were:

A. To investigate the effects of different anaesthetic combinations on physiological parameters including rectal temperature, pulse rate, respiration rate, duration of various stages of anaesthesia, blood oxygen saturation (SpO_2) and non-invasive blood pressure.

B. To assess the effects of anaesthetic drugs on the arterial blood gas parameters.

C. To determine the most suitable combinations of anaesthetics in semi-captive conditions.

We hypothesised that opioids would give more stable and prolonged anaesthesia than other drugs in the ungulates (West et al. 2007). However, the dosage calculation would be very critical as higher or lower dosages could lead to mild or deep anaesthesia (Kock & Burroughs 2012). Moreover, each combination will act on various physiological parameters and will give relatively close to average readings of parameters under study.

4. MATERIAL AND METHODS

4.1. Study site

Sir Bani Yas Island (Fig. 6) is 250 km West of Abu Dhabi and is 87 km² (8700 ha) in size. The island is declared as a protected conservation area. The Arabian Wildlife Park of 4100 hectares was developed in 2009. There are roughly over 16,000 individuals from 18 ungulate species (i.e. Arabian oryx, Arabian tahr, axis deer, Beisa oryx, Barbary sheep, blackbuck, Dorcas gazelle, waterbuck, reticulated giraffe, eland, fallow deer, gemsbok, llama, mountain gazelle, red deer, sand gazelle, scimitar-horned oryx, and urial sheep), three ratites (i.e. ostrich, emu, and rhea), four carnivore species (cheetah, hyaena, caracal cat, golden jackal), three reptile species (Greek tortoise, African tortoise, and Uromastyx lizard), and other species of natural fauna roaming free. Moreover, four species are indigenous to the UAE. Approximately 2.5 million trees were planted on the island in order to create a habitat for all the animals resembling their natural habitats (Dhaheri et al. 2017).

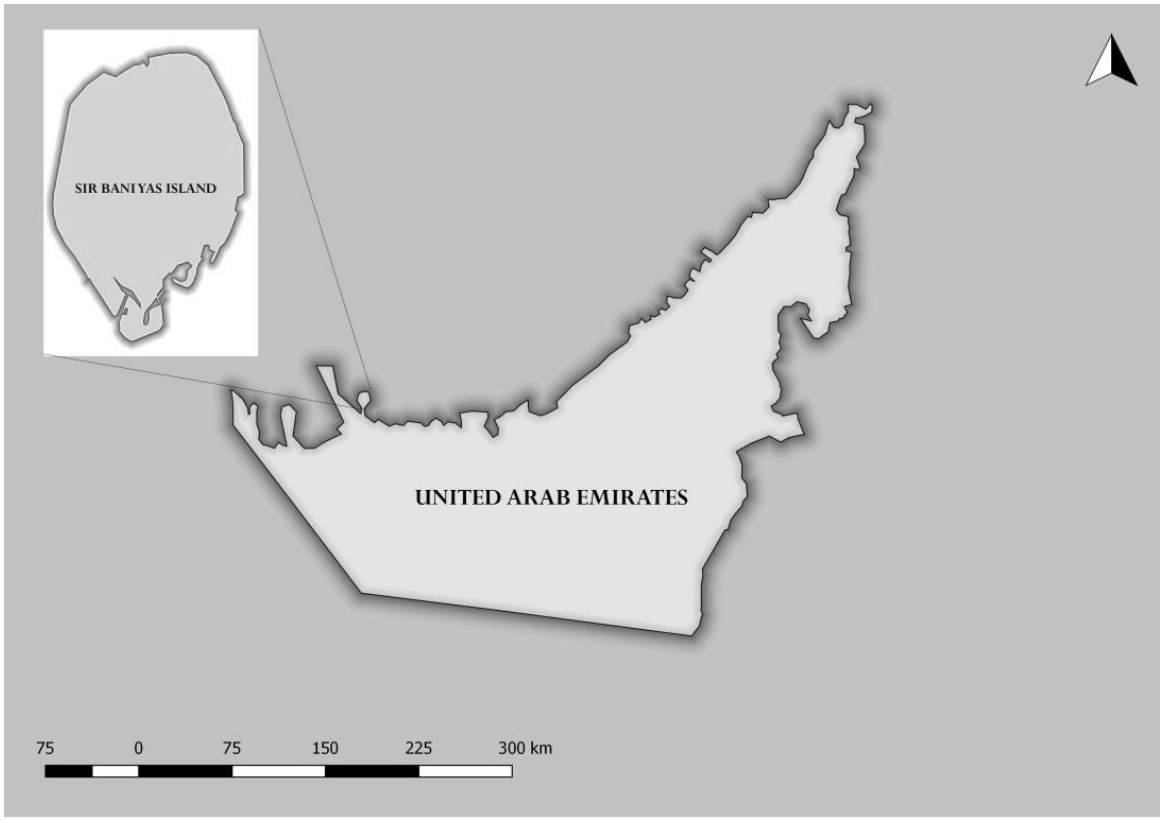

Figure 6: Map of Sir Bani Yas Island (Credit: Abid Mehmood)

4.2. Study animals and inclusion/exclusion criteria

In the current study, two carnivore and three ungulate species were studied (Table 1). The carnivores, especially cheetah, roam freely and predate on the ungulates. The hyaenas were either roaming in the park or were housed in enclosures of 0.56 ± 0.16 ha. The ungulates feed on fresh browse, pastures, and are also supplemented with herbivore concentrates and hay. A small population of eland were free-ranging in the park. However, approximately 90% of the population was in various management camps of an average size of 24 ha. The Barbary sheep males were freely roaming in the park. The females of Barbary sheep and Beisa oryx were in a camp of 213 ha. The males of Beisa oryx were kept in a management camp of 34 ha.

The animals were immobilised as part of various management interventions including translocations, separations and other veterinary procedures. All chemical restraint procedures were executed according to the highest animal care and management standards. Moreover, they were following animal collection management, care and research use guideline (BFM-WL-CI/0017-2014). Moreover, no animal individual was explicitly selected for study purposes. All the immobilisation procedures took place between 2014 and 2019. Where applicable, the protocols and animal selection were randomised, and the observers monitoring vital signs, quality of anaesthesia, and processing samples were double-blinded to avoid any biases. All the immobilised animals included in the study were healthy, young (not geriatric or pregnant), and were without any underlying medical conditions. They were categorised as ASA Class 1 (American Society of Anaesthesiologists) (Doyle & Garmon 2018). Any procedure with incomplete data was also excluded from the study.

Table 1: Details of studied species on Sir Bani Yas Island.

S.	Species	Bodyweight (kg) (mean ± SE)	Total population	N of animals in study	Sex Ratio (Male.Female)
1	Arabian striped hyaena	31.39 ± 0.36	15	15	6.9
2	Northeast African cheetah	46.84 ± 1.60	5	5	3.2
3	Patterson’s eland	521.95 ± 18.88	1090	40	20.20
4	Barbary sheep	136.71 ± 2.55	1120	56	56.0
5	Beisa oryx	138.31 ± 2.36	592	45	35.10

4.2.2. Drugs, combinations and target doses

The drug agonists used in the current study (Table 2) were injections of thiafentanil oxalate (Thianil; 10 mg/ml) antagonised with naltrexone hydrochloride (Trexonil; 50 mg/ml) from Wildlife Pharmaceuticals Pty Ltd, Mpumalanga, South Africa); medetomidine hydrochloride (Ilium Medetomidine; 1 mg/ml) antagonised with atipamezole hydrochloride (Ilium Atipamezole; 5 mg/ml), xylazine hydrochloride (Xylazil-100; 100 mg/ml), and ketamine hydrochloride (Ketamil injection; 100 mg/ml) from Troy Laboratories, NSW, Australia. Xylazine was antagonised either by yohimbine hydrochloride (Reverzine ^{TM} Injection, 10 mg/ml; Bayer, Pymble, NSW, Australia), atipamezole, or tolazoline hydrochloride (Akron, Inc. IL, USA).

Table 2: Various drug agonists and antagonists used during the chemical restraint of carnivores and ungulates at Sir Bani Yas Island, UAE.

Drugs	Abbreviation	Drug Category	Conc. (mg/ml)
Ketamine hydrochloride	K	Cyclohexylamines	100
Medetomidine hydrochloride	M	α-2 agonists	1
Medetomidine hydrochloride	M	α-2 agonists	10
Xylazine hydrochloride	X	α-2 agonists	100
Thiafentanil oxalate	T	μ-receptor agonists	10
Atipamezole hydrochloride	A	α-2 antagonists	5
Yohimbine hydrochloride	Y	α-2 antagonists	10
Tolazoline hydrochloride	TZ	α-2 antagonists	100
Naltrexone hydrochloride	N	μ-receptor antagonists	50

The target doses (Table 3) were established from literature and field experience. They were calculated for each procedure based on the estimated weight of the animals, as suggested by Burroughs et al. (2012) and Hahn et al. (2014). The doses administered were later calculated by taking the actual weight of the animals through a portable field weighing scale as suggested by Fournier et al. (1995) and Lescano et al. (2014).

4.2.3. Drug injection (darting)

Animals were sedated using a dart gun (Dan-Inject CO_2 Injection Rifle, No. 0471, Model JM), either from ranger's vehicle or hiding trailer. The average distance between

the operator of the injection and the animal was between 10 - 15 meters depending on species and its location. All the immobilisation procedures were performed early morning due to hot climatic conditions in the region. Moreover, the animals have different body temperatures during different times of the day and performing immobilisations at different times could provide biased body temperatures of the animals. The darts were placed intramuscularly (IM). The ideal site for dart placement was hindquarters as suggested by Kock & Burroughs (2012). However, in hyaenas, the neck was also a suitable darting site (Hahn et al. 2014). The data of only those procedures were included where the drug was injected from a single dart, and no supplementary top-up of medicine was required. Once the animal was unconscious, it was approached quietly. The state of unconsciousness was assessed by touching it through the bamboo stick. If there was no response the animal was held into sternal position for herbivores, as they tend to regurgitate, and lateral position for carnivore species as suggested by Kock & Burroughs (2012). The drug(s) antagonist(s) were administered (half intravenous and half IM) once there were signs of drug agonist(s) metabolism, e.g. pedal or palpebral reflexes and ear twitching.

Table 3: Study species and target doses of various drugs in selected combinations for assessment of various physiological parameters during chemical restraint.

Species	Combinations	No. of immobilisations (n)	Target doses (mg/kg)
Arabian striped hyaena	KM	34	K: 2.3 + M: 0.04
	KX	16	K: 5.0 + X: 1.0
Northeast African cheetah	KM	18	K: 1.9 + M: 0.03
Peterson's eland	TKX	20	T: 0.01 + K: 0.18 + M: 0.03
	TKM	20	T: 0.01 + K: 0.18 + X: 0.24
Barbary sheep	KX	29	K: 0.94 + X: 1.40
	KXM	27	K: 0.83 + X: 1.23 + M: 0.03
Beisa oryx	KX	20	K: 0.82 + X: 0.94
	KXM	25	K: 0.78 + X: 0.89 + M: 0.03

4.2.4. Duration and quality of different phases of chemical restraint

Each chemical restraint procedure was further into induction, anaesthesia maintenance, and recovery phases. Each of these phases was further classified into

different stages, and time durations were recorded (Table 4). The quality of different phases was assessed separately through a scoring system provided in Table 5.

4.2.5. Monitoring of the vital signs

The vital signs such as the respiration rate, pulse rate, rectal temperature, blood oxygen saturation (SpO_2), capillary refill time, and non-invasive blood pressure were monitored during anaesthesia maintenance phase at five-minute intervals as suggested by Janovsky et al. (2000b) and Lescano et al. (2014). The rectal temperature was measured through a rectal thermometer (NOVAMED Digital thermometer, UK) and the rectal thermometer probe of the pulse oximeter (Purescope Veterinary Patient Monitor IP-3000/4000 Series, Infunix Technology Co., Ltd. Korea). The pulse rate was monitored by cardiac auscultation, as suggested by Janovsky et al. (2000b). The respiration rate was recorded by direct observation of the movement of the thoracic cavity. The SpO_2 percentage was recorded by placing the probe of the pulse oximeter at the tongue of the animals. Moreover, the non-invasive blood pressure was measured by placing the cuff of pulse oximeter on the median palmar artery (proximal to the metacarpal pad) as instructed in the operational manual of the monitor. Capillary refill time was assessed by pressing the oral mucosa through thumb and then counting the required seconds to regain its colour as suggested by Lescano et al. (2014). In some procedures, oxygen was supplemented after fifteen minutes into the anaesthesia maintenance phase. The supplementary oxygen was administered using a medium feline respiratory mask attached to a medical oxygen cylinder fitted with a regulator (Midmark, England, United Kingdom) at the rate of two litres per minute to compensate hypoxaemia as suggested by Kock & Burroughs (2012).

4.2.6. Arterial blood gas analysis

The arterial blood gas analysis was performed where possible at fifteen minutes into the anaesthesia maintenance phase. If oxygen supplementation was performed, it was also analysed fifteen minutes after oxygen supplementation. The blood samples were taken from the femoral artery. The samples were immediately analysed with a point of care blood gas analyser (iSTAT® 1, Abbott USA) and CG8+ Cartridge as suggested by Lescano et al. (2014).

4.2.7. Haematology and biochemistry

Venous blood samples were taken to analyse the blood chemistry and haematology. The samples were taken from the jugular vein with 20 ml disposable handheld syringes. For blood biochemistry 6-8 ml blood was collected in serum gel tubes, 3-4 ml in heparin lithium tubes for cortisol hormone, and 3-4 ml in EDTA K_3 tubes for haematology. The blood in the serum gel and heparin tubes were centrifuged at 2000 rpm for 3-5 minutes and 1000 rpm for 2-3 minutes respectively. The samples were either analysed using Abbott CELL-DYN® 3700 Haematology Analyser or Abbott Architect c4000 clinical chemistry analyser; or through Central Veterinary Research Laboratory (paid services).

4.2.8. Data Analysis

The data were analysed for normality through the Shapiro–Wilk test. The data for the study of Arabian striped hyaena and Patterson's eland were analysed through the general linear models (GLM). The data for the effects of oxygen supplementation in the Northeast African cheetah and Arabian striped hyaena were analysed through Wilcoxon matched-pairs test.

Table 4: Various time variables for different phases of chemical restraint.

Phase	Time variables (minutes)	Description
Induction	Time to the first sign (Ataxia)	Time from the injection of the medicine to the first sign of the drugs taking effect such as ataxia
	Time of sternal position	Time from the injection of the medicine to the time when animal acquired a sternal position
	Time of head down	Time from the injection of the medicine to the time when the animal placed its head on the ground, unable to support the head
	Induction completion time	Time from the injection of the medicine to the time when the animals were entirely unconscious and safe to approach and handle
Anaesthesia maintenance time		Time from complete induction to the time when signs of drug metabolism were displayed such as a change in breathing, palpebral reflex, or body jerk
Recovery	Reversal time	Time from the administration of antagonist to the first sign of consciousness such as ear twitching, the return of swallow reflex or blinking
	Time to lift the head up after reversal	Time from the administration of antagonist to lift the head and maintain the head in normal posture
	Time to Sternal Position after reversal	Time from the administration of antagonist until the animal acquired a sternal position

Time to Stand up after reversal	Time from the administration of antagonist until the animal could stand up and maintain balance
Time to complete recovery	Time from the administration of antagonist until the animal stopped exhibiting any signs of the effects of anaesthetic drugs

Table 5: Description of quality scores for different phases of a chemical restraint procedure (modified from Lescano et al. 2014)

Score	Quality	Induction	Anaesthesia	Recovery
1	Excellent	Quick and smooth induction; absence of uncoordinated movement, stereotypic reaction, ptyalism, vomiting, and discomfort at the injection site	The absence of body movement, pedal and palpebral reflexes, muscle tone, response to an external stimulus	Quick and smooth recovery; absence of uncoordinated movement, ptyalism, vomiting, quick retraction of the tongue
2	Good	Quick induction but resistance to loss of balance, slight ptyalism, pacing, and licking	Ear twitching, no muscle tone, occasional (< 3 times) pedal and palpebral reflexes, occasional body twitching to an external stimulus	Quick recovery, slight struggle to balance, slight ptyalism, licking, and slightly weak coordination in hindquarters
3	Satisfactory	Moderate or Slow induction, pacing, ptyalism, slight discomfort at the injection site, panting and grunting	Ear and limb twitching, slight muscle tone, delayed pedal and palpebral reflexes, slight twitching to an external stimulus	Slow recovery, delayed retraction of the tongue, Ptyalism, struggle in standing and uncoordinated movements, erection of mane hair
4	Unsatisfactory	No induction or violent resistance, vomiting, panting, stereotypic pacing, grunting, excessive ptyalism, severe discomfort to the injection site and licking	Body movement, limb withdrawn, grunting sounds, increased muscle tone, blinking, twitching of ear, immediate pedal and palpebral reflexes, rapid response to an external stimulus	Delayed recovery, inability to retract tongue, gain balance and no palpebral reflexes, unable to stand, temporary loss of sensation in the hindquarter, shivering

5. RESULTS

5.1. Chemical immobilisation of Arabian striped hyaena with ketamine-medetomidine and ketamine-xylazine

All immobilisation procedures were successful with both combinations. The mean drug doses for both combinations are provided in Table 6. The quality of induction had a mean score and standard error (MS±SE) 1.4±0.1 and 1.1±0.1 in KM and KX, respectively. The mean scores of anaesthesia were 1.0±0.0 and 2.0±0.0 in KM and KX. The recovery of the animals in KM was 1.8±0.1, whereas, the quality of recovery in KX was 2.69±0.12 (Table 7).

Table 6: Drug doses for the immobilisation of Arabian striped hyaena with KM/KX.

Combinations	Drugs	Mean Dosage ± SE (mg/kg)
KM	K	2.27 ± 0.044
	M	0.04 ± 0.001
	A	0.21 ± 0.003
KX	K	4.95 ± 0.115
	X	0.99 ± 0.023
	A	0.09 ± 0.003
	(or) Y	0.23 ± 0.006

Table 7: Quality scores for immobilisation of Arabian striped hyaena with KM/KX.

Chemical Restraint Category	Scores					
	Median		Inter Quartile Range		Mean ± SE	
	KX	KM	KX	KM	KX	KM
Induction	1	1	1	1	1.3±0.1	1.4±0.1
Anaesthesia	1	1	0	0	2.0±0.0	1.0±0.0
Recovery (KM with atipamezole)	3	1	1	2	-	1.8±0.01
Recovery (KX with atipamezole)	2.5	-	1	-	2.5±0.17	-
Recovery (KX with yohimbine)	3	-	0	-	3±0	-

The durations for various variables for the immobilisation of Arabian striped hyaena with KM and KX combinations are provided in Table 8. Both combinations provided quick induction. The results of current study showed a significant difference for anaesthesia time ($F = 5.1$; $P < 0.05$), time to stand after administration of reversal ($F = 8.3$; $P < 0.05$) and complete recovery ($F = 7.9$; $P < 0.05$) between the two combinations. Between the sexes, there was a significant difference in the time to lift the head ($F = 4.2$; $P < 0.05$) and time to stand after administration of reversal ($F = 6.2$; $P < 0.05$). There was also a significant difference in the times to complete recovery between the reversals (atipamezole and yohimbine) in the KX ($F = 529.0$; $P < 0.05$).

Table 8: Time variables for Arabian striped hyaena immobilised with KM/KX.

Duration category	**Time Variables**	**KM (n = 34)** Mean ± SE (Minutes)	**KX (n=16)** Mean ± SE (Minutes)
From the administration of anaesthetics	Time to First Sign (ataxia)	4.56 ± 0.40	4.19 ± 0.33
	Time of Sternal Position	6.15 ± 0.65	5.75 ± 0.39
	Time of head down	6.80 ± 0.65	6.87 ± 0.43
	Induction Time	10.12 ± 0.65	9.37 ± 0.45
	Anaesthesia Time	59.5 ± 2.41	49.25 ± 1.31
From the administration of reversal	Time to First Reversal Sign	1.94 ± 0.22	2.13 ± 0.35
	Time to head up	3.70 ± 0.56	4.13 ± 1.13
	Time to Sternal Position	4.03 ± 0.54	5.63 ± 1.09
	Time to Stand	4.91 ± 0.60	10.38 ± 1.48
	Time to complete recovery	12.32 ± 1.37	21.25 ± 2.16

The mean changes in the pulse rate, respiration rate, systolic and diastolic blood pressure, blood oxygen saturation and rectal temperature during anaesthesia are given in Figure 1 of Annex – 9.1. The capillary refill time for all immobilisations was less than two seconds. There was no significant difference in the vital signs between males and females ($P > 0.05$). The results of the current study also revealed statistically significant differences between the combinations for rectal temperature, pulse rate, respiration rate, and blood oxygen saturation ($P < 0.05$) (Table 9).

Table 9: Vital signs of Arabian striped hyaena immobilised with KM/KX.

Variables	Units	KM (n = 34)	KX (n=16)
Rectal Temperature	°C	37.58± 0.29	36.00± 0.68
Pulse Rate	Beats / min	50.46± 1.90	61.14± 2.79
Respiration Rate	Breaths / min	29.44± 0.99	23.80± 1.57
SpO_2	%	89.59± 1.34	82.06±3.92
N.I.B.P (Systolic)	mmHg	145.36± 2.13	141.16±3.66
N.I.B.P (Diastolic)	mmHg	106.74± 1.97	102.43± 3.50

Note: SE for measurements of rectal temperature, pulse rate and respiration rate in an animal immobilised once (N=1) indicates SE of five repeated / consecutive measurements during one immobilisation event.

There was no statistically significant difference between combinations or sexes for pH (potential of hydrogen), pCO_2 (partial pressure of carbon dioxide), pO_2 (partial pressure of oxygen) BE (base excess); HCO_3 (bicarbonate), TCO2 (total carbon dioxide) sO2 (oxygen saturation), Na+ (sodium) K+ (potassium) iCa^{2+} (ionised calcium), Hct (haematocrit), and Hb (haemoglobin). The partial pressure of oxygen (pO_2) had a statistically significant difference between combinations ($F = 5.9$, $P < 0.05$). Most of the variables remained within described feline range except ionised Calcium (iCa^{++}) which was high; pO_2 and sO_2 were low (Table 10).

Table 10: Blood gas parameters of Arabian striped hyaena immobilised with KM/KX.

Variables	Units	Range*	KM (n = 21) Mean ± SE	KX (n=16) Mean ± SE
pH	-	7.25 - 7.40	7.28 ±0.01	7.26 ± 0.01
pCO_2	mmHg	33.0- 51.0	45.94 ±1.02	46.79 ± 2.08
HCO_3^-	mmol/L	13.0 - 25.0	21.73 ±0.67	22.22 ± 0.67
TCO_2	mmol/L	16 - 25	22.81 ±0.65	23.19 ± 0.61
BE	mmol/L	(-5) - (+2)	-5.52 ±0.69	-5.94 ± 0.38
pO_2	mmHg	90 - 110	53.00 ±2.54	44.44 ± 3.08
sO_2	%	> 90	80.57 ±2.39	69.44 ± 5.09
Na^+	mmol/L	147 - 162	144.38 ±0.25	143.50 ± 0.43
K^+	mmol/L	2.9 - 4.2	4.35 ±0.07	4.17 ± 0.08
iCa^{++}	mmol/L	1.20 - 1.32	1.43 ±0.01	1.45 ± 0.02
Hct	% PCV	24 - 40	38.33 ±0.96	39.88 ± 0.93
Hb	g/dL	8.0 - 13.0	12.98 ±0.32	13.21 ± 0.31

Note: SE for measurements of rectal temperature, pulse rate and respiration rate in an animal immobilised once (N=1) indicates SE of five repeated / consecutive measurements during one immobilisation event. pH, Potential of Hydrogen; pCO_2, Partial Pressure of Carbon Dioxide; pO_2, Partial Pressure of Oxygen; BE, Base Excess; HCO_3, Bicarbonate; TCO_2, Total Carbon Dioxide; sO_2, Oxygen Saturation; Na^+, Sodium; K^+, Potassium; iCa^{++}, Ionised Calcium; Hct, Haematocrit; Hb, Haemoglobin. ***** Feline range from I-Stat reference manual for CG8+ Cartridge

The statistical analysis of the blood chemistry and haematology variables between sexes showed a significant difference in platelet count, WBC, LDH and cortisol levels ($P < 0.05$), and statistically significant difference between the combinations for MCH, PHOS, ALT, ALP, CREA, TP, Glu, α-Amylase, and cholesterol ($P < 0.05$). All the variables were within the described range when compared with the range of values of striped hyaena extracted from ZIMS by Species 360 (Table 6 of Annex – 9.1).

5.2. Effects of oxygen supplementation on arterial blood gas and acid-base status in Arabian striped hyena immobilised with KM combination

The quality of induction, anaesthesia and recoveries was similar to the scores mentioned in Table 7 for KM combination under section 5.2. The combination provided quick induction, adequate depth and duration of anaesthesia, and smooth recoveries (Table 11). The results suggested that the pulse rate, respiration rate, and MAP had no statistically significant difference before and after oxygen supplementation ($P > 0.05$) (Table 12). However, the rectal temperatures and blood oxygen saturation percentage (SpO_2) had a statistically significant difference before and after oxygen supplementation (Table 22).

Table 11: Duration of induction, anaesthesia maintenance, and complete recovery of Arabian striped hyaena immobilised with KM.

Event description	Duration (min) (n = 11)
Induction	10.3 ± 0.5
Anaesthesia maintenance	57.3 ± 0.6
Full recovery	11.9 ± 0.4

The arterial oxygen saturation, arterial partial pressure, the ratio of PaO_2, and FiO_2 and A-a gradient of oxygen was below the reference range before oxygen supplementation (Table 13). The levels of $PaCO_2$ and total carbon dioxide (TCO_2) were near to the maximum values of reference ranges (Table 13). There was a significant increase in SaO_2, PaO_2, PaO_2/FiO_2, and A-a Gradient after oxygen supplementation ($P<0.05$) (Table 3 of Annex – 9.2). However, a significant increase ($P < 0.05$) in $PaCO_2$ and TCO_2 was also observed.

There was a significant decrease ($P < 0.05$) in the pH and a significant increase in bicarbonates before and after oxygen supplementation (Table 13). Moreover, the current iCa^{2+} level was above the reference range. Whereas, the K^+ concentration was marginally below the upper limit of the reference range (Table 13).

Table 12: Vital signs (mean ± SE) of Arabian striped hyaena before and after oxygen supplementation during field immobilisation with KM.

Vital signs	Units	Before oxygen supplementation (n = 11)	Oxygen supplementation (n = 11)
Heart rate (HR)	Beats/min	50 ± 2.4	49.7 ± 2.3
Respiration rate (RR)	Breaths/min	28.3 ± 1	26.8 ± 1.2
Oxygen saturation (SpO_2)	%	86.1 ± 1.3	98.2 ± 0.5
Mean arterial pressure (MAP)	mmHg	128.6 ± 2.9	123.2 ± 3.6
Rectal temperature	°C	38.2 ± 0.2	38 ± 0.2

Table 13: Blood-gas parameters of Arabian striped hyena before and after oxygen supplementation during field immobilisation with KM.

Parameters	Reference range	Before oxygen supplementation (Mean ± SE) (n = 11)	After oxygen supplementation (Mean ± SE) (n = 11)
SaO_2 (%)	>90	73.5 ± 4.9	90.8 ± 3.8
PaO_2 (mmHg)	90–110	47.3 ± 3.6	127.5 ± 12.6
$PaCO_2$ (mmHg)	33.0–51.0	49.3 ± 1.8	55.2 ± 1.9
TCO_2 (mmol/L)	16–25	23.1 ± 1.1	25.2 ± 1.0
PaO_2/FiO_2	>400	225.1 ± 17.0	455.2 ± 45.0
A-a Gradient	<15	43.3 ± 3.3	5.9 ± 13.5
pH	7.25–7.40	7.25 ± 0.01	7.23 ± 0.01
BE_{ecf} (mmol/L)	(-5) – (+2)	-5.7 ± 1.1	-5.3 ± 0.7
HCO_3^- (mmol/L)	13.0–25.0	21.5 ± 1.0	23.3 ± 1.0
Na^+ (mmol/L)	147–162	144.7 ± 0.6	144.5 ± 0.7
K^+ (mmol/L)	2.9–4.2	4.0 ± 0.1	4.1 ± 0.1
i Ca^{++} (mmol/L)	1.2–1.3	1.5 ± 0.01	1.5 ± 0.01

SaO_2, arterial oxygen saturation; PaO_2, the partial pressure of O_2; $PaCO_2$, the arterial partial pressure of CO_2; TCO_2, total CO_2; $PaO_2/FiO2$, the ratio of the partial pressure of O_2 and fraction of inspired O_2; A-a Gradient, alveolar-arterial gradient; BEecf, base excess (extracellular fluid); HCO_3^-, bicarbonates; Na^+, sodium; K^+, potassium; iCa^{++}, ionised calcium.

5.3. Effects of oxygen supplementation on blood gas variables in Northeast African cheetah (Acinonyx jubatus soemmeringii) immobilised with ketamine and medetomidine.

The actual immobilisation doses are provided in Table 14. The combination provided quick induction, adequate anaesthesia and smooth recoveries with time durations provided in Tables 15 and 16.

Table 14: Drug doses for the immobilisation of Northeast African cheetahs.

Combinations	Drugs	Mean Dosage ± SE (mg/kg)
KM	K	1.5 ± 0.1
	M	0.03 ± 0.001
	A	0.1 ± 0.01

Table 15: Quality scores for induction, anaesthesia and recovery in Northeast African cheetahs immobilised with KM.

Chemical Restraint Category	Scores (n = 18)		
	Median	Inter Quartile Ranges	Mean ± SE
Induction	1.00	0.25	1.22 ± 0.10
Anaesthesia	1.00	0.00	1.17 ± 0.09
Recovery	1.00	1.00	1.39 ± 0.12

Table 16: Duration (mean ± SE) of induction, anaesthesia and recovery in Northeast African Cheetahs immobilised with KM.

Phases	Variables	Time (minutes) (n = 18)
Induction	Ataxia	2.78 ± 0.33
	Sternal position	3.61 ± 0.37
	Head down	6.17 ± 0.48
	Induction time	8.50 ± 0.54
Anaesthesia	Anaesthesia time	45.94 ± 0.76
Recovery	Recovery time to the first sign	2.44 ± 0.18
	Recovery time to head up	3.78 ± 0.25
	Recovery time to Sternal Position	6.39 ± 0.35
	Time to stand	9.39 ± 0.36
	Time to full recovery	19.44 ± 0.53

There was no statistically significant difference in the vital signs, including pulse rate, respiration rate, systolic, diastolic, and mean arterial blood pressure ($P > 0.05$). However, there was a significant difference in rectal temperature and peripheral haemoglobin oxygen saturation ($P < 0.05$) (Table 17).

Table 17: Vital signs (mean ± SE) of Northeast African cheetah before and after oxygen supplementation during field immobilisation with KM.

Parameters	Before oxygen supplementation (n = 18)	After oxygen supplementation (n = 18)
Rectal temperature (°C)	38.5 ± 0.1	38.4 ± 0.1
Heart rate (beats/minutes)	64.9 ± 1.4	64.4 ± 2
Respiration rate (breaths/minute)	19.1 ± 0.7	18.6 ± 0.6
Haemoglobin oxygen saturation (%)	75.1 ± 1.8	97.7 ± 0.3
Non-invasive systolic blood pressure (mmHg)	172.4 ± 7.7	180.7 ± 5.1
Non-invasive diastolic blood pressure (mmHg)	128.6 ± 7	138.6 ± 5.1
Mean arterial blood pressure (mmHg)	143.2 ± 7.2	152.6 ± 5

Table 18: Blood-gas parameters (mean ± SE) of Northeast African cheetah before and after oxygen supplementation during field immobilisation with KM.

Parameters	Before oxygen supplementation (N = 18)	After oxygen supplementation (N = 18)
pH	7.31 ± 0.01	7.24 ± 0.01
PCO_2 (mmHg)	39.50 ± 0.65	50.16 ± 0.50
PO_2 (mmHg)	31.72 ± 0.59	91.67 ± 1.10
BE_{ecf} (mmol/L)	-8.11 ± 0.40	-5.56 ± 0.46
HCO_3 (mmol/L)	18.97 ± 0.38	21.06 ± 0.32
TCO_2 (mmol/L)	20.56 ± 0.35	21.61 ± 0.47
sO_2 (%)	50.56 ± 1.80	92.94 ± 1.22
Na (mmol/L)	159.11 ± 0.25	158.56 ± 0.27
K (mmol/L)	3.71 ± 0.04	3.60 ± 0.05
i Ca (mmol/L)	1.30 ± 0.01	1.29 ± 0.01
Hct % PCV	46.44 ± 0.61	46.83 ± 0.88
Hb (g/dL)	15.76 ± 0.19	15.70 ± 0.20

The results suggested a statistically significant difference for pH, $PaCO_2$, PaO_2, BE_{ecf}, HCO_3^-, Na^+, K^+, Hb, and SaO_2 ($P < 0.05$). The PaO_2 and SaO_2 values were low before oxygen supplementation and increased after oxygen supplementation (Table 1 of Annex – 9.3). There was a decrease in the pH, BE_{ecf}, Na^+, i Ca^{2+}, and K^+. While there was an increase in HCO_3^-, $PaCO_2$, TCO_2, and SaO_2 (Table 18). The PaO_2 and SaO_2 values were very low before oxygen supplementation. While Hct and Hb were high before and after oxygen supplementation compared to the reference ranges (Table 1 of Annex – 9.3).

5.4. Thermal and cardiopulmonary responses of Patterson's eland to the drug combinations of thiafentanil-ketamine with medetomidine/xylazine

All immobilisation procedures were successful without any failure or complications. However, excessive salivation was observed in all procedures with both combinations, especially with TKX. The mean actual doses for both combinations are provided in Table 19.

Table 19: Drug doses for the immobilisation of Patterson's eland.

Combinations	Drugs	Mean Dosage ± SE (mg/kg)
TKM	Thiafentanil	0.10 ± 0.0001
	Ketamine	0.18 ± 0.003
	Medetomidine	0.03 ± 0.0004
Reversals	Naltrexone	0.30 ± 0.004
	Atipamezole	0.07 ± 0.004
TKX	Thiafentanil	0.10 ± 0.0001
	Ketamine	0.18 ± 0.007
	Xylazine	0.24 ± 0.008
Reversals	Naltrexone	0.30 ± 0.003
	Yohimbine	0.11 ± 0.004

Both combinations provided quick induction, adequate anaesthesia and complete recovery (Table 20 and Table 21). The results showed a significant difference ($P < 0.05$) in the times to recumbency, head down, induction, and lifting head after the reversal (Table 1 of Annex – 9.4). There was no difference in anaesthesia maintenance and complete recovery times in both combinations (Table 1 of Annex – 9.4).

Table 20: Scores for the qualities of induction, anaesthesia and recovery in Patterson's eland immobilised with TKX and TKM.

Chemical Restraint Category	Scores (n = 20, each combination)					
	Median		Inter Quartile Ranges		Mean ± SE	
	TKX	TKM	TKX	TKM	TKX	TKM
Induction	1	1	1	0	1.3 ± 0.1	1.2 ± 0.1
Anaesthesia	1	1	0.75	0	1.2 ± 0.1	1.1 ± 0.1
Recovery	1	1	0	0	1.1 ± 0.1	1.1 ± 0.1

Table 21: Duration (mean ± SE) of induction, anaesthesia and recovery in Patterson's eland immobilised with TKX and TKM.

Variables	TKX (n = 20)	TKM (n = 20)
Ataxia (minutes)	3.15 ± 0.78	2.35 ± 0.13
Sternal recumbency (minutes)	7.85 ± 1.34	4.55 ± 0.72
Head down (minutes)	8.95 ± 1.35	4.55 ± 0.72
Complete induction (minutes)	11.8 ± 1.40	7.40 ± 0.80
Anaesthesia maintenance (minutes)	46.0 ± 1.29	46.10 ± 0.91
First effect of antagonists (minutes)	1.20 ± 0.09	1.10 ± 0.07
Head-up (minutes)	1.55 ± 0.15	1.45 ± 0.11
Standing up (minutes)	2.20 ± 0.27	2.35 ± 0.22
Complete recovery (minutes)	11.70 ± 0.48	11.15 ± 0.53

The results of the current study suggested a significant difference in rectal temperature, heart rate, respiration rate, and percutaneous haemoglobin oxygen saturation percentages (Table 22; Table 1 of Annex 9.4). The results of blood gas analysis showed a significant difference between the combinations for pH, partial pressure of carbon dioxide, the partial pressure of oxygen, base excess in the extracellular fluid, bicarbonates, total carbon dioxide, and haemoglobin ($P < 0.05$). The cortisol levels were higher in TKX compared to TKM (Table 23). All the haematological and biochemistry values were within the reported range in both combinations (Table 24).

Table 22: Vital signs (mean ± SE) of Patterson's eland immobilised with TKX and TKM.

Variables	TKX (n = 20)	TKM (n = 20)
Rectal Temperature (°C)	39.94 ± 0.20	40.24 ± 0.14
HR (beats minute^{-1})	61.42 ± 1.57	59.67 ± 1.83
f_R (breaths minute^{-1})	18.45 ± 0.84	16.11 ± 0.57
SpO_2 (%)	94.70 ± 1.67	93.48 ± 1.24
SAP (mmHg)	142.09 ± 3.27	145.03 ± 2.97
DAP (mmHg)	100.72 ± 2.96	96.67 ± 3.12
MAP (mmHg)	114.5 ± 2.9	112.8 ± 3.0

Table 23: Blood-gas variables (mean ± SE) of Patterson's eland immobilised with TKX and TKM.

Variables	TKX (n = 20)	TKM (n = 20)
pH	7.27 ± 0.02	7.34 ± 0.02
pCO_2 (mmHg)	43.40 ± 1.34	48.97 ± 1.44
pO_2 (mmHg)	90.50 ± 0.63	89.60 ± 0.68
BEecf (mmol/L)	-4.60 ± 1.68	0.35 ± 1.47
HCO_3^- (mmol/L)	22.11 ± 1.44	27.18 ± 1.11
TCO_2 (mmol/L)	23.15 ± 1.45	28.80 ± 1.13
sO_2 (%)	86.25 ± 1.59	88.30 ± 0.93
Na (mmol/L)	143.0 ± 0.59	143.25 ± 0.41
K (mmol/L)	4.47 ± 0.14	4.63 ± 0.10
Ca (mmol/L)	1.06 ± 0.02	1.03 ± 0.02
Hb (g/dL)	11.46 ± 0.30	9.69 ± 0.24
Cortisol (nmol/L)	23.96 ± 2.32	14.24 ± 0.95

Table 24: Haematological and blood biochemistry variables (mean ± SE) of Patterson's eland immobilised with TKX and TKM.

Variables (Units)	Range	TKX (n = 20)	TKM (n = 20)
		(Mean ± SE)	
RBC (10^{12} cells/L)	6.07 – 11.85	7.04 ± 0.14	7.20 ± 0.13
PCV (L/L)	0.280 – 0.521	0.37 ± 0.01	0.37 ± 0.01
MCV (fL)	35.3 – 57.1	52.86 ± 0.75	49.84 ± 0.60
MCH (pg)	12.7 – 18.8	16.96 ± 0.23	16.31± 0.21
MCHC (g/dl)	30.4 – 39.4	32.04 ± 0.29	32.53 ± 0.14
WBC (10^9/L)	2.7 – 18.0	5.47 ± 0.72	3.33 ± 0.26
NEU (%)	39.8 – 91.0	30.47 ± 5.43	52.18 ± 4.53
LYM (%)	6.0 – 47.0	63.53 ± 5.59	30.30 ± 2.28
MONO (%)	1.0 – 14.5	4.06 ± 0.77	2.36 ± 0.35
EOS (%)	0.0 – 9.6	1.17 ± 0.42	1.99 ± 0.23
BASO (%)	0.0 – 4.0	0.70 ± 0.12	0.21 ± 0.06
CK (U/L)	63 – 2315	394.95 ± 44.85	288.55 ± 18.92
LDH (U/L)	274 – 1320	1187.35 ± 59.23	1128.10 ± 32.15
Ca (mmol/L)	1.4 – 3.1	2.19 ± 0.04	1.99 ± 0.05
PHOS (mmol/L)	1.08 – 5.35	2.05 ± 0.16	2.48 ± 0.17
Iron (µmol/l)	2.3 – 50.6	26.25 ± 1.67	7.42 ± 0.35
AST (U/L)	48 – 259	127.70 ± 8.34	97.06 ± 3.26
ALT (U/L)	1 – 22	10.10 ± 0.62	7.01 ± 0.29
GGT (U/L)	5.0 – 55.0	16.15 ± 0.99	24.35 ± 2.26
CREA (µmol/L)	110 – 228	175.65 ± 6.71	191.80 ± 5.15
BUN (mmol/L)	3.2 – 16.6	7.52 ± 0.30	1.44 ± 0.48
TP (g/L)	49 – 91	79.35 ± 1.33	78.25 ± 1.23
Albumin (g/L)	23 – 56	43.35 ± 0.87	42.50 ± 0.34
Glucose (mmol/L)	3.6 – 11.7	7.61 ± 0.40	6.49 ± 0.48
Cortisol (nmol/L)	0.27 – 127.18*	23.96 ± 2.32	14.24 ± 0.95

* (Pennington et al. 2013); Reference range for Taurotragus derbianus extracted from ZIMS by Species 360; RCB, Red Blood Cell Count; PCV, Packed Cell Volume; MCV, Mean Cell Volume; MCH, Mean Cell Haemoglobin; WBC, White Blood Cell Count; NEU, Neutrophils; LYM, Lymphocytes; MONO, Monocytes; EOS, Eosinophils; BASO, Basophils; CK, Creatinine Kinase; LDH, Lactate Dehydrogenase; Ca, Calcium, PHOS, Phosphorus; AST, Aspartate transferase; ALT, Alanine Aminotransferase; CREA, Creatinine; GGT, Gamma-glutamyl transferase; BUN, Blood Urea Nitrogen; TP, Total Protein

5.5. Chemical immobilisation of Barbary sheep with ketamine-xylazine and ketamine-xylazine-medetomidine combinations

All 56 immobilisation procedures were successful. The mean actual drug doses are provided in Table 25. Both combinations provided rapid induction, adequate anaesthesia, and quick and smooth recoveries (Table 26). There was no statistically significant difference between the two combinations for the duration of ataxia, sternal recumbency, head down, complete induction, the first effect of antagonists, head-up, standing up, and complete recovery ($P > 0.05$) (Table 27). However, there was a significant difference in anaesthesia time between the two combinations ($P < 0.05$).

Table 25: Drug doses for the immobilisation of Barbary sheep.

Combinations	Drugs	Mean Doses ± SE (mg/kg)
KX	Ketamine	0.96 ± 0.04
	Xylazine	1.42 ± 0.05
Reversals	Tolazoline	4.6 ± 0.2
KXM	Ketamine	0.86 ± 0.04
	Xylazine	1.27 ± 0.04
	Medetomidine	0.03 ± 0.0001
Reversals	Tolazoline	4.0 ± 0.3
	Atipamezole	0.06 ± 0.003

Table 26: Scores for the qualities of induction, anaesthesia and recovery in Barbary sheep immobilised with KX (n = 29) and KXM (n = 27).

Chemical Restraint Category	Scores					
	Median		Inter Quartile Ranges		Mean ± SE	
	KX	KXM	KX	KXM	KX	KXM
Induction	1	1	1	1	1.3 ± 0.1	1.3 ± 0.1
Anaesthesia	1	1	0	0	1.2 ± 0.1	1.1 ± 0.1
Recovery	1	1	1	0	1.3 ± 0.1	1.0 ± 0.0

Table 27: Duration (mean ± SE) of induction, anaesthesia and recovery in Barbary sheep immobilised with KX (n = 29) and KXM (n = 27).

Variables	KX (n = 29)	KXM (n = 27)
Ataxia (minutes)	4.8 ± 0.7	5 ± 0.6
Sternal recumbency (minutes)	6.8 ± 0.8	6.6 ± 0.7
Head down (minutes)	7.2 ± 0.8	7.3 ± 0.7
Complete induction (minutes)	10.5 ± 1.2	10.2 ± 0.9
Anaesthesia maintenance (minutes)	49.7 ± 1.0	52.3 ± 0.8
First effect of antagonists (minutes)	4.3 ± 1.4	2.2 ± 0.2
Head-up (minutes)	5.1 ± 1.6	3.3 ± 0.3
Standing up (minutes)	6.3 ± 1.7	4.3 ± 0.4
Complete recovery (minutes)	7.5 ± 1.7	5.5 ± 0.4

There was no statistically significant difference between the two combinations for rectal temperature, pulse rate, respiration rate, systolic, diastolic, and mean arterial blood pressure ($P > 0.05$). However, there was a statistically significant difference between the two combinations for blood oxygen saturation percentage ($P < 0.001$) (Table 28).

Table 28: Vital signs (mean ± SE) of Barbary sheep immobilised with KX (n = 29) and KXM (n = 27).

Variables	KX (n = 29)	KXM (n = 27)
Rectal Temperature (°C)	41.5 ± 2.5	38.8 ± 0.2
Pulse rate (beats minute^{-1})	74 ± 0.5	73.2 ± 0.6
Respiration rate (breaths minute^{-1})	13.2 ± 0.1	12.9 ± 0.1
SpO_2 (%)	83.9 ± 0.4	90.0 ± 0.3
SAP (mmHg)	127.4 ± 0.2	127.4 ± 0.2
DAP (mmHg)	88.6 ± 0.2	88.3 ± 0.3
MAP (mmHg)	101.5 ± 0.2	101.3 ± 0.2

5.6. Chemical immobilisation of Beisa oryx with ketamine-xylazine and ketamine-xylazine-medetomidine combinations

All 45 immobilisation procedures were successful without any failure or complication. The mean actual drug doses are provided in Table 29. Both combinations provided rapid induction, adequate anaesthesia, and quick and smooth recoveries (Table 30). There was no statistically significant difference between the two combinations for the duration of sternal recumbency, head down, complete induction, the first effect of antagonists, head-up, standing up, and complete recovery ($P > 0.05$) (Table 31). However, there was a significant difference for ataxia and anaesthesia time between the two combinations ($P < 0.05$).

Table 29: Drug doses for the immobilisation of Beisa oryx

Combinations	Drugs	Mean Dosage ± SE (mg/kg)
KX	Ketamine	0.8 ± 0.02
	Xylazine	0.9 ± 0.02
Reversals	Tolazoline	4.3 ± 0.1
KXM	Ketamine	0.8 ± 0.02
	Xylazine	0.9 ± 0.02
	Medetomidine	0.03 ± 0.002
Reversals	Tolazoline	3.7 ± 0.2
	Atipamezole	0.07 ± 0.002

Table 30: Scores for the qualities of induction, anaesthesia and recovery in Beisa oryx immobilised with KX (n = 20) and KXM (n = 25).

Chemical restraint category	Scores					
	Median		Interquartile ranges		Mean ± SE	
	KX	KXM	KX	KXM	KX	KXM
Induction	1	1	1	1	1.3±0.1	1.4±0.1
Anaesthesia	1	1	0	0	1.2±0.1	1.2±0.1
Recovery	2	1	1	0	1.6±0.1	1.2±0.1

Table 31: Duration (mean ± SE) of induction, anaesthesia and recovery in Beisa oryx immobilised with KX (n = 20) and KXM (n = 25).

Variables	KX (n = 20)	KXM (n = 25)
Ataxia (minutes)	3.0 ± 0.4	5.1 ± 0.8
Sternal recumbency (minutes)	6.1 ± 0.6	7.7 ± 1.0
Head down (minutes)	7.7 ± 0.6	8.8 ± 1.1
Complete induction (minutes)	10.3 ± 0.7	11.2 ± 1.2
Anaesthesia maintenance (minutes)	51.0 ± 0.7	56.2 ± 0.5
First effect of antagonists (minutes)	2.2 ± 0.2	2.1 ± 0.2
Head-up (minutes)	2.9 ± 0	3.3 ± 0.2
Standing up (minutes)	4.4 ± 0.6	5 ± 0.3
Complete recovery (minutes)	5.9 ± 0.6	6.1 ± 0.5

There was no statistically significant difference between the two combinations for respiration rate and blood haemoglobin saturation percentage ($P > 0.05$). However, there was a statistically significant difference between the two combinations for rectal temperature, pulse rate, systolic, diastolic, and mean arterial blood pressure ($P < 0.05$) (Table 32).

Table 32: Vital signs (mean ± SE) of Beisa oryx immobilised with KX (n = 20) and KXM (n = 25).

Variables	KX (n = 20)	KXM (n = 25)
Rectal Temperature (°C)	39.5 ± 0.2	38.6 ± 0.1
Pulse rate (beats minute^{-1})	36.2 ± 0.8	41.0 ± 1.7
Respiration rate (breaths minute^{-1})	23.7 ± 1.3	22.9 ± 0.7
SpO_2 (%)	94.3 ± 0.8	94.6 ± 0.2
SAP (mmHg)	125.5 ± 3.3	137.4 ± 1.1
DAP (mmHg)	78.6 ± 2.3	87.1 ± 0.7
MAP (mmHg)	94.3 ± 2.6	103.9 ± 0.8

6. DISCUSSION

In the current study, the field immobilisation of two carnivores and three ungulate species was recorded. Various combinations and their effects on the physiological parameters were assessed. The chemical restraint of Arabian striped hyaena with KM and KX combinations was compared for the first time. Moreover, we reported the arterial blood gas and acid-base status before and after oxygen supplementation in Arabian striped hyaena and Northeast African cheetah for the first time. Two combinations i.e. TKX and TKM, were compared in Patterson's eland in the current study. Additionally, we compared two combinations i.e. KX and KXM in Barbary sheep and Beisa oryx. All combinations provided rapid induction, adequate anaesthesia, and smooth recoveries. The depth of anaesthesia was adequate to handle the animals, perform minor procedures such as vaccination, sample collection, crating, taking body measurements, and microchipping.

When immobilising a wild animal, quick induction is one of the desired results from a given combination of anaesthetic drugs. If induction is prolonged, it can result in undue stress, injuries, loss of animals in the habitat such as running into thick forest cover, hiding, entering a burrow, inter and intraspecific aggression and compromised the safety of personnel conducting immobilisation procedures. Rapid induction of wild animals enables monitoring of animals, lessening of injuries, trauma, hyperthermia, the risk to humans, and reduction in chances of animal escape or loss. Species such as carnivores and large ungulates can both pose a threat to themselves or personnel if rapid induction is not achieved (Pappas 2002; Burroughs et al. 2012; Hahn et al. 2014; Buijs et al. 2016; Barros et al. 2018).

Ketamine is one of the drugs widely used in many wild species. It is known as a general anaesthetic and analgesic, and it does not cause cardiopulmonary depression. It is an *N*-Methyl-D-aspartate antagonist. Ketamine causes anaesthetic and amnestic effects by inhibiting thalamocortical communication via interneurons (Ward et al. 2006). It results in loss of consciousness, analgesia, and muscular hypertonicity, and maintains cardiac output indirectly with negligible effects on respiratory function at regular doses. However, it causes high excitatory state, inadequate muscle relaxation and excessive salivation when used solely. It requires higher doses of ketamine to attain required

anaesthesia and analgesia when not used in combination with other drugs. It is not recommended as a sole agent in wild animals (Ward et al. 2006).

When ketamine is combined with medetomidine, the effects of medetomidine are improved, providing sedation that otherwise requires higher doses of medetomidine to achieve desired results (Jalanka & Roeken 1990). Medetomidine is more effective with higher potency than xylazine, as it produces more prolonged sedation and analgesia, has reduced emetic effects by suppressing norepinephrine in the central nervous system (Paddleford & Harvey 1999). When pharmacokinetics of medetomidine are compared, it has higher lipophilic characteristics and is rapidly absorbed and distributed (Jalanka & Roeken 1990).

In the current study on Arabian striped hyaena, up to 55 %, less dose of ketamine was required in KM combination as compared to KX combination (2.27 ± 0.04 mg/kg and 4.95 ± 0.11 mg/kg for KM and KX, respectively) as well as documented dose (2.5-3.0 mg/kg) for carnivores (Jalanka & Roeken 1990; Mehmood et al. 2019). Moreover, a lower dose of ketamine (1.5 ± 0.1 mg/kg) was required in the current study on immobilisation of cheetah with KM compared to the dose for carnivores. These results suggest that medetomidine enhanced the efficacy of ketamine more as compared to xylazine due to its selectivity and high affinity to adrenoceptors (Sinclair 2003). The resulting lower dose of ketamine improved the quality of immobilisation by both reducing the side effects associated with a high dose of ketamine as well as improving the quality of reversal and recovery as ketamine does not have an antagonist (Jalanka & Roeken 1990). A similar reduction in the dose of ketamine was reported in snow leopards with approximately 25 % reduction in the ketamine dose (Jalanka & Roeken 1990). In our study on Barbary sheep, less dose of ketamine was required in KXM (0.96 ± 0.04) as compared to (0.86 ± 0.04). However, in the chemical restraint of Beisa oryx, the same dose of ketamine was required in both KX and KXM combinations.

In the current study on hyaena, KM produced adequate anaesthesia and recovery as compared to KX. Both KX and KM provided rapid induction without any stereotypic reactions, loss of balance, and ptyalism. There was one case of vomiting in hyaena that was immobilised with KX (Annex 9.1). Vomit could be associated with the emetic nature of xylazine in hyaenas and other carnivores (Bufalari et al. 2007; Hahn et al. 2014).

Similarly, other studies on cougars, otters, lions, donkeys, and marmosets reported good quality scores when these species were sedated with ketamine in combination with medetomidine or other α-2 adrenoceptors such as xylazine (Selmi et al. 2004; Soto-Azat et al. 2004; Wenger et al. 2010; Bakker et al. 2013; Lescano et al. 2014; Maney et al. 2018).

KM combinations in cheetah and hyaena provided higher scores compared to KX for the quality and depth of anaesthesia without and body movement, jerks, pedal or palpebral reflexes, muscle tone, and response to external stimulus. It could be associated with medetomidine that produces sedative and analgesic effects, and good myorelaxation in carnivores (Jalanka & Roeken 1990). The KX combination in our study on hyaena produced adequate anaesthesia depth with occasional reflexes to external stimulus and ear twitching. These occasional body movements can be associated with the presence of ketamine when combined with xylazine (Mulder 1978; Ward et al. 2006; Mehmood et al. 2019). The mean anaesthesia duration in both carnivore species studied was higher compared to previously reported studies (Jalanka & Roeken 1990). However, dexmedetomidine in combination with ketamine, was reported to produce higher quality and duration of anaesthesia as compared to KM in Golden-headed tamarinds (Selmi et al. 2004). In our study on Barbary sheep and Beisa oryx, both KX and KXM combinations provided excellent to good quality scores for induction, anaesthesia, and recovery phases. However, the addition of medetomidine to KX improved the quality scores in KXM for anaesthesia in Barbary sheep.

We immobilised Patterson's eland with TKX and TKM to compare for a most suitable combination among the two. Eland is difficult to sedate and usually requires high doses of immobilisation drugs (Burroughs et al. 2012). However, in the current study on Patterson's eland, we required a lower dose of thiafentanil as compared to previous literature (Annex 9.4) (Burroughs et al. 2012). Thiafentanil may have been potentiated due to the addition of ketamine and α-2 adrenergic agonists (xylazine or medetomidine) (Pérez 2013). Both combinations (TKX and TKM) provided quick ataxia and inductions in the eland, which can be associated with the action of thiafentanil, known to provide rapid induction when compared to other derivatives of morphine. Moreover, the induction was rapid in TKM, which may again be associated with the lipophilic properties of medetomidine (Burroughs et al. 2012). The duration of induction with TKM was longer

in the current studies as compared to the previously reported duration of induction with TKM in axis deer. However, the induction was smooth without any complication in eland compared to the axis deer (Smith et al. 2006).

Rapid and smooth recoveries are essential during the chemical restraint of wild animals as recovering animals are not able to defend themselves and are susceptible to injuries or mortalities inflicted by conspecifics (Rockhill et al. 2011). Jalanka & Roeken (1990) reported ataxic recoveries associated with higher doses of ketamine in KX and KM combinations in carnivores, suggesting residual motor impairment. In our studies on both hyaena and cheetah with KM, the recoveries were quick and smooth. However, the immobilisation of hyaena with KX provided comparatively slower recoveries with delayed retraction of tongue, weak coordination, and ptyalism. Reversals with atipamezole were more rapid and smoother as compared to yohimbine (Mehmood et al. 2019). It could be due to the higher high alpha-2/alpha-1selectivity ratio of 8526 of atipamezole as compared to 40 of yohimbine (Jalanka & Roeken 1990; Mehmood et al. 2019). Additionally, yohimbine partially antagonises xylazine that may lead to slower recoveries (Paddleford & Harvey 1999). However, both combinations provided shorter recovery times when compared to nine minutes in snow leopards (Jalanka & Roeken 1990).

Similarly, the reversals of KX and KXM provided quick recoveries in Barbary sheep and Beisa oryx. In Barbary sheep KMX provided quicker and smoother recoveries (Tables 25 and 26). In Beisa oryx, the recoveries were smoother with KXM while they were marginally quicker with KX (Tables 29 and 30). The smoother recoveries in KXM can be attributed to the addition of atipamezole to tolazoline. Moreover, similar trends were observed in the eland with TKX and TKM combinations. Where TKM provided quicker and smoother recoveries due to atipamezole as compared to reversal with yohimbine in TKX. Moreover, no re-sedation occurred after the administration of the reversals, as naltrexone inhibits re-narcotisation (West et al. 2007).

The α-2 adrenoceptors are reported to cause the loss of thermoregulatory control that may result in hyperthermia or hypothermia (Jalanka & Roeken 1990; Fernandez-Moran et al. 2001a). The pattern of rectal temperatures at five-minute intervals in Arabian striped hyaena reveal that the temperatures slightly decreased in KM and increase in KX overtime

(Mehmood et al. 2019). Although the temperature in both combinations remained within the reference range (Hahn et al. 2014; Sahu et al. 2018). Similarly, the rectal temperature was significantly lower (but within the reference range) in our study on hyaena immobilised with KM, at later stages of anaesthesia (before and after oxygen supplementation). That could be attributed to the hypothermic effects of medetomidine (Jalanka & Roeken 1990; Fernandez-Moran et al. 2001a). However, the rectal temperatures (38.5 °C) for cheetahs immobilised with KM in the current study were marginally above the temperature range (38.0 ± 0.4 °C – 38.1 ± 0.8 °C) previously reported for cheetah with KM combination (Stagegaard et al. 2017).

Giant eland is reported to be vulnerable to hyperthermia when chemically immobilised (Pye et al. 2001). The observed rectal temperatures in the current study on the immobilisation of Patterson's eland with TKX and TKM were within the reference range (37.6 °C to 42.2 °C) for eland. It can be attributed to the reduced times to achieve ataxia and induction due to the presence of thiafentanil, that is known to produce quick induction. The rectal temperatures were higher in both Barbary sheep and Beisa oryx, immobilised with KX as compared to KXM, in the current study. The addition of medetomidine may have contributed towards a decrease in rectal temperatures in both species. When using α-2 adrenoceptors, caution shall be taken to dart the animals at cooler times of the day e.g. mornings and evenings, especially in the hot climatic areas, such as the UAE. Moreover, the animals shall not be subjected to undue physical stress and prolonged chasing, as these may contribute towards the development of hyperthermia (Jalanka & Roeken 1990).

The α-2 adrenoceptors are known to cause bradycardia, a reduced heart rate than the normal (Jalanka & Roeken 1990; Fernandez-Moran et al. 2001b; Wenger et al. 2010). When ketamine is used in combination with α-2 adrenoceptors, especially xylazine, it reduced the bradycardic effects of xylazine through cardiotonic action reconciled by vagolytic action (Bharathidasan et al. 2014; Mehmood et al. 2019).

In the current study on Arabian striped hyaena, the heart rates were lower in KM combination as compared to the KX. However, their heart rates were within the reported range for hyaena in both combinations. Moreover, the temperature slightly decreased at later stages of anaesthesia in both combinations (Mehmood et al. 2019). Moreover,

medetomidine is also known to cause bradycardia in felines (West et al. 2007). It is reported that cheetahs exhibit bradycardia when they are chemically sedated. Although no complication related to bradycardia was reported in these studies. The heart rate in the current study on cheetah in similar conditions was within the reported ranges (59 – 69 beats/minute) (Stegmann & Jago 2006; Stagegaard et al. 2017).

In the current study on eland, the heart rates were marginally lower in TKM as compared to TKX. Although these were within the reported heart rates (50-70 beats per minutes) for eland (Pye et al. 2001). Impala, when chemically restrained with the combination of thiafentanil and medetomidine also exhibited similar trends of heart rate (Meyer et al. 2008). Similarly, Barbary sheep immobilised with KXM showed lower heart rates compared to KX combination that could be attributed to the bradycardic effects of medetomidine (Jalanka & Roeken 1990; Fernandez-Moran et al. 2001b; Wenger et al. 2010). However, the heart rates of Beisa oryx showed significantly higher heart rates in KXM (41.0 ± 1.7 beats/minute) as compared to KX combination (36.2 ± 0.8). We could not attribute any reason for this variation in the Beisa oryx.

Medetomidine is known to cause hypotension, with an initial increase in blood pressure followed by hypotension or normotension (Jalanka & Roeken 1990). In our studies on hyaena similar pattern was observed. The MAP decreased before and after oxygen supplementation in hyaena immobilised with KM relating to the effects of medetomidine. Moreover, in cheetah immobilised in the current study with KM, the MAP was lower compared to the previously reported MAP (Stegmann & Jago 2006). However, the MAP increased at later stages of anaesthesia (after oxygen supplementation) and we could not attribute any reason to the cause of that increase. Similarly, the MAP was lower in TKM as compared to TKX in the eland. The current study on Barbary sheep also suggested lower MAP in KXM compared to KX. However, in the Beisa oryx, the reverse effect was observed as KXM had higher MAP compared to KX.

Anaesthetic and analgesic drugs such as cyclohexylamines (e.g. ketamine), α-2 adrenoceptors (e.g. medetomidine and xylazine), and opioids (e.g. thiafentanil) are known to cause respiratory depression. Ketamine causes respiratory depression by affecting the muscarinic-nicotinic cholinergic activity of the nervous system (Jalanka & Roeken 1990). The respiratory rates were significantly lower in KX combination as compared to KM in

Arabian striped hyaena (Mehmood et al. 2019). Moreover, the respiration pattern was more stable with KM. However, there was no significant difference in the respiration rates in hyaena and cheetah before and after oxygen supplementation. There was no significant difference between KX and KXM combinations in Barbary sheep and Beisa oryx. However, in eland, the respiration rates were significantly lower in TKM combination as compared to TKX combination. Ketamine is reported to ameliorate the respiratory depression of medetomidine (Burroughs 1993; Fernandez-Moran et al. 2001b). There are reports of respiratory depression caused by medetomidine in dogs through the activation of the α-2 receptor in the locus coeruleus (Lerche & Muir 2004).

Hypoxaemia, reduced oxygen concentration in blood, is a serious complication in wildlife chemical immobilisation practices and the animal are more susceptible when they are breathing room air (West et al. 2007). The α-2 adrenergic agonists are known to depress the respiratory centres and sensitivity to carbon dioxide in many wildlife species. Xylazine has shown central hypoxaemia due to pulmonary alterations (Celly et al. 1997). Opioids also contribute to the respiratory depression by affecting the ability of the animals to regulate gaseous exchange and cardiac output that may lead to hypoxaemia (Lian et al. 2016). In the current study, Beisa oryx immobilised with KX and KXM did not show a significant difference in SpO_2 percentage and no hypoxaemia was recorded. However, hypoxaemia was recorded in Barbary sheep immobilised with KX combination as compared to KXM. Moreover, no hypoxaemia was recorded in eland immobilised with TKX and TKM combinations (Annex 9.4).

The SpO_2 percentage suggested hypoxaemia with KX and KM in hyaena, and with KM in cheetah. The α-2 adrenoceptors agonists are known to cause an oxygen depression and increase in CO_2 followed by subsequent compensation in the felids (Jalanka & Roeken 1990). Hypoxaemia can be a severe complication and may become life-threatening if it is not compensated or treated accordingly. Oxygen supplementation can compensate for hypoxaemia. However, it is incredibly essential to monitor its efficacy (Annex 9.2). A reliable tool to monitor arterial blood gas and acid-base status is arterial blood gas analysis that is widely used in the discipline of human medicine (Hennessey & Japp 2016).

In our study on the efficacy of oxygen supplementation in KM combination for hyaena and cheetah suggested that oxygen supplementation compensated hypoxaemia (Annex 9.2 and 9.3). There was a significant difference in both current studies between SaO_2 and PaO_2 before and after oxygen supplementation. Moreover, higher $PaCO_2$ and TCO_2 indicated hypoventilation that contributes to hypercarbia (West et al. 2007). Anaesthetic drugs suppress the sensory receptors responsible for detecting carbon dioxide and thus leading to the retention of carbon dioxide (Deem et al. 1998). There was a decrease in pH before and after oxygen supplementation in both studies. This decrease in pH corresponding to the increase in $PaCO_2$, suggesting respiratory acidosis. Moreover, a significant increase in the bicarbonates can be attributed to a reaction to an increase in $PaCO_2$ (Hennessey & Japp 2016). Although oxygen supplementation corrected hypoxemia, pH levels did not return to normal. In clinical conditions, pH does not usually return to normal range and complete compensation does not occur (Sood et al., 2010).

In the current study on Arabian striped hyaena, the cortisol levels were significantly higher in females (Mehmood et al. 2019). In spotted hyaena, the cortisol levels in adult or dominant females are usually higher than males and female cubs (Van Jaarsveld & Skinner 1992). The mean cortisol level for both sexes was higher compared to the reported values for spotted hyaena (87.96±74.03 ng/ml and 101.83±71.79 ng/ml for males and females respectively). It suggests a response to immobilisation stress during the initial phases of the immobilisation. Due to unavailability of samples at the later stages of immobilisation, it was difficult to assess whether the initial stress to capture was suppressed or not (Van Jaarsveld & Skinner 1992; Sheriff et al. 2011). The study on eland suggests that there was a significant difference in both combinations for cortisol levels; suggesting TKM had lower cortisol concentrations compared to TKX. The α-2 adrenergic agonists, especially medetomidine, are known to obtund the stress response via inhibition of the adrenomedullary, sympathoadrenal, and nociceptive functions (Benson et al. 2000).

Although, medetomidine is known for its adrenolytic properties where PCV decreases due to pooling of RBCs in the spleen (Jalanka & Roeken 1990); the erythrocytes, PCV and haematocrit values were within the prescribed range for striped hyaena in the current study. α2-adrenergic insulin inhibition in the beta cells of the pancreas and higher production of glucose in the liver frequently causes hyperglycaemia with medetomidine (Mehmood et al. 2019). Moreover, in tigers higher dosage of xylazine caused

hyperglycaemia (Jalanka & Roeken 1990). No hyperglycaemia was observed in our studies. The clinical analysis of blood also showed that there was no chronic kidney or liver disorder as ketamine is not recommended for renal or hepatic dysfunctional patients (Jalanka & Roeken 1990).

The limitations of the study included lack of data on samples for cortisol at later stages of immobilisation, low number of animals in cheetah studies and unavailability of invasive blood pressure (IBP) that could have provided reliable blood pressure observations.

7. CONCLUSIONS

This research aimed to assess how various anaesthetic drugs affect the cardio-respiratory, thermal, acid-base, and blood gas variables in carnivores (i.e. Arabian striped hyaena, Northeast African cheetah) and ungulates (i.e. Patterson's eland, Barbary sheep, and Beisa oryx) suggested that KM combination was more suitable for the immobilisation of Arabian striped hyaena and Northeast African cheetah, as it provided better quality and timings of induction, anaesthesia and recovery as compared to KX. However, both species immobilised with KM developed hypoxemia and respiratory acidosis. The results of this study showed that oxygen supplementation adequately treated hypoxemia, and a compensatory response was observed with an increase in the bicarbonate levels. Therefore, oxygen supplementation is recommended during the immobilisation of Arabian striped hyena and Northeast African cheetah in field conditions.

Moreover, the immobilisation of Patterson's eland with TKX and TKM revealed that both combinations provided rapid and reliable immobilisation adequate to permit short minimally invasive procedures in eland. Although, there was a mild hypoxaemia observed in TKM combination as compared to TKX, although the values were within prescribed range for the elands. Additionally, KXM was found to be a suitable combination in Barbary sheep as compared to KX, by providing rapid induction, adequate anaesthesia, and quick and smooth recoveries. Whereas, KX in Beisa oryx was found suitable combination as it provided quicker ataxia and induction times.

The limitations of the studies include a few numbers of individuals of cheetah, few ABG analysis for each immobilisation. Furthermore, invasive blood pressure could have

provided more reliable BP measurements. To better understand the implications of KXM and KX combinations on Barbary sheep and Beisa oryx, further studies are suggested to explore acid-base and blood gas status of animals during immobilisation with these combinations.

The findings of the current study reported KM for the first time as a suitable combination in Arabian striped hyaena. Moreover, the results provide valuable insight into the effects of various combinations on several critical physiological parameters. These findings will help practitioners to take necessary precautions and will provide a baseline for the evaluation of immobilisation in studied species.

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9. ANNEXES

9.1. Comparison of physiological responses of Arabian striped hyaena (*Hyaena hyaena sultana*) to effective immobilisations with ketamine-medetomidine and ketamine-xylazine in (semi-) captive conditions.

Citation: Mehmood A, Abid S, Hejcmanová P, Asadi MA, Kabeer B, Jilani MJ, Bilal S, Ashraf MW. 2019. Comparison of physiological responses of Arabian striped hyaena (*Hyaena hyaena sultana*) to effective immobilisations with ketamine-medetomidine and ketamine-xylazine in (semi-) captive conditions. PeerJ 7:20.

9.2. Arterial blood gas and acid-base status, before and after oxygen supplementation, in Arabian striped hyena (*Hyaena hyaena sultana*) during field immobilisation.

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9.3. Effects of oxygen supplementation on blood gas variables in Northeast African cheetah (*Acinonyx jubatus soemmeringii*) immobilised with ketamine and medetomidine.

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9.4. Thermal and cardiopulmonary responses of Patterson's eland (*Taurotragus oryx pattersonianus*) to the drug combinations of thiafentanil-ketamine with medetomidine/xylazine.

Citation: Mehmood A, Hejcmanová P. 2020. Thermal and cardiopulmonary responses of Patterson's eland (*Taurotragus oryx pattersonianus*) to the drug combinations of thiafentanil-ketamine with medetomidine/xylazine. Veterinary Anaesthesia and Analgesia (re-submitted after revision).

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Comparison of physiological responses of Arabian striped hyaena (*Hyaena hyaena sultana*) to effective immobilisations with ketamine-medetomidine and ketamine-xylazine in (semi-) captive conditions

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ABSTRACT

Chemical immobilisation is an integral component for the conservation of wild animals and can be stressful if proper protocols are not administered. References on the immobilisation of Arabian striped hyaena (*Hyaena hyaena sultana*) are scarce. The current study was designed to evaluate the physiological and clinical responses of Arabian striped hyaena, immobilised with ketamine-medetomidine (KM) and ketamine-xylazine (KX); and to compare immobilisation effectiveness of the two combinations in a cross-sectional clinical study. A total of 15 (six males, nine females) (semi-) captive and adult Arabian striped hyaena with an average weight of 31.39 ± 0.36 kg were immobilised 50 times for annual vaccination and translocation purposes from January 2014 till March 2018 on Sir Bani Yas Island, United Arab Emirates. A total of 34 immobilisations were executed with (Mean ± SE) 2.27 ± 0.044 mg/kg ketamine and 0.04 ± 0.001 mg/kg medetomidine; while 16 with 4.95 ± 0.115 mg/kg ketamine and 0.99 ± 0.023 mg/kg xylazine. The drugs were remotely delivered intramuscular. The evaluation of physiological and clinical parameters included monitoring of vital signs through pulse oximetry, blood gas analysis of arterial blood through Istat blood gas analyser, and blood biochemistry and haematology. The quality of induction, anaesthesia and recovery was also assessed. Atipamezole (0.21 ± 0.003 mg/kg) was used to antagonise the effects of KM and 0.09 ± 0.003 mg/kg atipamezole or by 0.23 ± 0.006 mg/kg yohimbine for KX. Data were analysed using the general linear model and inferential statistics. KM was more effective in induction (scores; KM = 1.41 ± 0.10; KX = 1.31 ± 0.12), anaesthesia (KM = 1.00 ± 0.00; KX = 2.0 ± 0.0) and recovery (KM = 1.76 ± 0.15; KX = 2.69 ± 0.12) phases as compared to KX. There was a significant difference ($P < 0.05$) amongst the two combinations for anaesthesia time (KM = 59.5 ± 2.41; KX = 49.25 ± 1.31 min.), time to stand after reversal (KM = 4.91 ± 0.60; KX = 10.38 ± 1.48 min.) and full loss of the signs of anaesthetics (KM = 12.32 ± 1.37; KX = 21.25 ± 2.16 min.) along

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with rectal temperature (KM = 37.58 ± 0.29; KX = 36.00 ± 0.68 °C), pulse rate (KM = 50.46 ± 1.90; KX = 61.14 ± 2.79 beats/min), respiration rate (KM = 29.44 ± 0.99; KX = 23.80 ± 1.57 breaths/min.) and partial pressure of oxygen (KM = 89.59 ± 1.34; KX = 82.06 ± 3.92%). The blood oxygen saturation by oximeter indicated hypoxaemia in KX (82.06 ± 3.92), supported by the data from blood gas analyser. KM combination was more suitable for the immobilisation of Arabian striped hyaena, providing a better quality of induction, anaesthesia and recovery compared to KX. However, we strongly suggest further investigation to see the effects of oxygen supplementation for the compensation of hypoxaemia.

Subjects Conservation Biology, Veterinary Medicine, Zoology
Keywords Blood gas analysis, Immobilisation, Vital signs, Induction, Anaesthesia, Carnivores, Hyaena

INTRODUCTION

The Arabian striped hyaena (*Hyaena hyaena sultana*) is the most critical large scavenger found in the tropical grassland and woodland ecosystems (*Kruuk, 1976*). The striped hyena is a carnivore with a broad head, long ears and somewhat pointed muzzle. The body slopes down from head to tail bearing black stripes on pale or pale grey underfur (*Rosevear, 1974*). Hyaenas resemble dogs in their appearance, but the skull, teeth and other anatomical resemblances bring them closer to felines. Therefore, they are placed in separate family Hyaenidae in the suborder Feliformia (cats and cat-like carnivores) and are closely related to domestic cats than dogs (*Prater, 1971*; *Wilson & Mittermeier, 2009*; *Hahn et al., 2014*). *H. hyaena* is categorised as Near Threatened in the Red List of Threatened Species by IUCN (*AbiSaid & Dloniak, 2015*). *H. h. sultana* is distributed from Oman, Saudi Arabia, and the United Arab Emirates (UAE) until Yemen; in the UAE, it was last sighted in the wild in 1996 (*Hellyer & Aspinall, 2005*).

Chemical immobilisation and anaesthesia is an integral component of conservation, diagnostic and surgical procedures in wild animal species (*Wack, 2003*). Animal capture and handling are one of the most stress-inducing events for wild animals. The effects of stress on the blood haematology and biochemistry of the animal are directly related to the capture technique employed and are much reduced in chemical capture as compared to the physical capture (*Marco & Lavin, 1999*).

Ketamine hydrochloride has been widely used for the immobilisation of wild carnivores. It is used as an anaesthetic in combination with other drugs and has an eclectic range of benefits such as broader safety margin, low cost, high rate of absorption, lower cardiovascular and respiratory effects, and its international accessibility (*Burroughs, 1993*). However, ketamine has some disadvantages such as the requirement of a higher dose, excessive salivation, muscular hypertonicity and contractions with poor muscle relaxation, damage to retina and mydriasis, occasionally a transient and minor respiratory depression, rough inductions and recoveries, and non-availability of the antagonist (*Adams, 2001*). As the pharyngeal and laryngeal reflexes are not affected by ketamine, excessive salivation

is not a threatening condition. However, if deemed necessary to avoid the possibility of aspiration pneumonia, salivary hypersecretion can be controlled by administration of atropine sulphate (*Heinz et al., 2006*; *Thomas, 2013*).

Ketamine combinations with α2-adrenergic agonists such as medetomidine or xylazine can reduce the dosage of ketamine, suppress its side effects and provide anxiolysis and muscle relaxation (*Knight, 1980*; *Adams, 2001*; *Sinclair, 2003*). Xylazine is an α2-adrenergic agonist that depresses the central nervous system, induces sedation and has a myorelaxation effect. The side effects of xylazine include regurgitation especially in carnivores, reduced blood pressure, heart rate and rectal temperature, excessive salivation, and may cause abortion if the female animal is pregnant (*Knight, 1980*; *Shindle & Tewes, 2000*; *Rockhill et al., 2011*). Medetomidine is a highly specific α2-adrenergic agonist that has ten times more affinity and 200 times more selectivity for adrenoceptors and induces elongated duration of analgesia and anaesthesia than xylazine (*Sinclair, 2003*). The undesired effects of medetomidine include hypertension frequently followed by peripheral hypotension, hypothermia, respiratory depression and bradycardia (*Jalanka & Roeken, 1990*). The effects of medetomidine and xylazine can be reversed using potent and selective α2-adrenoceptor antagonist such as atipamezole hydrochloride. The effects of xylazine can also be reversed using yohimbine hydrochloride (*Jalanka & Roeken, 1990*; *Hahn et al., 2014*).

Spotted hyaena have been immobilised successfully with ketamine at a dosage rate of 7–15 mg/kg (*Smuts, 1973*), xylazine one mg/kg with one mg/kg phencyclidine (*Young & Whyte, 1973*), zoletil four mg/kg (*Van Jaarsveld & Skinner, 1991*), xylazine 10.7 ± 1.9 mg/kg with ketamine 0.5 ± 0.1 mg/kg (*Van Jaarsveld, McKenzie & Meltzer, 1984*) and ketamine four to six mg/kg with xylazine one mg/kg (*Hahn et al., 2014*) in numerous studies. However, the references on the immobilisation of Arabian striped hyaena are scarce. In the present study, we investigated the two combinations of ketamine with xylazine and medetomidine and assessed the quality of induction, anaesthesia, and recovery stages along with the effects of these drugs on the physiological and clinical parameters through monitoring of vital signs, blood haematology, biochemistry, blood gas analysis and behavioural response of the animals to immobilisation.

MATERIALS AND METHODS

Study area

The study was conducted in Sir Bani Yas Island, which is 250 km West of Abu Dhabi and is 87 km^2 (8,700 hectares) in size. The entire island is protected; an Arabian Wildlife Park consisting of 4,100 ha which was developed in 2009. Roughly over 16,000 individuals belonging to various species inhabit the island, with 2.5 million planted trees providing a suitable habitat to the animals (*Dhaheri et al., 2017*).

Study animals

A total of 15 (six males and nine females) (semi-) captive adult *H. h. sultana*, with an average weight and standard error of 31.39 ± 0.36 kilograms, were managed under conservation breeding project at the island. A total of 50 immobilisations

Table 1 Details of investigated animals and reasons for immobilisation of *Hyaena hyaena sultana* immobilised with Ketamine-Medetomidine and Ketamine-Xylazine combinations in (semi-) captive conditions.

Animal ID	Sex	Age	Average weight (kg) ± SE	Number of immobilisations	Health condition/reasons for immobilisation (*n*)
Aramis	Male	Adult	34.50 ± 3.37	3	HV
Arnold	Male	Adult	36.70 ± 3.20	5	TL (1); HV (4)
Athos	Male	Adult	30.85 ± 0.73	3	HV
Bravo	Male	Adult	31.10 ± 1.63	5	TL (1); HV (4)
Buwaytir	Male	Adult	29.85 ± 1.23	3	TL (1); HV (2)
Porthos	Male	Adult	30.17 ± 1.83	3	TL (1); HV (2)
Alpha	Female	Adult	28.83 ± 1.43	3	TL (2); HV (1)
Chips	Female	Adult	31.13 ± 1.01	4	TL (1); HV (3)
D'Artagnan	Female	Adult	30.70 ± 0.89	5	TL (1); HV (4)
Dopey	Female	Adult	30.83 ± 1.48	3	HV (3)
Gadget	Female	Adult	30.57 ± 1.43	3	HV
Luna	Female	Adult	32.43 ± 0.57	2	HV
Phiri	Female	Adult	29.65 ± 1.22	5	TL (2); HV (3)
Sirius	Female	Adult	34.00 ± 0.00	1	HV
Starsky	Female	Adult	31.25 ± 0.75	2	TL

Note:
TL, translocations; HV, health check and vaccination.

(ketamine-medetomidine (KM): $n = 34$ and ketamine-xylazine (KX): $n = 16$) were performed on these 15 animals as part of routine animal management and veterinary interventions for vaccinations (necessary protocol for the whole collection) and translocations. Additionally, 18 immobilisations were performed for treatments from January 2014 till March 2018 on Sir Bani Yas Island, UAE (Table 1). The animals were free roaming in 4,100 ha park or were housed in enclosures of an average size of 0.57 ± 0.16 ha (mean ± SE). The animals and protocols (KM or KX) were randomly selected by using simple random sampling (*Suresh, Suresh & Thomas, 2011*). However, due to a shortage of xylazine during the study, more immobilisations had to be performed with KM combination. The 18 immobilisations of injured animals with KM combination, rated as ASAPS Class 2E according to American Society of Anaesthesiologists (*Doyle & Garmon, 2018*), were excluded from the analysis due to biased numbers of patients, as a single patient (out of four patients) received 12 out of 18 treatments and pre-anaesthesia excitement and trauma affecting the quality of induction, anaesthesia and recovery phases. All the immobilisations were performed according to the highest animal care and management standards and institutional animal health care and management plan (BFM-WL-CI/0017-2014). No patient was explicitly selected for study purposes; therefore, ethical committee approval was not required.

Chemical restraint

The animals were darted either from a vehicle or a hiding trailer depending on the conditions, ensuring that the animal was not excited prior to the drug delivery. Due to the high temperatures in the region, all the immobilisations were either early morning or late

Table 2 Dosage of drug agonists and antagonists in two combinations for the immobilisation of *Hyaena hyaena sultana* immobilised with Ketamine-Medetomidine and Ketamine-Xylazine combinations in (semi-) captive conditions.

Combinations	Drugs	Concentration (mg/ml)	Mean dosage ± SE (mg/kg)	Standard dose (ml)
Ketamine + Medetomidine (KM)	Ketamine	100	2.27 ± 0.044	0.7
	Medetomidine	1	0.04 ± 0.001	1.3
	Atipamezole (Reversal)	5	0.21 ± 0.003	1.3
Ketamine + Xylazine (KX)	Ketamine	100	4.95 ± 0.115	1.5
	Xylazine	100	0.99 ± 0.023	0.3
	Atipamezole (Reversal)	5	0.09 ± 0.003	0.6
	(or) Yohimbine (Reversal)	10	0.23 ± 0.006	0.7

evening. The ideal darting site was hindquarters. However, depending on the orientation of the animal, the neck area was also found suitable (*Hahn et al., 2014*). The intramuscular (IM) drug injection was executed through Dan-Inject CO_2 Injection Rifle (No. 0471, Model JM) using a dart syringe of three ml with a plain (1.5 × 20 mm) Dan-Inject needle. To avoid data biasness, it was made sure that all injections were completely delivered IM so that a second dart or supplementary dosage was not required. It was made sure that the animals were not near their burrows while roaming in 4,100 ha area and the burrows were closed in the smaller enclosures to halt the entry of drugged animals into the burrows. In the two combinations, the drugs used were ketamine hydrochloride (Ketamil Injection), medetomidine hydrochloride (Ilium Medetomidine Injection), and xylazine hydrochloride (Ilium Xylazil—100) from Troy Laboratories, Australia. The target dosage in KM was 2.33 mg/kg and 0.04 mg/kg, while five mg/kg and one mg/kg for KX calculated from literature for hyaena species (*Kock & Burroughs, 2012*; *Hahn et al., 2014*).
The drug dosages were calculated by visual weight estimation and previous weighing records of the animals according to Table 2, whereas the actual weight was measured using a portable weighing scale and actual administered drug dose was calculated (*Fournier et al., 1995*; *Lescano et al., 2014*). Once the procedure was finished, the effects of α2-adrenergic agonist were reversed using atipamezole hydrochloride (Ilium Atipamezole Injection; Troy Laboratories, Glendenning, NSW, Australia), or yohimbine hydrochloride (Reverzine™ Injection; Bayer, Pymble, NSW, Australia). Xylazine is usually reversed with yohimbine, but it was not available during some of the immobilisations, and we had to work with the opportunity in line with institutional and veterinary requirements at those times. Therefore, partial reversals of KX combination were with atipamezole and partial with yohimbine. The observers recording vital signs, quality of anaesthesia, and taking and processing blood samples were double-blinded to avoid biases.

Duration and quality of the anaesthesia

The time was recorded from the delivery of dart until induction and later anaesthesia and recovery. After the induction animal was approached silently and was checked for the state of anaesthesia by touching it through a bamboo stick. If there was no response, the animal was placed in a lateral position as they have a simple stomach, and in the lateral

Table 3 Scoring Table for Induction, Immobilisation Quality and Recovery for immobilisation of *Hyaena hyaena sultana* immobilised with KM and KX combinations (modified from *Lescano et al., 2014*).

Score	Quality	Induction	Anaesthesia	Recovery
1	Excellent	Quick and smooth induction; absence of uncoordinated movement, stereotypic reaction, ptyalism, vomiting, and discomfort at the injection site	The absence of body movement, pedal and palpebral reflexes, muscle tone, response to an external stimulus	Quick and smooth recovery; absence of uncoordinated movement, ptyalism, vomiting, quick retraction of the tongue
2	Good	Quick induction but resistance to loss of balance, slight ptyalism, pacing, and licking	Ear twitching, no muscle tone, occasional (<3 times) pedal and palpebral reflexes, occasional body twitching to an external stimulus	Quick recovery, slight struggle to balance, slight ptyalism, licking, and slightly weak coordination in hindquarters
3	Satisfactory	Moderate or Slow induction, pacing more than 50 m, ptyalism, slight discomfort at the injection site, panting and grunting	Ear and limb twitching, slight muscle tone, delayed pedal and palpebral reflexes, slight twitching to an external stimulus	Slow recovery, delayed retraction of the tongue, Ptyalism, struggle in standing and uncoordinated movements, erection of mane hair
4	Unsatisfactory	No induction or violent resistance, vomiting, panting, stereotypic pacing, grunting, excessive ptyalism, severe discomfort to the injection site and licking	Body movement, limb withdrawn, grunting sounds, increased muscle tone, blinking, twitching of ear, immediate pedal and palpebral reflexes, rapid response to an external stimulus	Delayed recovery, inability to retract tongue, gain balance and no palpebral reflexes, unable to stand, temporary loss of sensation in the hindquarter, shivering

Note:
The scoring for induction, immobilisation and recovery was assessed separately based on observations at each stage during an immobilisation procedure.

position, their respiration remains effective (*Kock & Burroughs, 2012*). The chemical restraint procedure was divided into three categories viz. induction, anaesthesia and recovery. For each category, times in minutes for different events were recorded. For induction, the times from injection of anaesthetics to the time of first sign of the drug taking effect (ataxia), sternal position, head down and completion of induction were recorded. Anaesthesia period was considered the duration in which animal had sufficient loss of body movements or sudden jerks, the absence of pedal and palpebral reflexes, no muscle tone and no response to an external stimulus such as injections and sample collection and was calculated as the time from the completion of induction till administration of the reversal. The reversal was administered when the animals exhibited the signs of drugs being metabolised and losing their effects, such as pedal of palpebral reflexes, ear twitching, and grunting sounds. For recovery, the times were recorded for the duration from the administration of reversal till first sign of consciousness (ear twitching, blinking of eyes, retraction of the tongue), lifting head up, going to sternal position and complete recovery (*Fournier et al., 1995*; *Lescano et al., 2014*). The quality of induction, anaesthesia and recovery were assigned a numerical score from 1 (excellent) to 4 (unsatisfactory) as described by *Lescano et al. (2014)*. The description for each score is presented in Table 3.

Monitoring of the vital signs

During anaesthesia phase, the rectal temperature, pulse rate, respiration rate, non-invasive blood pressure, capillary refill time, and blood oxygen saturation (SpO_2) were recorded at 5-min intervals (*Janovsky et al., 2000*; *Lescano et al., 2014*). Changes in body temperature

were measured through rectal thermometer (NOVAMED Digital thermometer, Surrey, England, UK) and rectal thermometer probe of the pulse oximeter (Purescope Veterinary Patient Monitor IP-3000/4000 Series; Infunix Technology Co., Ltd., Seoul, South Korea). Pulse rate was monitored by cardiac auscultation and by a pulse oximeter to cross-verify by manual observations (*Janovsky et al., 2000*). Respiration rate was monitored manually through direct observation of the movement of the thoracic cavity. The blood oxygen level was measured through pulse oximeter with the probe placed at the tongue of the animals. Systolic and diastolic non-invasive blood pressure was measured through pulse oximeter by placing the cuff on the median palmar artery (proximal to the metacarpal pad) as instructed in the operational manual of the monitor (Infunix Technology Co., Ltd., Seoul, South Korea). Capillary refill time was assessed by pressing the oral mucosa through thumb and then counting the required seconds to regain its colour (*Lescano et al., 2014*).

Blood gas analysis

The blood was taken from the femoral artery after 15 min into the anaesthesia and immediately analysed using I-Stat handheld blood gas analyser (iSTAT® 1, Abbott Laboratories, Lake Bluff, IL, USA) and i-STAT® CG8+ Cartridge. The variables that were measured included pH, partial pressure of carbon dioxide, partial pressure of oxygen (pO_2), blood concentrations of sodium, potassium, glucose (Glu) and ionised calcium (iCa), total carbon dioxide, bicarbonate, base excess, haematocrit (Hct), haemoglobin (Hb), and haemoglobin oxygen saturation (sO_2) (*Lescano et al., 2014*). Due to lack of available reference range for parameters mentioned above, feline range provided with the test kit was used as hyaenas are more closely related to felids, especially their physiological responses to xylazine and ketamine (*Wilson & Mittermeier, 2009*; *Hahn et al., 2014*; *ABAXIS, 2018*).

Blood haematology and biochemistry analysis

Blood biochemistry and haematology was performed on the blood samples drawn from the jugular vein using 20 ml disposable syringes. Blood (six to eight ml) was collected in serum gel tubes for blood biochemistry, three to four ml in heparin lithium tubes for cortisol hormone, and three to four ml in EDTA K_3 tubes for haematology. The blood in the serum gel and heparin tubes was centrifuged at 2,000 rpm for 3–5 min and 1,000 rpm for 2–3 min, respectively. The samples were either analysed using Abbott CELL-DYN® 3700 Haematology Analyser or Abbott Architect c4000 clinical chemistry analyser; or through Central Veterinary Research Laboratory (paid services). The study variables included; red blood cells count (RBC), Hb, packed cell volume (PCV), mean cell volume, mean cell haemoglobin (MCH), platelets, white blood cell count (WBC), neutrophils, lymphocytes, monocytes, eosinophils, basophils, creatinine kinase, lactate dehydrogenase (LDH), calcium (Ca), phosphorus (PHOS), aspartate transferase (AST), alanine transferase (ALT), alkaline phosphate (ALP), total bilirubin, creatinine (CREA), blood urea nitrogen, total protein (TP), albumin, uric acid, triglycerides, cholesterol, α-Amylase, Glu, and cortisol. The parameters mentioned above were compared to the references ranges of striped hyaena provided in ZIMS by Species 360.

Table 4 Time variables of various chemical restraint stages for *H. h. sultana* immobilised with Ketamine-Medetomidine and Ketamine-Xylazine combinations in (semi-) captive conditions.

Time variables	KM (n = 34) Mean ± SE (Minutes)	KX (n = 16) Mean ± SE (Minutes)	*F* statistics for factors with $P < 0.05$
Time to first sign (ataxia)*	4.56 ± 0.40	4.19 ± 0.33	–
Time of sternal position*	6.15 ± 0.65	5.75 ± 0.39	–
Time of head down*	6.80 ± 0.65	6.87 ± 0.43	–
Induction time*	10.12 ± 0.65	9.37 ± 0.45	–
Anaesthesia time	59.5 ± 2.41	49.25 ± 1.31	$F_{DRUG} = 5.1$
Time to first reversal sign+	1.94 ± 0.22	2.13 ± 0.35	–
Time to head up+	3.70 ± 0.56	4.13 ± 1.13	$F_{SEX} = 4.2$
Time to sternal position (Reversal)+	4.03 ± 0.54	5.63 ± 1.09	–
Time to stand+	4.91 ± 0.60	10.38 ± 1.48	$F_{DRUG} = 8.3$; $F_{SEX} = 6.2$
Time to complete recovery+	12.32 ± 1.37	21.25 ± 2.16	$F_{DRUG} = 7.9$

Notes:
* Time from administration of anaesthetics
+ Time from administration of reversal.

Data analyses

The recorded data for vital signs ($n = 34$ for KM and $n = 16$ for KX); blood gas and clinical biochemistry analyses ($n = 21$ for KM and $n = 16$ for KX) were assessed through Shapiro–Wilk test for normal distribution and then were analysed through general linear model using STATISTICA software, with main effects, individuals as random, combination and sex were fixed.

RESULTS

All 50 immobilisation events were successful with both combinations at induction, anaesthesia and recovery phases. The quality of induction was between excellent and good with the mean score and standard error (MS ± SE) 1.41 ± 0.10 and 1.31 ± 0.12 in KM and KX, respectively. The quality of anaesthesia was excellent in KM (1.00 ± 0.00) as compared to KX, where it was scored as good (2.0 ± 0.0). The recovery of the animals in KM was between excellent and good (1.76 ± 0.15), whereas, the quality of recovery in KX was between good and satisfactory (2.69 ± 0.12), especially reversing with yohimbine (3.00 ± 00) as compared to atipamezole (2.50 ± 0.17).

The time variables of both combinations for different stages of immobilisation are provided in Table 4. There was a significant difference amongst the two combinations for anaesthesia time ($F = 5.1$; $P < 0.05$), time to stand after administration of reversal ($F = 8.3$; $P < 0.05$) and complete recovery ($F = 7.9$; $P < 0.05$). Moreover, there was a significant difference between the sexes in the time to lift the head ($F = 4.2$; $P < 0.05$) and time to stand after administration of reversal ($F = 6.2$; $P < 0.05$). Females took less time compared to males. However, we could not establish any reason why females were quicker to lift the head and stand compared to males, and there was no significant difference in time to full recovery between the sexes. The two reversing agents for KX showed a significant difference in time to full recovery ($F = 529.0$; $P < 0.05$).

The mean variations of pulse rate, respiration rate, systolic and diastolic non-invasive blood pressure, blood oxygen saturation and rectal temperature throughout anaesthesia are presented in Fig. 1. The capillary refill time for all immobilisations was less than 2 s. There was no significant difference in the vital signs between males and females ($P > 0.05$). However, Rectal temperature, pulse rate, respiration rate and blood oxygen saturation were significantly different ($F = 7.5$, 6.4, 6.8 and 4.6, respectively, with $P < 0.05$) between the combinations (Table 5).

The blood gas analysis results showed no statistically significant difference between sexes or drug combinations except pO_2 that had statistically significant difference among combinations ($F = 5.9$, $P < 0.05$). The comparison of variables from blood gas analysis to the range value of felines suggested that most of the variables remained within described range except ionised Calcium (iCa^{++}) which was high; pO_2 and sO_2 were low.

The results of the clinical variables are given in Table 6. The statistical analysis of the variables between sexes showed a significant difference in platelet count, WBC, LDH and cortisol levels ($P < 0.05$). There was also a statistically significant difference between the combinations for MCH, PHOS, ALT, ALP, CREA, TP, Glu, α-Amylase, and cholesterol ($P < 0.05$). The mean values were compared with the range of values of striped hyaena extracted from ZIMS by Species 360, to assess the condition of the animals. All the variables were within the described range (Table 6).

DISCUSSION

Chemical restraint with KX and KM combinations and their effects on the physiological and clinical parameters of Arabian striped hyaena are reported for the first time in the current study. With both combinations, the immobilisation was achieved rapidly for handling and execution of desired procedures such as vaccination, translocation or treatment according to described dosages (Table 2). It is incredibly essential that carnivores especially hyaenas assume quick induction due to their affinity to enter a burrow, hide, run deep into the forest or mountainous area, or intraspecific aggression and safety of both humans and animals. Quick induction for the immobilisation of wild animals facilitates monitoring of animals, reduction of injuries, stress, hyperthermia; and chances of animal escape or hiding (*Hahn et al., 2014*; *Barros et al., 2018*).

Ketamine is regarded as a general anaesthetic that has analgesic activity and no cardiopulmonary depression but has poor muscle relaxation and high excitatory state in animals with excessive salivation when administered alone. The amnestic and anaesthetic effects of ketamine are caused due to overstimulation and disruption of the nervous system. Ketamine alone is not recommended, especially in canids due to its over-excitatory effects and prolonged recoveries (*Ward et al., 2006*).

We required a lower dosage of ketamine than recommended (2.5–3.0 mg/kg) for carnivores with KM (*Jalanka & Roeken, 1990*). The actual dosage for ketamine in the current study for KM was 55% lower than the KX, suggesting that medetomidine is highly effective in enhancing the potency of ketamine. It could be attributed to the high affinity and selectivity of medetomidine to adrenoceptors than xylazine (*Sinclair, 2003*). Therefore, a lower dosage of ketamine was required that enhanced the quality of

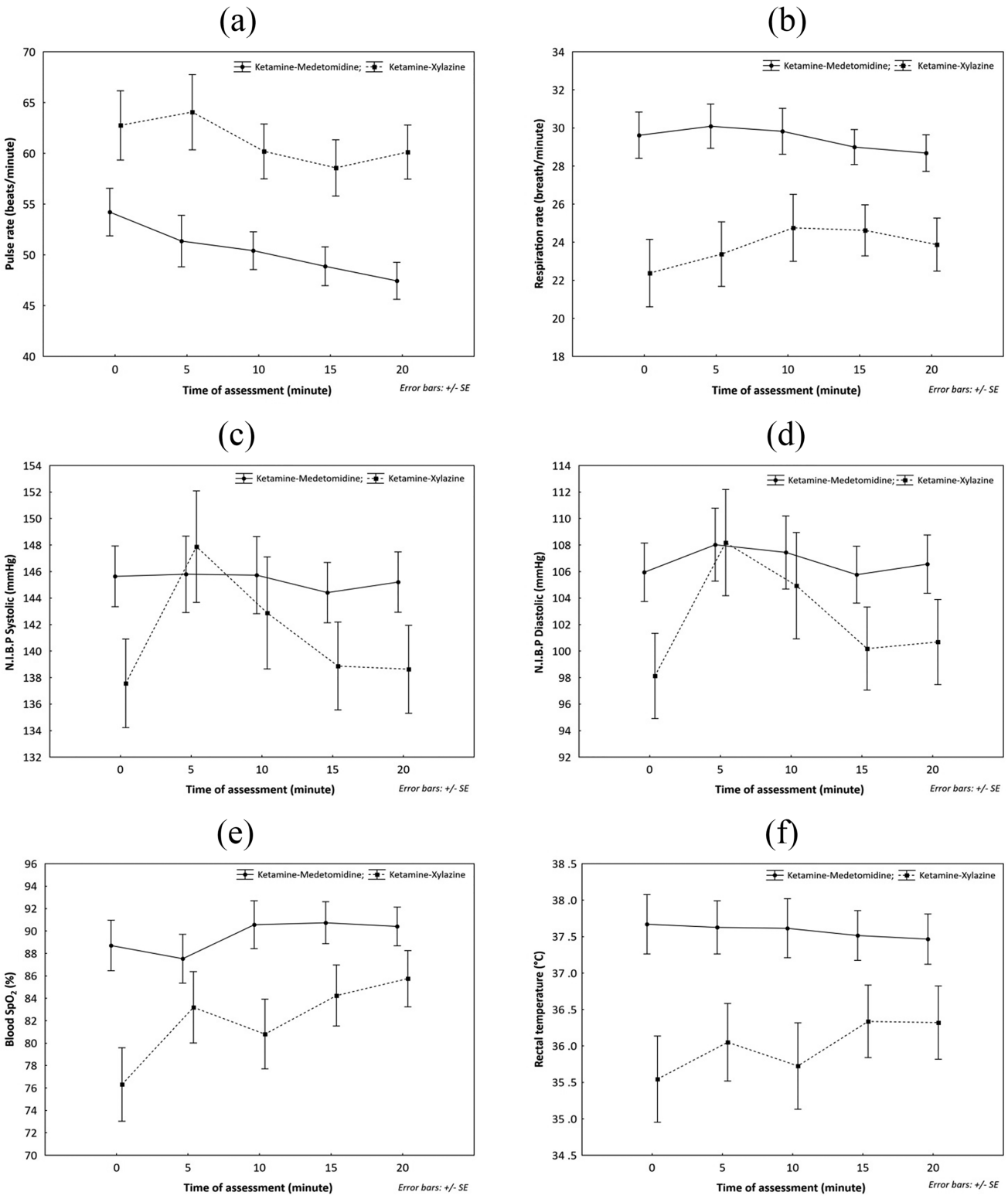

Figure 1 Comparison of mean variation of vital signs during anaesthesia of *Hyaena hyaena sultana* using combinations of Ketamine-Medetomidine and Ketamine Xylazine in (Semi-) Captive conditions; (A) pulse rate; (B) respiration rate; (C) N.I.B.P. systolic; (D) N.I.B.P. diastolic; (E) Blood SpO_2; (F) rectal temperature.
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Table 5 Comparison of physiological variables of vital signs and blood gas analysis during chemical restraint of *H. h. sultana* immobilised with KM and KX combinations in (semi-) captive conditions.

Vital sign variables	Units	Range	KM (n = 34) Mean ± SE	KX (n = 16) Mean ± SE	F statistics for factors with $P < 0.05$
Rectal temperature	°C	–	37.58 ± 0.29	36.00 ± 0.68	$F_{DRUG} = 7.5$
Pulse rate	Beats/min	–	50.46 ± 1.90	61.14 ± 2.79	$F_{DRUG} = 6.4$
Respiration rate	Breaths/min	–	29.44 ± 0.99	23.80 ± 1.57	$F_{DRUG} = 6.8$
SpO_2	%	–	89.59 ± 1.34	82.06±3.92	$F_{DRUG} = 4.6$
N.I.B.P (Systolic)	mmHg	–	145.36± 2.13	141.16±3.66	–
N.I.B.P (Diastolic)	mmHg	–	106.74± 1.97	102.43± 3.50	–
		Range*	**KM (n = 21)**	**KX (n = 16)**	
pH	–	7.25–7.40	7.28 ± 0.01	7.26 ± 0.01	–
pCO_2	mmHg	33.0–51.0	45.94 ± 1.02	46.79 ± 2.08	–
HCO_3^-	mmol/l	13.0–25.0	21.73 ± 0.67	22.22 ± 0.67	–
TCO_2	mmol/l	16–25	22.81 ± 0.65	23.19 ± 0.61	–
BE	mmol/l	(−5)–(+2)	−5.52 ± 0.69	−5.94 ± 0.38	–
pO_2	mmHg	90–110	53.00 ± 2.54	44.44 ± 3.08	$F_{DRUG} = 5.9$
sO_2	%	>90	80.57 ± 2.39	69.44 ± 5.09	–
Na^+	mmol/l	147–162	144.38 ± 0.25	143.50 ± 0.43	–
K^+	mmol/l	2.9–4.2	4.35 ± 0.07	4.17 ± 0.08	–
iCa^{++}	mmol/l	1.20–1.32	1.43 ± 0.01	1.45 ± 0.02	–
Hct	% PCV	24–40	38.33 ± 0.96	39.88 ± 0.93	–
Hb	g/dl	8.0–13.0	12.98 ± 0.32	13.21 ± 0.31	–

Notes:
SE for measurements of rectal temperature, pulse rate and respiration rate in an animal immobilised once (n = 1) indicates SE of five repeated/consecutive measurements during one immobilisation event. pH, potential of hydrogen; pCO_2, partial pressure of carbon dioxide; pO_2, partial pressure of oxygen; BE, base excess; HCO_3, bicarbonate; TCO_2, total carbon dioxide; sO_2, oxygen saturation; Na^+, sodium; K^+, potassium; iCa^{++}, ionised calcium; Hct, haematocrit; Hb, haemoglobin.
* Feline range from I-Stat reference manual for CG8+ Cartridge.

immobilisation by lowering the side effects of ketamine as well as enhancing reversal quality as ketamine does not have a reversal. Studies on snow leopard also suggested a reduction of ketamine doses up to 25% (*Jalanka & Roeken, 1990*).

The addition of ketamine also enhances the effect of medetomidine, which produces sedation depending on its dose and requires high dosage for complete immobilisation. The dosage of medetomidine (0.04 ± 0.001 mg/kg) was lower than the recommended dosage (0.06–0.1 mg/kg) for carnivores (*Jalanka & Roeken, 1990*) but was within the recommended dose for hyaena (*Kock & Burroughs, 2012*). Moreover, the dosage of ketamine in KX was closer (4.95 ± 0.115 mg/kg and) in the current study compared to that used in hyaenas (5–10 mg/kg) (*Kock & Burroughs, 2012*). The xylazine dose (0.99 ± 0.023 mg/kg) in our combination was within the dosage range of 0.5–1.0 mg/kg for spotted hyaena and 1.0 mg/kg for jaguar (*Burroughs, 1993*; *Kock & Burroughs, 2012*; *Bharathidasan et al., 2014*).

Due to high potency, suppression of norepinephrine in the nervous system, reduced emetic effects, longer sedative and analgesic duration of medetomidine, it is preferred over xylazine (*Paddleford & Harvey, 1999*). The pharmacokinetics of medetomidine reveal its rapid absorption and distribution due to its high lipophilic characteristics

Table 6 Comparison of clinical variables during chemical restraint of *H. h. sultana* immobilised with Ketamine-Medetomidine and Ketamine-Xylazine combinations in (semi-) captive conditions.

Variables	Units	Range*	KM (n = 21) Mean ± SE	KX (n = 16) Mean ± SE	F statistics for factors with $P < 0.05$
RBC	10^{12} cells/l	4.54–9.80	7.38 ± 0.13	7.11 ± 0.21	–
Hb	g/dl	9.8–17.6	15.39 ± 0.27	14.72 ± 0.43	–
PCV	l/l	–	0.45 ± 0.01	0.43 ± 0.02	–
MCV	fl	8.3–73.4	61.20 ± 0.77	60.21 ± 0.91	–
MCH	pg	15.8–24.6	21.29 ± 0.16	20.56 ± 0.20	F_{DRUG} = 6.04
MCHC	g/dl	28.4–38.1	34.70 ± 0.60	34.21 ± 0.68	–
PLT	10^9/l	–	223.33 ± 6.3	210.75 ± 9.26	F_{SEX} = 6.05
IRON	µmol/l	–	17.9 ± 1.04	18.94 ± 1.37	–
WBC	10^9/l	5.4–17.1	8.15 ± 0.22	7.15 ± 0.40	F_{SEX} = 13.7; F_{INDIV} = 2.6
NEU	%	53.6–88.5	70.30 ± 1.70	64.94 ± 1.83	–
LYM	%	6.9–38.0	25.57 ± 1.67	30.42 ± 2.10	–
MONO	%	0.9–10.6	3.59 ± 0.31	2.79 ± 0.25	–
EOS	%	0.0–8.0	2.21 ± 0.62	1.81 ± 0.54	–
BASO	%	0.0–3.6	0.02 ± 0.01	0.11 ± 0.07	–
CK	U/l	53–567	143.8 ± 10.9	262.0 ± 73.38	–
LDH	U/l	394–1810	579.8 ± 19.4	585.25 ± 28.18	F_{SEX} = 7.4
Ca	mmol/l	2.0–2.8	2.48 ± 0.02	2.44 ± 0.02	F_{INDIV} = 3.08
PHOS	mmol/l	0.60–1.70	1.48 ± 0.04	1.26 ± 0.03	F_{DRUG} = 28.8
AST/GOT	U/l	41–113	102.3 ± 7.16	99.56 ± 7.48	–
ALT/GPT	U/l	21–91	46.57 ± 2.54	37.25 ± 3.87	F_{DRUG} = 8.08
ALP	U/l	10.0–48.0	15.05 ± 1.09	12.88 ± 0.49	F_{DRUG} = 4.9
TBIIL	µmol/l	0.0–6.8	1.11 ± 0.10	1.38 ± 0.09	–
CREA	µmol/l	62–150	82.76 ± 2.94	92.56 ± 4.52	F_{DRUG} = 5.4
BUN	mmol/l	3.9–13.4	11.89 ± 1.01	11.54 ± 0.47	–
TP	g/l	53–74	67.52 ± 0.64	64.94 ± 0.44	F_{DRUG} = 9.6
ALB	g/l	15–32	29.95 ± 0.49	30.63 ± 0.43	–
Glu	mmol/l	3.5–12.1	8.03 ± 0.46	9.99 ± 0.60	F_{DRUG} = 4.4
α-Amylase	U/l	346–911	694.5 ± 22.3	519.13 ± 21.80	F_{DRUG} = 15.9
Cholesterol	mmol/l	3.8–11.0	5.16 ± 0.22	4.16 ± 0.15	F_{DRUG} = 13.7; F_{INDIV} = 2.6
UA	µmol/l	0–36	9.48 ± 0.44	8.06 ± 0.49	–
TG	mmol/l	0.4–2.2	1.44 ± 0.23	1.22 ± 0.07	–
Cortisol	nmol/l	–	477.4 ± 43.9	475.13 ± 54.23	F_{SEX} = 8.9

Note:

* Reference range for striped hyaena extracted from ZIMS by Species 360. RBC, red blood cell count; Hb, haemoglobin; PCV, packed cell volume; MCV, mean cell volume; MCH, mean cell haemoglobin; PLT, platelets; IRON, iron; WBC, white blood cell count; NEU, neutrophils; LYM, lymphocytes; MONO, monocytes; EOS, eosinophils; BASO, basophils; CK, creatinine kinase; LDH, lactate dehydrogenase; Ca, calcium, PHOS, phosphorus; AST, aspartate transferase; ALT, alanine transferase; ALP, alkaline phosphate; TBIL, total bilirubin; CREA, creatinine; BUN, blood urea nitrogen; TP, total protein; ALB, albumin; Glu, glucose; UA, uric acid; TG, triglycerides.

(*Jalanka & Roeken, 1990*). KM combination was found more effective in anaesthesia and recovery phases compared to KX combination according to the scoring system provided in Table 3.

The induction with both combinations was between excellent and good, giving smooth induction, the absence of stereotypic reactions, with no to little ptyalism and resistance

to loss of balance. However, there was a single case of vomiting with KX that can be related to xylazine being emetic in Hyaenidae and other carnivores (*Bufalari et al., 2007*; *Hahn et al., 2014*). The studies on donkeys, captive cougars, Southern river otters, free-ranging African lions, and marmosets had excellent to good scores using ketamine with medetomidine or other drugs such as xylazine (*Selmi et al., 2004a*; *Soto-Azat, Reyes & Medina-Vogel, 2004*; *Wenger et al., 2010*; *Bakker et al., 2013*; *Lescano et al., 2014*; *Maney et al., 2018*). The quality and depth of the anaesthesia were excellent with KM combination providing deep anaesthesia, no body movements or sudden jerks, the absence of pedal and palpebral reflexes, no muscle tone and no response to an external stimulus such as injections and sample collection. It could be attributed to the clinical analgesic, sedative and myrelaxation properties of medetomidine in felids and canids (*Jalanka & Roeken, 1990*). The good quality of anaesthesia was similarly reported in marmosets using KM, wild dogs using KM with atropine, and wild leopards using KX (*Ward et al., 2009*; *Belsare & Athreya, 2010*; *Bakker et al., 2013*). On the other hand, KX combination provided good anaesthesia with occasional ear and body twitching, pedal and palpebral reflexes to the external stimuli (*Mulder, 1978*; *Ward et al., 2006*). Occasional body movements are associated with the effects of ketamine when used in combination with xylazine. With both combinations, good myorelaxation was achieved to even perform dental examination (*Mulder, 1978*; *Jalanka & Roeken, 1990*; *Ward et al., 2006*).
The average anaesthesia time in both combinations was higher as compared to the described useful anaesthesia time (*Jalanka & Roeken, 1990*). The study on Golden-headed tamarinds, however, showed that the combination of dexmedetomidine with ketamine provided higher quality and duration of anaesthesia compared to KM (*Selmi et al., 2004b*).

Residual motor impairment is apparent when a higher dosage of ketamine in KX and KM combinations are used in carnivores, leading to ataxic recoveries (*Jalanka & Roeken, 1990*). Recovery in KM combinations was between excellent and good. The animals were quick to recover, without vomiting, and with quick retraction of the tongue, slight struggle to balance, slight ptyalism and occasionally weak coordination in hindquarters. Due to the lower dosage of ketamine in KM, the animals were able to recover smoothly and quickly. The recovery in KX combination was mostly satisfactory providing slow recovery, delayed retraction of the tongue, ptyalism, weak coordination or uncoordinated movements and erection of mane.

The reversals with atipamezole were more effective as compared to yohimbine, which coincides with the study of *Jalanka & Roeken (1990)*, who described a rapid reversal with atipamezole; as it has a high alpha-2/alpha-1selectivity ratio of 8,526 compared to 40 of yohimbine. Moreover, yohimbine is known only to partially antagonise the effects of xylazine, leading to delayed recoveries (*Paddleford & Harvey, 1999*). Atipamezole also showed a significant difference in time to full recovery and produced shorter recovery times compared to yohimbine. The times to arousal or first reversal sign with both combinations were markedly shorter than that in snow leopards (9 min) (*Jalanka & Roeken, 1990*). It is essential in wild conditions where the post-anaesthetic period for animals is very crucial as they can be a victim of aggression from other conspecifics that reversal is quick and smooth (*Rockhill et al., 2011*).

The respiration rate in KX was lower than KM; as ketamine causes dose-dependent respiratory depression due to an imbalance of muscarinic—nicotinic cholinergic activity of the brain centre (*Jalanka & Roeken, 1990*). The respiration was occasionally shallow and fast during the middle of anaesthesia compared to the whole duration in KX, and with time, the respiratory rate decreased in both combinations. The respiratory depression is associated with α2-adrenergic agonist and ketamine may ameliorate the respiratory depression when used with medetomidine (*McKenzie et al., 1993*; *Fernandez-Moran et al., 2001*). KM provided a stable respiratory pattern throughout the immobilisation.

Both xylazine and medetomidine are known to cause initial oxygen depression and increased CO_2 and subsequent compensation in cats and snow leopards (*Jalanka & Roeken, 1990*). The blood oxygen saturation monitored by oximeter indicated a significant difference between the combinations with KX showing signs of hypoxaemia. The blood gas analysis also indicated severe hypoxaemia with KX combination, although, KM was also below the described range. Oxygen supplementation is essential and useful intervention when working with KX and KM on Arabian hyaenas. Blood gas analysis revealed mild metabolic acidosis. In wild felids, the metabolic acidosis is supposed to be normal due to their high dietary protein intake (*Fahlman et al., 2005*; *Wenger et al., 2010*).

Hypoxaemia is a complication commonly associated with wildlife immobilisation, especially when animals are breathing normal air (*West, Heard & Cauklett, 2007*). α2-adrenergic agonists are known to depress the respiratory centres and sensitivity to carbon dioxide in many species. The administration of xylazine has shown central hypoxaemia due to pulmonary alterations (*Celly et al., 1997*). Hypoxaemia can be a serious complication if coupled with hyperthermia and may lead to the death of the animal if not compensated (*West, Heard & Cauklett, 2007*). However, there was no case of hyperthermia recorded with studied combinations.

The pattern of rectal temperature fluctuation during the anaesthesia (Fig. 1) reveals that the temperature starts to decrease in animals sedated with KM and slightly increases in KX which coincides with the reported disadvantages of xylazine that causes loss of thermoregulatory control and causes hyperthermia (*Jalanka & Roeken, 1990*; *Fernandez-Moran et al., 2001*). In the current study, no case of hyperthermia was recorded, and the rectal temperature in both combinations was within the reported range (*Hahn et al., 2014*; *Sahu, Sahoo & Mohapatra, 2018*). It is incredibly essential to administer KX when the external temperatures are not high, and animals are not subjected to undue physical exertion and stress; as external temperatures and prolonged chasing before dart can cause hyperthermia (*Jalanka & Roeken, 1990*).

Bradycardia is an intrinsic symptom with the agonist of α2-adrenoceptors, especially medetomidine (*Jalanka & Roeken, 1990*; *Fernandez-Moran et al., 2001*; *Wenger et al., 2010*). The heart rate was significantly lower in KM as compared to KX, but the means with both combinations were within the described range for hyaenas. Both combinations showed slight signs of bradycardia during the later phases of immobilisation, where the heart rate decreased gradually but not below the normal range. When ketamine is used with xylazine, it suppresses the bradycardic effect of xylazine through cardiotonic action

reconciled by vagolytic action (*Bharathidasan et al., 2014*). The administration of anticholinergic drugs such as atropine is reported to reduce bradycardia and salivation in hyaenas when immobilised with KX (*Hahn et al., 2014*). However, in the current study, we did not administer atropine. Additionally, ventricular arrhythmias are reported in dogs and wolves when atropine is used with KM (*Kreeger, 1996*). Another prominent effect of medetomidine is hypotension that is marked with an initial increase and then hypo- or normotension that was also observed in the current study (*Jalanka & Roeken, 1990*).

Although, medetomidine is known for its adrenolytic properties where PCV decreases due to pooling of RBCs in the spleen (*Jalanka & Roeken, 1990*); the erythrocytes, PCV and Hct values were within the prescribed range for striped hyaena in the current study. α2-adrenergic insulin inhibition in the beta cells of the pancreas and higher production of Glu in the liver frequently causes hyperglycaemia with medetomidine. Moreover, in tigers higher dosage of xylazine caused hyperglycaemia (*Jalanka & Roeken, 1990*). No hyperglycaemia was observed in our studies. The clinical analysis of blood also showed that there was no chronic kidney or liver disorder as ketamine is not recommended for renal or hepatic dysfunctional patients (*Jalanka & Roeken, 1990*). Cortisol levels were significantly higher in females. In spotted hyaena, the cortisol levels in adult or dominant females are usually higher than males and female cubs (*Van Jaarsveld & Skinner, 1992*). The mean cortisol level for both sexes was higher compared to the reported values for spotted hyaena (87.96 ± 74.03 ng/ml and 101.83 ± 71.79 ng/ml for males and females, respectively). It suggests a response to immobilisation stress during the initial phases of the immobilisation. Due to unavailability of samples at the later stages of immobilisation, it is difficult to assess whether the initial stress to capture was suppressed or not (*Van Jaarsveld & Skinner, 1992*; *Sheriff et al., 2011*).

The limitations of the study included lack of data on effects of oxygen supplementation to the tested combination for compensation of hypoxaemia, and samples for cortisol at later stages of immobilisation, and unavailability of yohimbine and xylazine during the study that led to more immobilisations with KM.

CONCLUSIONS

In conclusion, we found KM combination more suitable for the immobilisation of Arabian striped hyaena, providing better quality and timings of induction, anaesthesia and recovery as compared to KX. KX also provided, in general, adequate immobilisation of the animals and can be alternatively used with the provision of supplementary oxygen, as complication of hypoxaemia was common in both combinations. However, certain other parameters with KX, especially time to full recovery can be critical if there are other animals around and can harm the recovering individual. We found severe hypoxaemia with KX, although there was hypoxaemia in KM to some extent. It urges a further study with the provision of supplementary oxygen to assess its effectiveness in the reduction of hypoxaemia. Other limitations included unavailability of yohimbine and xylazine during the study that led to more immobilisations with KM.

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Competing Interests

The authors declare that they have no competing interests.

Author Contributions

- Abid Mehmood conceived and designed the experiments, performed the experiments, analyzed the data, contributed reagents/materials/analysis tools, prepared figures and/or tables, authored or reviewed drafts of the paper, approved the final draft, gathering sources and planning.
- Sadia Abid conceived and designed the experiments, performed the experiments, analyzed the data, contributed reagents/materials/analysis tools, prepared figures and/or tables, authored or reviewed drafts of the paper, approved the final draft.
- Pavla Hejcmanová conceived and designed the experiments, performed the experiments, analyzed the data, contributed reagents/materials/analysis tools, prepared figures and/or tables, authored or reviewed drafts of the paper, approved the final draft.
- Muhammad Arslan Asadi conceived and designed the experiments, performed the experiments, analyzed the data, authored or reviewed drafts of the paper, approved the final draft.
- Bilal Kabeer conceived and designed the experiments, performed the experiments, analyzed the data, authored or reviewed drafts of the paper, approved the final draft.
- Muhammad Jawad Jilani conceived and designed the experiments, performed the experiments, analyzed the data, authored or reviewed drafts of the paper, approved the final draft.
- Sadaf Bilal conceived and designed the experiments, performed the experiments, analyzed the data, authored or reviewed drafts of the paper, approved the final draft.

- Muhammad Waseem Ashraf conceived and designed the experiments, performed the experiments, authored or reviewed drafts of the paper, approved the final draft.

Animal Ethics

The following information was supplied relating to ethical approvals (i.e., approving body and any reference numbers):

No approval from the Institutional Animal Care Committee was required as data was collected during routine veterinary procedures according to the animal collection management plan BFM-WL-CI/0017-2014.

Data Availability

The following information was supplied regarding data availability:

Mehmood, Abid (2019): assessment data. figshare. Dataset. https://doi.org/10.6084/m9.figshare.8174180.v2

Mehmood, Abid (2019): Vital signs 19092018.xlsx. figshare. Dataset. https://doi.org/10.6084/m9.figshare.7729535.v2.

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9.2. Arterial blood gas and acid-base status, before and after oxygen supplementation, in Arabian striped hyena (*Hyaena hyaena sultana*) during field immobilisation.

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Abid Mehmood

From: Rao Zahid Abbas <editor@pvj.com.pk>
Sent: Saturday, April 25, 2020 9:09 PM
To: Abid Mehmood
Subject: Re: Short Communication "Arterial blood gas and acid-base status, before and after oxygen supplementation, in Arabian striped hyena (Hyaena hyaena sultana) during field immobilization"

Dear Author,

Thank you very much for showing interest in our journal "Pakistan Veterinary Journal" (www.pvj.com.pk) having impact factor 1.360 (JCR-2018). Your submission's safe arrival is hereby acknowledged. Your manuscript has been allotted Manuscript # PVJ-20-211; **please write this manuscript # in the subject of each email** in your all future correspondence. Authors of manuscripts originating other than Pakistan have to pay processing fee (US$ 300) on acceptance of their article.
Regards,

Dr. RAO ZAHID ABBAS
Editor
Pakistan Veterinary Journal
University of Agriculture
Faisalabad, Pakistan

On Mon, Apr 20, 2020 at 4:01 PM Abid Mehmood <abid@wildbiodiversity.org> wrote:

> Dear Dr. Rao Zahid Abbas/Dr. Faqir Muhammad,
>
> I hope my email finds you well, healthy, and safe during the current pandemic.
>
> Kindly find attached short communication titled "Arterial blood gas and acid-base status, before and after oxygen supplementation, in Arabian striped hyena (*Hyaena hyaena sultana*) during field immobilization." It is submitted to your journal for publication from the original research work of my Ph.D. research studies at the Czech University of Life Sciences Prague. The studies focus on the chemical immobilization of Arabian striped hyena that is a near threatened subspecies. During field immobilization with ketamine-medetomidine combination, Arabian striped hyaena developed hypoxemia that can lead to further complications and organ failure. In the current study, we analyzed the arterial blood for blood gas and acid-base status to evaluate the efficacy of oxygen supplementation to correct hypoxemia and assess changes in acid-base status. Moreover, the authors declare no conflict of interest.
>
> We are looking forward to hearing from you.

Short Communication

Arterial blood gas and acid-base status, before and after oxygen supplementation, in Arabian striped hyena (*Hyaena hyaena sultana*) during field immobilization

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Statement of Novelty:

The Arabian striped hyena is a near threatened subspecies. During field immobilization with ketamine-medetomidine combination, Arabian striped hyaena developed hypoxemia that can lead to further complications and organ failure. In the current study, we analyzed the arterial blood for blood gas and acid-base status to evaluate the efficacy of oxygen supplementation to correct hypoxemia and assess changes in acid-base status.

ABSTRACT

The α2-adrenergic agonists such as medetomidine, cause respiratory depression in wild animals. It further leads to hypoxemia and later organ failure. The current study was designed to investigate the efficacy of oxygen supplementation to treat hypoxemia and its effects on blood gas and acid-base status. Twelve Arabian striped hyenas (*Hyaena hyaena sultana*) were immobilized with ketamine-medetomidine, and arterial blood samples were analyzed fifteen minutes into anesthesia and fifteen minutes after oxygen supplementation. Oxygen was supplemented at 2 L/min. The results suggested hypoxemia and respiratory acidosis before oxygen supplementation. There was a significant difference ($P<0.05$) in arterial oxygen saturation, the partial pressure of O_2, the arterial partial pressure of CO_2, bicarbonates, and pH before and after oxygen supplementation. The results suggest that the oxygen may adequately treat hypoxemia during field immobilization of hyena with ketamine-medetomidine.

Keywords: Arterial blood gas, acid-base balance, hyaena, chemical immobilization

INTRODUCTION

Arabian striped hyena (*Hyaena hyaena sultana*) is a vital scavenger and predator in the region, and its population is rapidly declining (AbiSaid and Dloniak, 2015). Ex-situ conservation initiatives for the species require regular veterinary interventions and restraint. Various drugs are used to immobilize hyenas such as ketamine, medetomidine, and xylazine (Kock and Burroughs, 2012). Drug combinations such as ketamine-medetomidine, provide adequate immobilization and anesthetic depth to execute desired procedures such as vaccination, morphometric measurements, collaring, sample collection, and minor surgeries in the field conditions (Mehmood *et al*., 2019).

However, anesthetic drugs, such as α2-adrenergic agonists, cause respiratory depression by affecting respiratory centers in the nervous system (Kock and Burroughs, 2012; Mehmood *et al*., 2019). Furthermore, it leads to hypoxemia and then to hypoxia, organ failure, and even mortality (Mehmood *et al*., 2019). These drugs also reduce the sensitivity of receptors to detect an increase in the CO_2 levels leading to hypercapnia. An increase in CO_2 is also accompanied by a fall in pH, causing acidosis (Hennessey and Japp, 2016). Arterial blood gas analysis (ABG) provides valuable information about blood gas and acid-base status and assists in determining timely intervention to treat any complications (Hennessey and Japp, 2016). The current study was designed to assess the efficacy of oxygen supplementation to correct hypoxemia and the changes in arterial blood variables.

MATERIALS AND METHODS

Study Area and Animals

For the current study, twelve (semi-) captive Arabian striped hyaena (eight females and four males), housed in large enclosures in a 4100 ha Arabian Wildlife Park (Sir Bani Yas Island, Abu Dhabi, United Arab Emirates), were sedated for the routine animal health check and vaccination procedures. The procedures were executed from January until June 2019, according to the Institutional Animal Care and Use Committee (Ref. BNR/SBY/00172014). One procedure was excluded due to incomplete data. The average weight of the animals was 31.6 ± 0.4 kg (mean ± SE, throughout the document).

Chemical restraint protocol

The animals were darted with a dart gun from hiding trailer on the neck region, early mornings, with 2.2 ± 0.03 mg/kg ketamine hydrochloride and 0.04 ± 0.0005 mg/kg medetomidine hydrochloride (Troy Laboratories, Australia) (Mehmood *et al*., 2019). After

completion of the required procedures, drug agonist effects were reversed with 0.2 ± 0.003 mg/kg atipamezole hydrochloride (Mehmood *et al*., 2019).

Monitoring

Time durations were recorded for complete induction (drug injection until complete unconsciousness), anesthesia maintenance, and complete recovery (the ability to walk). The anesthesia maintenance stage was subdivided into the non-oxygen supplementation phase and oxygen supplementation phase. Moreover, the vital signs such as heart rate, respiration rate, rectal temperature, blood oxygen saturation, and mean arterial pressure (non-invasive) were recorded at an equal interval of five minutes, three times before and after oxygen supplementation each. The vital signs were recorded with a pulse oximeter (Purescope Veterinary Monitor, Infunix Technology Co., Ltd., South Korea). Oxygen was provided at a rate of 2 L/min through a feline respiratory mask attached with a regulator to a medical oxygen cylinder (Midmark, United Kingdom) (Mehmood *et al*., 2019).

Arterial blood gas analysis

Arterial blood samples from the femoral artery were drawn once before oxygen supplementation and fifteen minutes after oxygen supplementation. The samples were immediately analyzed through a point of care I-Stat veterinary blood gas analyzer (Abbott Laboratories, USA). CG8+ analysis cartridge kit was used in all procedures and results were compared to the reference range provided with the kit manual (Mehmood *et al*., 2019). The alveolar-arterial (A-a gradient) gradient was calculated by the following equations (Sood *et al*., 2010);

$P_AO_2 = FiO_2\ (760-47) - PaCO_2/0.8$

A-a gradient = $P_AO_2 - PaO_2$

Where, FiO_2 is a fraction of inspired oxygen, and $PaCO_2$ is the partial pressure of CO_2, and PaO_2 is the partial pressure of oxygen.

Statistical analysis

The blood gas parameters were assessed for normality and were then subjected to the Wilcoxon matched-pairs test through STATISTICA® statistical package.

RESULTS AND DISCUSSION

The combination of ketamine medetomidine provided quick induction (10.3 ± 0.5 min) and recoveries (11.9 ± 0.4 minutes). Moreover, the combination provided adequate depth and duration of anesthesia for required procedures (Table 1). The quick induction and long duration of anesthesia may be associated with the high potency, sedative, and analgesic effects of medetomidine, through the suppression of norepinephrine (Mehmood *et al*., 2019).

Medetomidine is known to decrease heart rates, causing bradycardia. However, the addition of ketamine to α2-adrenoceptors compensates bradycardia through vagolytic action (Bharathidasan *et al*., 2014; Mehmood *et al*., 2019). There was no statistically significant difference between the heart rates and non-invasive mean arterial blood pressures before and after oxygen supplementation (Table 2). Heart rates in the current study were similar to previously reported heart rates of 50.46 ± 1.90 beats/min (Mehmood *et al*., 2019).

The rectal temperatures before (38.2 ± 0.2 °C) and after (38.0 ± 0.2 °C) oxygen supplementation had a statistically significant difference (Table 2). The α2-adrenoceptors can cause a rise in body temperature, leading to hyperthermia (Fernandez-Moran *et al*., 2001). Although, in the current study, neither hyperthermia nor hypothermia was observed.

The respiration rate did not significantly differ before (28.3 ± 1 breath/min) and after (26.8 ± 1.2 breath/min) oxygen supplementation (Table 2). Whereas, the blood oxygen

saturation percentage (SpO_2) measured by pulse oximeter showed a statistically significant difference before (86.1 ± 1.3 %) and after (98.2 ± 0.5 %) oxygen supplementation (Table 2). The α2-adrenoceptors, such as medetomidine, are known to cause respiratory depression by affecting the muscarinic and nicotinic cholinergic activity leading to hypoxemia (Mehmood *et al*., 2019).

Hypoxemia is one of the serious side effects with the use of anesthetic drugs and may become a life-threatening complication if it is not treated or compensated. Oxygen supplementation can compensate for the decreased oxygen levels in the patient, but thorough monitoring is necessary to assess its efficacy. The arterial blood gas analysis is one of the essential tools to evaluate the status of arterial blood gases and the acid-base homeostasis. It is widely used in human medicine practice (Hennessey and Japp, 2016).

In the present study, the arterial oxygen saturation (SaO_2) (73.5 ± 4.9 %) and arterial partial pressure of oxygen (PaO_2) (47.3 ± 3.6 mmHg) suggested hypoxemia before oxygen saturation when compared to the reference range (Table 2). Similarly, the ratio of PaO_2 and FiO_2 (225.1 ± 17.0) and A-a gradient (43.3 ± 3.3) suggest hypoxemia. The higher levels of $PaCO_2$ (49.3 ± 1.8 mmHg) and total carbon dioxide (TCO_2) (23.1 ± 1.1 mmHg) suggest hypoventilation, as the values were marginally below the maximum range according to the reference range (Table 2). The higher values of the A-a gradient also suggest a V-Q mismatch i.e., ventilation and perfusion at alveoli are not proportional (Day, 2002). After oxygen supplementation, there was a significant increase in SaO_2 (90.8 ± 3.8 %), PaO_2 (127.5 ± 12.6 mmHg), PaO_2/FiO_2 (455.2 ± 45.0), and A-a Gradient (5.9 ± 13.5), suggesting compensation of hypoxemia (Table 2).

Although oxygen supplementation improved the oxygen levels significantly, there was also a significant increase in $PaCO_2$ (55.2 ± 1.9 mmHg) TCO_2 (25.2 ± 1.0 mmol/L). The

increases in $PaCO_2$ levels may be due to the suppression of sensory receptors that detect carbon dioxide levels. Thus, the carbon dioxide is retained, and its levels start increasing (Deem *et al*., 1998).

The acid-base status showed a significant decrease in the pH before (7.25 ± 0.01) and after (7.23 ± 0.01) oxygen supplementation, indicating acidosis. The decrease in pH corresponds to the increase in $PaCO_2$, suggesting respiratory acidosis. Moreover, a significant increase in the bicarbonates can be attributed to a reaction to an increase in $PaCO_2$ (Hennessey and Japp, 2016). Generally, acidosis causes an increase in ionized calcium. In the current study, iCa^{2+} was above the reference range (hypercalcemia) (Hennessey and Japp, 2016). Acidosis also causes hyperkalemia (increase in potassium concentration). The K^+ concentration was marginally below the upper limit of the reference range (2.9-4.2 mmol/L). It is due to the increased reabsorption of K^+ in the collecting ducts (Hamm et al., 2013). Although oxygen supplementation corrected hypoxemia, pH levels did not return to normal. In clinical conditions, pH does not usually return to normal range and complete compensation does not occur (Sood *et al*., 2010).

Conclusions

The findings of the current study suggest that Arabian striped hyena immobilized with ketamine medetomidine developed hypoxemia and respiratory acidosis. The oxygen supplementation adequately treated hypoxemia, and a compensatory response was observed with an increase in the bicarbonate levels. Oxygen supplementation is recommended during the immobilization of Arabian striped hyena in field conditions.

Acknowledgments:

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Authors' contributions:

AM: Design of the study, data collection, statistical analysis, and preparation of the manuscript; PH: assessment of study design, statistical analysis, statistical inferences, preparation of the manuscript and critical review.

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Table 1 Durations of induction, anesthesia maintenance, and complete recovery in Arabian striped hyaena during field immobilization with a ketamine-medetomidine drug combination

Event description	Duration (min) (n = 11)
Induction [1]	10.3 ± 0.5
Anesthesia maintenance [2]	57.3 ± 0.6
Full recovery [3]	11.9 ± 0.4

[1] from the injection of drug agonists to complete loss of consciousness

[2] from induction until the administration of reversal after the appearance of signs of drug metabolism, e.g., ear twitching

[3] from the administration of reversal until fully coordinated walking

198

199 **Table 2** Mean heart rates, respiration rates, rectal temperature, blood oxygen saturation
200 percentage, and non-invasive mean arterial blood pressure of Arabian striped hyaena,
201 before and after oxygen supplementation, during field immobilization with a ketamine-
202 medetomidine drug combination

Vital signs	**Units**	**Before oxygen supplementation (Mean ± SE) (n = 11)**	**Oxygen supplementation (Mean ± SE) (n = 11)**	**Test statistics * (P < 0.05)**
Heart rate (HR)	Beats/min	50 ± 2.4	49.7 ± 2.3	-
Respiration rate (RR)	Breaths/min	28.3 ± 1	26.8 ± 1.2	-
Oxygen saturation (SpO_2)	%	86.1 ± 1.3	98.2 ± 0.5	***Z = 2.9; P = 0.003***
Mean arterial pressure (MAP)	mmHg	128.6 ± 2.9	123.2 ± 3.6	-
Rectal temperature	°C	38.2 ± 0.2	38 ± 0.2	***Z = 2.2; P = 0.02***

203

204 * Wilcoxon matched-pairs test

205

206

207 **Table 3** Arterial blood gas analysis of Arabian striped hyena before and after oxygen

208 supplementation during field immobilization with ketamine-medetomidine

Parameters	Reference range	Before oxygen supplementation (Mean ± SE) (n = 11)	After oxygen supplementation (Mean ± SE) (n = 11)	Test statistics * (P < 0.05)
Blood gas parameters				
SaO_2 (%)	>90	73.5 ± 4.9	90.8 ± 3.8	**Z = 2.8; P = 0.004**
PaO_2 (mmHg)	90–110	47.3 ± 3.6	127.5 ± 12.6	**Z = 2.9; P = 0.003**
$PaCO_2$ (mmHg)	33.0–51.0	49.3 ± 1.8	55.2 ± 1.9	**Z = 2.9; P = 0.003**
TCO_2 (mmol/L)	16–25	23.1 ± 1.1	25.2 ± 1.0	**Z = 2.8; P = 0.005**
PaO_2/FiO_2	>400	225.1 ± 17.0	455.2 ± 45.0	**Z = 2.9; P = 0.003**
A-a Gradient	<15	43.3 ± 3.3	5.9 ± 13.5	**Z = 2.6; P = 0.007**
Acid-base parameters				
pH	7.25–7.40	7.25 ± 0.01	7.23 ± 0.01	**Z = 2.9; P = 0.003**
BE_{ecf} (mmol/L)	(-5) – (+2)	-5.7 ± 1.1	-5.3 ± 0.7	-
HCO_3^- (mmol/L)	13.0–25.0	21.5 ± 1.0	23.3 ± 1.0	**Z = 2.9; P = 0.003**
Na^+ (mmol/L)	147–162	144.7 ± 0.6	144.5 ± 0.7	-
K^+ (mmol/L)	2.9–4.2	4.0 ± 0.1	4.1 ± 0.1	-
i Ca^{++} (mmol/L)	1.2–1.3	1.5 ± 0.01	1.5 ± 0.01	-

209

210 SaO_2, arterial oxygen saturation; PaO_2, the partial pressure of O_2; $PaCO_2$, the arterial partial
211 pressure of CO_2; TCO_2, total CO_2; $PaO_2/FiO2$, the ratio of the partial pressure of O_2 and
212 fraction of inspired O_2; A-a Gradient, alveolar-arterial gradient; BEecf, base excess
213 (extracellular fluid); HCO_3^-, bicarbonates; Na^+, sodium; K^+, potassium; iCa^{++}, ionized
214 calcium.

9.3. Effects of oxygen supplementation on blood gas variables in Northeast African cheetah (*Acinonyx jubatus soemmeringii*) immobilised with ketamine and medetomidine.

Citation: Mehmood A, Abid S, Bilal S, Asadi MA, Kabeer B, Jilani MJ, Hejcmanová P. 2020. Effects of oxygen supplementation on blood gas variables in Northeast African cheetah (*Acinonyx jubatus soemmeringii*) immobilised with ketamine and medetomidine. Veterinary Anaesthesia and Analgesia (re-submitted after revision).

VETERINARY ANAESTHESIA AND ANALGESIA

Effects of oxygen supplementation on blood gas variables in Northeast African cheetah (*Acinonyx jubatus soemmeringii*) immobilised with ketamine and medetomidine

Journal:	*Veterinary Anaesthesia and Analgesia*
Manuscript ID	VAA-20-0065
Article Type:	Short Communication
Keywords:	Hypoxaemia, blood gas analysis, oxygen supplementation, acidosis, cheetah

Word count (Abstract): 297 words

Word count (Introduction-conclusions)**:** 2000 words

Abstract

Objective To investigate the effects of oxygen supplementation on blood gas variables in Northeast African cheetah (*Acinonyx jubatus soemmeringii*) immobilised with ketamine/medetomidine

Study design Non-randomised prospective study

Animals Five (three males and two females) adult cheetahs with an average weight and standard error of 46.8 ± 1.6 kg

Methods During routine health checks, 18 chemical restraint procedures were performed. Animals were immobilised with 1.5 ± 0.05 mg kg^{-1} ketamine, and 0.028 ± 0.001 mg kg^{-1} medetomidine, delivered intramuscularly. Medetomidine was antagonised with 0.14 ± 0.005 mg kg^{-1} atipamezole. Vital signs were monitored at five minutes intervals with a pulse oximeter, and blood was taken from the femoral artery for blood gas analyses before and after oxygen supplementation. All animals developed hypoxaemia and were treated with oxygen supplementation at 2 litres $minute^{-1}$. The data were analysed using the Wilcoxon matched pairs test.

Results The mean times of ataxia, induction, anaesthesia, and full recovery were 2.78 ± 0.33 minutes, 8.5 ± 0.5 minutes, 45.9 ± 0.8 minutes, and 19.4 ± 0.5 minutes, respectively. Haemoglobin oxygen saturation (SpO_2), the partial pressure of oxygen (PaO_2), and oxygen saturation (SaO_2) (75 ± 1.8 %, 31.7 ± 0.6 mmHg, and 50.6 ± 1.8 %, respectively) indicated hypoxaemia. After oxygen supplementation, there was a significant difference in SpO_2 (97.7 ± 0.3 %), PaO_2 (91.67 ± 1.10 mmHg), and SaO_2 (92.9 ± 1.2 %) ($p < 0.001$). There was a decrease in the pH, base excess (BE_{ecf}), sodium (Na^+), ionised calcium (i Ca^{++}), and

potassium (K^+). However, there was an increase in bicarbonates (HCO_3^-), partial pressure of carbon dioxide $PaCO_2$, and SaO_2.

Conclusions and clinical relevance the protocol, although good for intended procedures, caused hypoxaemia. Oxygen supplementation corrected hypoxaemia, but it caused acidosis. Oxygen must be supplemented with caution in cheetahs immobilised with KM.

Keywords hypoxaemia, blood gas analysis, oxygen supplementation, acidosis

Introduction

Northeast African Cheetah (*Acinonyx jubatus soemmeringii*) is a subspecies of the cheetah and is listed as Vulnerable (Durant et al. 2015). Ketamine hydrochloride (K) can produce adequate analgesia and cataleptic anaesthesia by acting on the central nervous system. Medetomidine (M) is a potent and highly specific α2-adrenergic agonist and has high selectivity and affinity for adrenoceptors. Medetomidine is usually used in combination with ketamine for safe, lengthy and reversible immobilisation of various carnivores including cheetahs (Kock & Burroughs 2012).

Both K and M are known to cause respiratory depression and development of hypoxaemia in carnivores (Stegmann & Jago 2006; Kock & Burroughs 2012). Hypoxaemia ($PaO_2 < 90$ mmHg) is caused by the depression of the function of the medulla oblongata. It leads to an undetected increase in carbon dioxide levels in the bloodstream due to hypoventilation and ventilation-perfusion mismatching (West et al. 2007). If left untreated, it may lead to hypoxia leading to capture myopathy and organ failure (West et al. 2007). Oxygen supplementation is one of the interventions against hypoxaemia in wild carnivores (Kock & Burroughs 2012). The recent study was executed to investigate the changes in arterial blood gas variables after oxygen supplementation in the Northeast African Cheetahs immobilised with KM combination.

Materials and methods

Study animals

All restraint procedures were performed with the approval of Institutional Animal Care and Use Committee of BNR, SBY, Ref. no. BNR/SBY/00172014. The data was collected during routine veterinary interventions, and no immobilisation was performed exclusively for study purpose. Five Northeast African cheetahs (three males, two females), freely roaming in a 4,100 hectares wildlife park in Sir Bani Yas Island, were included in the study. The mean weight of the animals (± standard error) was 46.84 ± 1.60 kg. All animals were used to the human presence nearby. Eighteen successful chemical immobilisations were performed from January 2017 till May 2019. Immobilisation events with incomplete drug administration, a requirement of supplementary dose or incomplete data collection were not included in the current study.

Drugs and immobilisation procedure

Drugs used in the combination were ketamine hydrochloride (Ilium Ketamil, 100 mg ml^{-1}), medetomidine hydrochloride (Domitor, 1 mg ml^{-1}), and atipamezole hydrochloride (Ilium Atipamezole, 5 mg ml^{-1}) by Troy Laboratories, NSW, Australia. The dosage of the agonists was chosen based on previous experience and literature. It was calculated as 1.9 mg kg^{-1} ketamine, 0.03 mg kg^{-1} medetomidine, and antagonist 0.15 mg kg^{-1} atipamezole of the estimated weight (Kock & Burroughs 2012; West et al. 2007). The actual dose administered was later calculated by taking the animal weight using a field weighing scale.

Rangers observed the animals, and it was made sure that darting did not take place on the day the cheetahs had hunted as a full stomach may cause vomiting and may block the respiratory passage and cause choking (Kock & Burroughs 2012). All animals were darted

early morning and hindquarters were found to be the most suitable site for dart administration (Kock & Burroughs 2012). The drugs were delivered through a Dan-Inject 3 ml dart with 1.5 × 20 mm plain needle via Dan-Inject CO_2 powered rifle, model no. 0471 JM. It was made sure that the animals were not subjected to undue stress before or during the darting procedure.

Physiological and clinical data collection

The durations for various anaesthetic events were recorded such as induction time (time from delivery of drugs to the state of complete unconsciousness), anaesthesia maintenance time (duration from induction to the administration of reversal), and recovery (duration from the administration of reversal to complete consciousness and ability to walk). The anaesthesia maintenance stage was further divided into two phases, i.e. an initial non-oxygen supplementation phase and an oxygen supplementation phase. After fifteen minutes into the anaesthesia phase, supplementary oxygen was administered using a medium feline respiratory mask attached to a medical oxygen cylinder fitted with a regulator (Midmark, England, United Kingdom) at the rate of 2 litres $minute^{-1}$ to compensate hypoxaemia (Kock & Burroughs 2012). Both phases comprised of three readings of vital signs at five-minute intervals.

Oxygen saturation, non-invasive blood pressure, pulse rate (PR) and respiration rate (f_R), and rectal temperatures (T) were observed using a Purescope Veterinary Patient Monitor IP-3000/4000 (Infunix Technology Co., Ltd., Seoul, Korea). After fifteen minutes (without oxygen supplementation) and after thirty minutes (with oxygen supplementation) into the anaesthesia maintenance stage, the arterial blood from the femoral artery was analysed through a handheld blood gas analyser (iSTAT® 1, Abbott, IL, USA); by using CG8+ Cartridge kit (Mehmood et al. 2019). Atipamezole was administered (half intravenously and

half intramuscularly) when initial signs of recovery were observed (such as pedal or palpebral reflexes and ear twitching) to assess the duration of anaesthesia.

Statistical analyses

The data were analysed through the Shapiro-Wilk test to assess its normal distribution. The vital signs and blood gas parameters before and after oxygen supplementation were analysed through Wilcoxon matched pairs test and time variables through general linear model, using STATISTICA 10 software. The results are reported as mean ± standard error.

Results

The mean actual immobilisation dose was 1.5 ± 0.1 mg kg^{-1}, and 0.03 ± 0.001 mg kg^{-1} of ketamine and medetomidine, respectively. The effects of medetomidine were reversed with 0.1 ± 0.01 mg kg^{-1} atipamezole. The mean times to first sign of drug effects (ataxia), induction, anaesthesia maintenance, and full recovery were 2.8 ± 0.3 minutes, 8.5 ± 0.5 minutes, 45.9 ± 0.8 minutes, and 19.4 ± 0.5 minutes, respectively, providing quick immobilisation and complete recovery (Appendix A).

No statistically significant difference was observed for the recorded vital signs except T and peripheral haemoglobin oxygen saturation (SpO_2) percentage ($Z = 2.4$; $p = 0.01$ and $Z = 3.7$; $p = 0.0001$ respectively) (Table 1).

The analysis of blood gas showed a statistically significant difference ($p < 0.05$) in parameters before and after oxygen supplementation such as potent of hydrogen (pH), the arterial partial pressure of carbon dioxide ($PaCO_2$), the arterial partial pressure of oxygen (PaO_2), base excess in extracellular fluid (BE_{ecf}), bicarbonates (HCO_3^-), sodium (Na^+), potassium (K^+), haemoglobin (Hb), and arterial oxygen saturation (SaO_2). Mean PaO_2 and SaO_2 values were low before oxygen supplementation (31.7 ± 0.6 mmHg and 50.6 ± 1.8 %,

119 respectively). After oxygen supplementation, the mean PaO_2 and SaO_2 values increased (91.7
120 ± 1.1 mmHg and 92.9 ± 1.2 %). There was a decrease in the pH, BE_{ecf}, Na^+, ionised calcium
121 (i Ca^{++}), and K^+. However, there was an increase in HCO_3^-, $PaCO_2$, total carbon dioxide
122 (TCO_2), and SaO_2. (Table 1). Before oxygen supplementation, PaO2 and SaO_2 were very low
123 compared to the reference range for felines. Whereas, Haematocrit (Hct) and Hb were high
124 before and after oxygen supplementation. The pH and BE_{ecf} were slightly below the reference
125 range after oxygen supplementation (Table 1).

126 ## Discussion

127 The actual dose of ketamine was lower (1.5 ± 0.1 mg kg^{-1}) compared to the documented
128 dosage of 1.90 - 5 mg kg^{-1} (Kock & Burroughs 2012; West et al. 2007). Ketamine can
129 produce analgesia and cataleptic anaesthesia by interfering with the function of nervous
130 system. With its high selectivity and affinity for adrenoceptors, medetomidine complements
131 the anaesthetic and analgesic effects of ketamine and ketamine enhances the sedative effects
132 of medetomidine. Medetomidine also lowers the required dosage of ketamine, resulting in a
133 reduction of over-excitatory state and recovery times and reversal quality (Jalanka & Roeken
134 1990).

135 Medetomidine is known to cause hypothermia in wild animals (Jalanka & Roeken 1990).
136 However, all the animals included presented a temperature (38.5 °C) marginally above the
137 one reported (38.0 ± 0.4 °C – 38.1 ± 0.8 °C) in cheetah immobilised with Ketamine-
138 Medetomidine (Stagegaard et al. 2017). Bradycardia, reduced heart rate, is regarded as one of
139 the side effects of Medetomidine in felines (West et al. 2007). Cheetah is reported to have
140 reduced HR during chemical immobilisation. However, none of the studies reported
141 complications associated with bradycardia. The mean HR of Northeast African cheetahs in
142 the current study (64.46 beats $minute^{-1}$) was similar to previously reported in similar

143 conditions (59 – 69 beats minute^{-1}) (Stegmann & Jago 2006; Stagegaard et al. 2017). MAP in
144 the current study was lower (143.2 ± 7.2 and 152.6 ± 5 mmHg before and after oxygen
145 supplementation) compared to the previous reports (176.3 ± 24.8) (Stegmann & Jago 2006).
146 Medetomidine is known to causes an initial increase and then a decrease in blood pressure
147 (Jalanka & Roeken 1990). However, in the current study, there was an increase in MAP after
148 oxygen supplementation. The reason for this increase was not known.

149 The mean SpO_2 (75 ± 2 %) before oxygen supplementation suggested hypoxemia in all
150 the cases immobilised with KM combination. The mean SaO_2 (50.6 ± 1.8 %) from blood gas
151 analysis also showed hypoxemia against the reference range (>90). Similarly, PaO_2 (31.7 ±
152 0.6 mmHg) was low. It can be associated with the suppressing effects of medetomidine and
153 ketamine on the respiratory centre of the brain (Jalanka & Roeken 1990).

154 After oxygen supplementation, the SpO_2 increased to 97.7 ± 0.3 %, showing
155 improvement and a significant difference before and after oxygen supplementation.
156 Moreover, SaO_2 (92.9 ± 1.2 %) and PaO_2 (91.7 ± 1.1 mmHg) also improved significantly
157 after oxygen supplementation. It suggests that oxygen supplementation effectively corrected
158 hypoxaemia in all procedures. However, respiratory acidosis was observed with an increase
159 in $PaCO_2$ (50.2 ± 0.5 mmHg) and a decrease in pH (7.2 ± 0.01). Hypoventilation (the
160 retention of CO_2) can lead to an increase in $PaCO_2$, contributes to hypercarbia (West et al.
161 2007). There was no significant change in f_R before or after oxygen supplementation that
162 could be attributed to the rise in $PaCO_2$. The rise in $PaCO_2$ can be due to the suppression of
163 the response of medullary respiratory centre in the nervous system and peripheral
164 chemoreceptors to detect a rise in carbon dioxide levels (Deem et al. 1998).

165 Although the HCO_3^- value was within the reference range (13.0–25.0 mmol litre^{-1}), a
166 statistically significant increase in HCO_3^- after oxygen supplementation suggested

compensation response to increased $PaCO_2$ (Hennessey & Japp 2016). The BE_{ecf} (-8.1 ± 0.4 mmol $litre^{-1}$) also suggested metabolic acidosis that improved after oxygen supplementation.

Acidosis generally causes hyperkalaemia, i.e. increase in the concentration of K^+ in the extracellular fluids. The reduction in the activity of secretory apical K^+ channels in the principal cells of the collecting ducts decreases the secretion of K^+ and its increased reabsorption in the collecting duct (Hamm et al. 2013). In the current study, although there was a statistically significant decrease in the K^+ levels after oxygen supplementation, hypokalaemia was not recorded. There was a statistically significant decrease in Na^+ concentration after oxygen supplementation, but no clinically significant difference was recorded. Moreover, no hyponatremia (i.e. lower sodium concentration) was observed. Acidosis also caused iCa^{++} to increase and in some cases, lead to hypercalcemia. However, the iCa^{++} levels were within the reference range, despite the development of acidosis (Hennessey & Japp 2016).

Although there was no statistically significant difference in Hct value before or after oxygen supplementation, the Hct value was above the reference range. The Hct value increases during hypoxaemic conditions (Hennessey & Japp 2016). However, even after the correction of hypoxaemia with oxygen supplementation, the Hct values did not return to the normal range. The results of the current study suggest that although oxygen supplementation corrected hypoxaemia, acidosis occurred with increased $PaCO_2$. However, an increase in HCO_3^- in response to acidosis suggested compensation response. The limitations of the study included a small number of individuals and non-randomisation of oxygen supplementation.

Conclusions

The current study concludes that although the KM combination, at the doses used in this study, was adequate for the restraint and handling of the Northeast African cheetahs for

191 desired short procedures, hypoxaemia occurred in all cases. Moreover, supplementary oxygen
192 compensated hypoxaemia but acidosis occurred, and $PaCO_2$ levels increased. The
193 compensatory response was observed by an increase in HCO_3^-. Oxygen supplementation shall
194 be carefully administered considering the finding of this study as it may cause academia.
195 Further studies are suggested by a higher number of individuals to assess the efficacy of
196 oxygen supplementation.

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Table 1 Comparison of vital signs and blood gas parameters before and after administration of supplemental oxygen in Northeast African Cheetahs (*Acinonyx jubatus soemmeringii*) chemically restraint with ketamine and medetomidine

Parameters	Feline Reference range*	Before oxygen supplementation (Mean ± SE) (N = 18)	After oxygen supplementation (Mean ± SE) (N = 18)	Test statistics (Wilcoxon matched pairs test)
T (°C)	-	38.5 ± 0.1	38.4 ± 0.1	**Z = 2.4; *p* = 0.01**
HR (beats minute^{-1})	-	64.9 ± 1.4	64.4 ± 2	Z = 0.9; *p* = 0.3
f_R (breaths minute^{-1})	-	19.1 ± 0.7	18.6 ± 0.6	Z = 1.1; *p* = 0.3
SpO_2 (%)	-	75 ± 2	98 ± 0.3	**Z = 3.7; *p* = 0.0001**
SAP (mmHg)	-	172.5 ± 7.7	180.7 ± 5.1	Z = 0.6; *p* = 0.5
DAP (mmHg)	-	128.6 ± 7	138.6 ± 5.1	Z = -0.9; *p* = 0.3
MAP (mmHg)	-	143.2 ± 7.2	152.6 ± 5	**Z = 2.1; *p* = 0.03**
pH	7.25–7.40	7.3 ± 0.01	7.2 ± 0.01	**Z = 3.7; *p* = 0.0001**
$PaCO_2$ (mmHg)	33.0–51.0	39.5 ± 0.6	50.2 ± 0.5	**Z = 3.7; *p* = 0.0001**
PaO_2 (mmHg)	90–110	31.7 ± 0.6	91.7 ± 1.1	**Z = 3.7; *p* = 0.0001**
BE_{ecf} (mmol litre^{-1})	(-5) – (+2)	-8.1 ± 0.4	-5.6 ± 0.5	**Z = 3.6; *p* = 0.0002**
HCO_3^- (mmol litre^{-1})	13.0–25.0	19 ± 0.4	21.1 ± 0.3	**Z = 3.6; *p* = 0.0003**
TCO_2 (mmol litre^{-1})	16–25	20.6 ± 0.3	21.6 ± 0.5	Z = 1.7; *p* = 0.09
SaO_2 (%)	>90	50.6 ± 1.8	92.9 ± 1.2	**Z = 3.7; *p* = 0.0001**
Na^+ (mmol litre^{-1})	147–162	159.1 ± 0.2	158.6 ± 0.3	**Z = 2.5; *p* = 0.01**
K^+ (mmol litre^{-1})	2.9–4.2	3.7 ± 0.04	3.6 ± 0.05	**Z = 3.1; *p* = 0.002**
i Ca^{++} (mmol litre^{-1})	1.20–1.32	1.3 ± 0.01	1.3 ± 0.01	Z = 1.4; *p* = 0.2
Hct (% PCV)	24–40	46.4 ± 0.6	46.8 ± 0.9	Z = 0.7; *p* = 0.5
Hb (g/dL)	8.0–13.0	15.8 ± 0.2	15.7 ± 0.2	**Z = 2.1; *p* = 0.03**

***** From CG8+ cartridge (iSTAT) reference manual

Note: Mean ± SE for N = 1 is for three observation each before and after oxygen supplementation; T, rectal temperature; HR, heart rate; f_R, respiration rate; SpO_2, blood oxygen saturation; SAP, non-invasive systolic arterial blood pressure; DAP, non-invasive diastolic arterial blood pressure; MAP, non-invasive mean arterial blood pressure; pH, Potent of Hydrogen; $PaCO_2$, arterial partial pressure of carbon dioxide; PaO_2, partial pressure of oxygen; BE_{ecf}, base excess in extracellular fluid; HCO_3^-, bicarbonate; TCO_2, total carbon dioxide; SaO_2,arterial oxygen saturation; Na^+, sodium; K^+, potassium; iCa^{++}, ionised calcium; Hct, haematocrit; Hb, haemoglobin.

Appendix A Times of various anaesthetic events including induction, anaesthesia, and recovery in Northeast African Cheetahs (*Acinonyx jubatus soemmeringii*) chemically restraint with ketamine and medetomidine

Variables	**Time (minutes) (n = 18)**	**Test statistics (GLM) (for individuals)**
	Mean ± SE	
Ataxia	2.8 ± 0.3	F = 0.2; P = 0.5
Sternal position	3.6 ± 0.4	F = 0.4; P = 0.8
Head down	6.2 ± 0.5	F = 0.5; P = 0.8
Induction time	8.5 ± 0.5	F = 0.2; P = 0.9
Anaesthesia time	45.9 ± 0.8	F = 0.9; P = 0.5
Recovery time to the first sign	2.4 ± 0.2	F = 0.5; P = 0.7
Recovery time to head up	3.8 ± 0.3	F = 1.8; P = 0.2
Recovery time to Sternal Position	6.4 ± 0.4	F = 0.3; P = 0.9
Time to stand	9.4 ± 0.4	F = 1.5; P = 0.3
Time to full recovery	19.4 ± 0.5	F = 0.3; P = 0.9

Note: Ataxia (lack of body coordination), induction time (time from delivery of drugs to the state of complete unconsciousness), anaesthesia maintenance time (duration from induction to the administration of reversal), and recovery (duration from the administration of reversal to complete consciousness and ability to walk)

9.4. Thermal and cardiopulmonary responses of Patterson's eland (*Taurotragus oryx pattersonianus*) to the drug combinations of thiafentanil-ketamine with medetomidine/xylazine.

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VETERINARY ANAESTHESIA AND ANALGESIA

Thermal and cardiopulmonary responses of Patterson's eland (*Taurotragus oryx pattersonianus*) to the drug combinations of thiafentanil-ketamine with medetomidine/xylazine

Journal:	*Veterinary Anaesthesia and Analgesia*
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Abstract

Objective To assess immobilisation and restraint of Patterson's eland and to evaluate the thermal and cardiorespiratory responses with thiafentanil-ketamine-medetomidine (TKM) and thiafentanil-ketamine-xylazine (TKX) combinations

Study design Randomised clinical trial

Animals A group of 40 adult semi-captive Patterson's eland, 20 males and 20 females, with an average weight (± standard error) of 624.45 ± 14.80 kg and 419.45 ± 11.76 kilograms, respectively.

Methods A total of 10 males and 10 females were immobilised with each combination from November 2016 to February 2019. The drug dosages were 0.01 mg kg^{-1} thiafentanil, 0.18 mg kg^{-1} ketamine, 0.03 mg kg^{-1} medetomidine, and 0.24 mg kg^{-1} xylazine along with the drug antagonist dosages of 0.30 mg kg^{-1} naltrexone, 0.10 mg kg^{-1} atipamezole, and 0.10 mg kg^{-1} yohimbine. The time variables for various chemical restraint phases were recorded along with the thermal and cardiopulmonary responses with the aid of a pulse oximeter. Blood gas analysis was done with an I-Stat handheld blood gas analyser. Data were analysed with inferential statistics and GLM.

Results TKM provided rapid induction (7.40 ± 0.80 minutes), while both combinations provided adequate anaesthesia and quick recoveries. There was a significant difference in rectal temperature, heart rate, respiration rate, and SpO_2 between sexes and combinations ($p < 0.05$). Blood gas analysis showed a significant difference between the combinations for pH, pCO_2, pO_2,

HCO_3, TCO_2, and haemoglobin. The lower values of blood oxygen saturation suggested mild hypoxaemia in both combinations. The cortisol level was higher in TKX as compared to TKM (23.96 ± 2.32 and 14.24 ± 0.95 nmol/L for TKX and TKM, respectively; $p < 0.05$).

Conclusions and clinical relevance Both combinations provided rapid induction, adequate anaesthesia and complete recoveries, without post immobilisation complications and re-sedation, and can be used for effective immobilisation of eland for short veterinary interventions.

Keywords α-2 adrenergic agonist, eland, opioids, pulse oximeter, thiafentanil oxalate

Introduction

Patterson's eland (*Taurotragus oryx pattersonianus*) is a subspecies of the Common Eland. If anaesthesia is not induced rapidly, eland can be extremely dangerous as they can jump and run excessively resulting in hyperthermia, myopathy, and injuries (Burroughs et al. 2012). The development of safe immobilisation protocols that allow quick induction, short processing time, and a rapid reversal might be necessary to allow safe handling and restraint of wild animals (Mehmood et al. 2019).

Potent opioids are frequently used and reliable immobilisation drugs for anaesthesia of wild and captive antelopes. Thiafentanil oxalate is a mu (μ) receptor agonist opioid and delivers shorter induction times, less cardiopulmonary depressant effects, is less potent and has a higher therapeutic index, compared to carfentanil and etorphine (Lian et al. 2016). It may be combined with ketamine and α-2 adrenergic agonists such as medetomidine or xylazine to improve muscle relaxation and reduce induction time with minimal disorder of physiological variables (Pérez 2013). Thiafentanil has been used in several herbivores such as moose, steenbok, wildebeest, giant eland and impala (Burroughs et al. 2012). Although the use of thiafentanil, medetomidine, and xylazine is reported for eland (Burroughs et al. 2012), there is no comprehensive comparison to assess the efficacy and cardiopulmonary effects of these drugs in the eland. The present study was conducted to assess the immobilisation of Patterson's eland and to evaluate the thermal, cardiorespiratory, and stress responses with thiafentanil-ketamine-medetomidine (TKM) and thiafentanil-ketamine-xylazine (TKX) combinations.

Materials and Methods

Study animals

A group of 40 adult (5-7 year old) eland (20 males and 20 females) were immobilised between November 2016 and February 2019 for routine translocations and veterinary operations with the approval of Institutional Animal Care and Use Committee of BNR-SBY, Ref. no. BNR/SBY/00182014. The males and females weighed 624.45 ± 14.80 kg (Mean ± Standard Error) and 419.45 ± 11.76 kg, respectively. All the animals were healthy and young (not geriatric or pregnant) without any symptoms of any underlying disease and were rated as ASA (American Society of Anaesthesiologists) Class 1 (Doyle & Garmon 2018).

Chemical restraint

Drugs used were thiafentanil oxalate (Thianil 10 mg mL^{-1}) antagonised with naltrexone hydrochloride (Trexonil, 50 mg mL^{-1}) from Wildlife Pharmaceuticals Pty Ltd, Mpumalanga, South Africa); medetomidine hydrochloride (Ilium Medetomidine, 10 mg mL^{-1}) antagonised with atipamezole hydrochloride (Ilium Atipamezole, 5 mg mL^{-1}), xylazine hydrochloride (100 mg mL^{-1}, Xylazil-100), and ketamine hydrochloride (100 mg mL^{-1}, Ketamil Injection) from Troy Laboratories, NSW, Australia. Xylazine was antagonised with yohimbine hydrochloride (Reverzine™ Injection; Bayer, Pymble, NSW, Australia). The target drug agonist dosages of 0.01 mg kg^{-1} (4-6 mg) thiafentanil, 0.18 mg kg^{-1} (70-140 mg) ketamine, 0.03 mg kg^{-1} (12-20 mg) medetomidine, 0.24 mg kg^{-1} (100-160 mg) xylazine, 0.30 mg kg^{-1} (120-175 mg) naltrexone, 0.10 mg kg^{-1} (20-55 mg) atipamezole, and 0.10 mg kg^{-1} (40-80 mg) yohimbine were developed according to the weight and sex of the animals, previous field experience, and literature (Burroughs et al. 2012). The actual drug dose administered was later calculated after weighing the animals (Mehmood et al. 2019; Lescano et al. 2014). A simple random sampling technique was used for the choice of drug combinations and animals. Each anaesthetic event was assigned a computer-generated random number in drug groups, and the animals were immobilised in the order from

least to the greater random number. The observers collecting and processing samples and monitoring physiological variables were double-blinded (Mehmood et al. 2019). The drug was delivered intramuscularly (IM) in the hindquarters through Dan-Inject CO2 Injection Rifle (Model No. 0471 JM, Dan-inject, Kolding, Denmark) (Burroughs et al. 2012).

Monitoring

Time variables recorded were; times from administration of anaesthetic drugs to ataxia, sternal recumbency, head-down, and completion of induction. The time of anaesthesia maintenance was calculated from induction until the administration of drug antagonists. The time variables after administration of drug antagonists included the times to the first effect of antagonists (ear twitching and blinking), head-up, standing up, and complete recovery (Mehmood et al. 2019). After the animal entered an unconscious state, it was immediately placed in sternal recumbency (Burroughs et al. 2012). Rectal temperature, non-invasive blood pressure, and blood oxygen saturation (SpO_2) were recorded with a pulse oximeter (Purescope Veterinary Monitor IP-3000/4000 Series, Infunix Technology Co. Ltd, Korea). Mean arterial pressure (MAP) was calculated from systolic and diastolic arterial pressures. The movement of the thoracic cavity was observed to record the respiration rate (f_R) minute^{-1}. Heart rate (HR) was recorded by observing thoracic auscultation (minute^{-1}) (Mehmood et al. 2019). Data on physiological variables was recorded five times at an interval of every five minutes on a data sheet. After 15 minutes into the anaesthesia maintenance phase, arterial blood was drawn from the femoral artery and analysed via a iSTAT® point of care blood gas analyser (Abbott, Illinois, USA) with CG8 plus analysis cartridge kit (Mehmood et al. 2019).

Data Analyses

The data were subjected to a general linear model (GLM) after its assessment for normality via a Shapiro-Wilk normality test using the statistical software, STATISTICA version 10 (Stat Soft Inc. Oklahoma, USA).

Results

All 40 animals were immobilised successfully without any failure or post restraint complications. The mean dose of thiafentanil was 0.010 ± 0.0001 mg kg^{-1} antagonised with 0.30 ± 0.004 mg kg^{-1} (TKM) and 0.30 ± 0.003 mg kg^{-1} naltrexone (TKX). The mean dose of ketamine was 0.176 ± 0.003 mg kg^{-1} and 0.176 ± 0.007 for TKM and TKX, respectively. The mean doses of medetomidine and xylazine were 0.033 ± 0.0004 mg kg^{-1} and 0.235 ± 0.008 mg kg^{-1}, respectively. Medetomidine was antagonised with 0.07 ± 0.004 mg kg^{-1} atipamezole and xylazine with 0.11 ± 0.004 mg kg^{-1} yohimbine.

Both combinations provided quick induction, adequate anaesthesia and complete recovery. However, there was a significant difference in the times to recumbency ($F = 5.42$; $p = 0.02$), head down ($F = 7.85$; $p = 0.008$), induction ($F = 7.13$; $p = 0.01$) and lifting head after the reversal ($F = 4.72$; $p = 0.03$). The results are summarised in Table 1. TKM provided quicker recumbency and induction times than TKX. The anaesthesia maintenance and complete recovery times were similar in both combinations (Table 1).

There was a significant difference in rectal temperature, heart rate, respiration rate, and percutaneous haemoglobin oxygen saturation percentages (Table 1). All the immobilised animals had excessive salivation, higher in TKX combination. Blood gas analysis showed a significant difference between the combinations for pH, partial pressure of carbon dioxide (TKX = 43.40 ± 1.34; TKM = 48.97 ± 1.44), the partial pressure of oxygen, base excess in the extracellular fluid

120 (TKX = -4.60 ± 1.68; TKM = 0.35 ± 1.47), bicarbonates (TKX = 22.11 ± 1.44; TKM = 27.18 ±
121 1.11), total carbon dioxide (TKX = 23.15 ± 1.45; TKM = 28.80 ± 1.13), and haemoglobin (TKX
122 = 11.46 ± 0.30; TKM = 9.69 ± 0.24). Lower values of blood oxygen saturation suggested mild
123 hypoxaemia. Cortisol levels were higher in TKX compared to TKM (Table 1). There were a
124 gradual decrease in temperature and heart rate and an increase in oxygen saturation and respiration
125 rate (Appendix 1).

126 **Discussion**

127 Eland, when compared to other species, e.g. impala, require significantly higher doses of sedatives
128 and tranquilisers to permit adequate immobilisation (Burroughs et al. 2012). In the current study,
129 two combinations of opioids, cyclohexylamines, and alpha-2 adrenoceptor agonists (TKM and
130 TKX) were compared. The dose of thiafentanil required to permit immobilisation was reduced
131 when compared to previously reported results in eland (Burroughs et al. 2012). In the present
132 study, the addition of cyclohexylamine, ketamine, and α-2 adrenergic agonists, medetomidine or
133 xylazine, may have potentiated the immobilisation provided by thiafentanil (Pérez 2013).
134 Ketamine an *N*-Methyl-D-aspartate antagonist, inhibits thalamocortical communication via
135 interneurons, resulting in dissociative anaesthesia characterised by loss of consciousness,
136 analgesia, and muscular hypertonicity, and maintains cardiac output indirectly with negligible
137 effects on respiratory function at normal doses.

138 In the current study, both combinations provided rapid ataxia and induction. Thiafentanil is
139 known to provide rapid induction compared to other morphine derivatives. TKM provided swift
140 sternal recumbency and complete induction characterised by absence of pedal and palpebral
141 reflexes, and response to external stimuli. Various studies measured the quality of anaesthesia by

142 loss of reflexes and autonomic responses. However, these criteria may mislead, and the animal
143 may suffer pain without an ability to respond. Moreover, cyclohexylamines and opioids may also
144 interfere with certain physiological responses such as heart rate and blood pressure, thus not
145 reflecting true state and depth of anaesthesia (Whelan & Flecknell 1992).

146 Ketamine is identified as improving the effects of α-2 adrenergic agonists, especially
147 medetomidine (Mehmood et al. 2019). Medetomidine has higher lipophilic properties that cause
148 its quick absorption and effects. The animals achieved sternal recumbency characterised by low
149 complete relaxation of the neck, permitting the head to rest on the ground. The time to achieving
150 this was shorter in animals darted with TKM compared to TKX. This may be associated with the
151 presence of α-2 adrenergic agonists that are known to have analgesic, myorelaxant and sedative
152 properties (Burroughs et al. 2012). Both combinations provided adequate anaesthetic depth for
153 blood and faecal collection. The induction times were longer as compared to previous studies with
154 TKM on Axis deer, although the elands had smooth induction without any complication as
155 compared to the rough induction reported for Axis deer (Smith et al. 2006).

156 In the current study, no re-sedation occurred after the administration of reversals. Naltrexone
157 is known to inhibit re-narcotisation. However, xylazine is known to cause re-sedation attributed to
158 yohimbine which partially antagonises the effects of xylazine due to its low alpha-2/alpha-1
159 selectivity ratio. Moreover, residual motor impairment is associated with the use of ketamine that
160 does not have an antagonist and may result in rough recoveries if reversals for other drugs are
161 given too early (Mehmood et al. 2019).

162 A study suggested the vulnerability of giant eland to hyperthermia (Pye et al. 2001). In the
163 current study, hyperthermia was not observed as the temperatures were within the reported range

of 37.6 °C to 42.2 °C, which can be associated with reduced ataxia and induction times. The time of the immobilisation event is also critical and may significantly contribute towards hyperthermia, especially in hot climatic zones. Therefore, immobilising animals early morning may also have contributed to the reduction of hyperthermia.

The heart rate was slightly lower in TKM compared to TKX; however, it was not below the described range (50-70 beats per minutes) (Pye et al. 2001). Both medetomidine and xylazine are known to cause bradycardia. When ketamine is combined with xylazine, this side effect is reduced by vagolytic and cardiotonic actions. Similar findings were described for impala immobilised with thiafentanil and medetomidine (Meyer et al. 2008).

Opioids are recognised as contributing to respiratory depression by rendering the animals incapable of regulating gas exchange, cardiac output, ventilation and dilation of the spleen, leading to hypoxaemia (Lian et al. 2016). Moreover, α-2 adrenergic agonists also affect the normal cardiorespiratory function. There was a significant difference in respiration rate where TKM showed lower breaths per minute compared to TKX. Studies conducted on dogs also suggest respiratory depression mediated by medetomidine via the activation of the α-2 receptor in the locus coeruleus (Lerche & Muir 2004).

There was a significant difference in both combinations for cortisol levels; suggesting TKM had lower cortisol concentrations compared to TKX. The α-2 adrenergic agonists, especially medetomidine, are known to obtund the stress response via inhibition of the adrenomedullary, sympathoadrenal, and nociceptive functions (Benson et al. 2000).

184 The limitations of the study include single blood gas analysis for each immobilisation. More
185 samples could provide better understanding of blood gas parameters at later stages of anaesthesia.
186 Invasive blood pressure (IBP) could have also provided reliable observations.

187 **Conclusions**

188 Both immobilisation protocols provided rapid and reliable sedation adequate to permit short
189 minimally invasive procedures. Rapid recovery from anaesthesia was achieved using antagonists,
190 and no residual effects were noted. Animals immobilised with TKM showed rapid induction,
191 exhibited statistically but not clinically significant hypoxaemia and respiratory depression when
192 compared to TKX (although the values were within the acceptable range for the eland).

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Table 1 Time variables, vital signs, and blood gas parameters during field immobilisation of Patterson's Eland (*Taurotragus oryx pattersonianus*) with Thiafentanil-Ketamine-Medetomidine and Thiafentanil-Ketamine-Xylazine combinations

Variables/Parameters	**TKX (n = 20)**	**TKM (n = 20)**	**GLM** (P<0.05)
	(Mean ± SE)		
Ataxia (minutes)	3.15 ± 0.78	2.35 ± 0.13	
Sternal recumbency (minutes)	7.85 ± 1.34	4.55 ± 0.72	*C: (F = 5.42; p = 0.02)*
Head down (minutes)	8.95 ± 1.35	4.55 ± 0.72	*C: (F = 7.85; p = 0.008)*
Complete induction (minutes)	11.8 ± 1.40	7.40 ± 0.80	*C: (F = 7.13; p = 0.01)*
Anaesthesia maintenance (minutes)	46.0 ± 1.29	46.10 ± 0.91	
First effect of antagonists* (minutes)	1.20 ± 0.09	1.10 ± 0.07	
Head-up (minutes)	1.55 ± 0.15	1.45 ± 0.11	*SC: (F = 4.72; p = 0.03)*
Standing up (minutes)	2.20 ± 0.27	2.35 ± 0.22	
Complete recovery (minutes)	11.70 ± 0.48	11.15 ± 0.53	
Rectal Temperature (°C)	39.94 ± 0.20	40.24 ± 0.14	*S: (F = 8.5; p = 0.006)*
HR (beats minute^{-1})	61.42 ± 1.57	59.67 ± 1.83	*S: (F = 12.6; p = 0.001)*
f_R (breaths minute^{-1})	18.45 ± 0.84	16.11 ± 0.57	*S: (F = 14.7; p = 0.0004)* *C: (F = 7.98; p = 0.007)*
SpO_2 (%)	94.70 ± 1.67	93.48 ± 1.24	*S: (F = 16.6; p = 0.0002)*
SAP (mmHg)	142.09 ± 3.27	145.03 ± 2.97	
DAP (mmHg)	100.72 ± 2.96	96.67 ± 3.12	
MAP* (mmHg)	114.5 ± 2.9	112.8 ± 3.0	
pH	7.27 ± 0.02	7.34 ± 0.02	*C: (F = 5.9; p = 0.020)*
pCO_2 (mmHg)	43.40 ± 1.34	48.97 ± 1.44	*S: (F = 27.06; p = 0.000008)* *C: (F = 13.91; p = 0.0006)*
pO_2 (mmHg)	90.50 ± 0.63	89.60 ± 0.68	*C: (F = 109.01; p = 0.000)*
BEecf (mmol/L)	-4.60 ± 1.68	0.35 ± 1.47	*C: (F = 5.02; p = 0.03)*
HCO_3^- (mmol/L)	22.11 ± 1.44	27.18 ± 1.11	*C: (F = 8.24; p = 0.006)*
TCO_2 (mmol/L)	23.15 ± 1.45	28.80 ± 1.13	*S: (F = 4.58; p = 0.03)* *C: (F = 10.41; p = 0.002)*
sO_2 (%)	86.25 ± 1.59	88.30 ± 0.93	
Na (mmol/L)	143.0 ± 0.59	143.25 ± 0.41	
K (mmol/L)	4.47 ± 0.14	4.63 ± 0.10	
Ca (mmol/L)	1.06 ± 0.02	1.03 ± 0.02	*S: (F = 9.87; p = 0.003)*

Hb (g/dL)	11.46 ± 0.30	9.69 ± 0.24	*C: (F = 23.64; p = 0.00002)*
Cortisol (nmol/L)	23.96 ± 2.32	14.24 ± 0.95	*S: (F = 4.17; p = 0.04)* *C: (F = 17.61; p = 0.0001)*

TKX, Thiafentanil-Ketamine-Medetomidine; TKM, Thiafentanil-Ketamine-Medetomidine; F, F value of General linear model; Sexes, S; Combinations, C; Sexes*Combinations, SC; SE for measurements of vital signs in an animal immobilised once (N=1) indicates SE of 5 repeated / consecutive measurements during one immobilisation event; HR, heart rate; f_R, respiration rate; SpO_2, blood oxygen saturation; SAP, non-invasive systolic arterial blood pressure; DAP, non-invasive diastolic arterial blood pressure; MAP, non-invasive mean arterial blood pressure pCO_2, partial pressure of carbon dioxide; pO_2, partial pressure of oxygen; BEecf, base excess in extracellular fluid; HCO_3^-, bicarbonate; TCO_2, total carbon dioxide; sO_2,oxygen saturation; Na, sodium; K, potassium; Ca, ionised calcium; Hb, haemoglobin. * ear twitching or blinking

Appendix 1 Cardiorespiratory variables at different time intervals during field immobilisation of Patterson's Eland (*Taurotragus oryx pattersonianus*) with Thiafentanil-Ketamine-Medetomidine and Thiafentanil-Ketamine-Xylazine combinations

Variables	**Drugs**	Time into the anaesthesia maintenance (Mean ± SE)				
		5 min	10 min	15 min	20 min	25 min
T (°C)	TKX	40.0 ± 0.2	40.0 ± 0.2	39.9 ± 0.2	39.9 ± 0.2	39.9 ± 0.2
	TKM	40.3 ± 0.1	40.4 ± 0.1	40.2 ± 0.1	40.2 ± 0.1	40.1 ± 0.1
HR (beats minute^{-1})	TKX	63.0 ± 1.7	61.9 ± 2.1	61.5 ± 1.7	61.3 ± 1.6	59.6 ± 1.5
	TKM	61.1 ± 2.0	60.1 ± 2.0	59.8 ± 2.0	59.1 ± 1.8	58.4 ± 2.0
f_R (breaths minute^{-1})	TKX	20.4 ± 1.4	18.0 ± 0.9	18.0 ± 0.7	17.9 ± 0.8	18.1 ± 0.8
	TKM	16.0 ± 0.8	16.4 ± 0.6	16.4 ± 0.6	15.7 ± 0.5	16.1 ± 0.5
SpO_2 (%)	TKX	92.8 ± 2.4	92.7 ± 2.3	94.3 ± 1.7	96.4 ± 1.4	97.4 ± 1.1
	TKM	89.9 ± 3.1	90.1 ± 2.5	92.8 ± 1.7	96.9 ± 0.5	97.9 ± 0.6
SAP (mmHg)	TKX	142.0 ± 3.7	144.2 ± 4.8	142.9 ± 4.9	141.3 ± 2.9	140.1 ± 2.2
	TKM	147.8 ± 4.0	143.8 ± 4.0	144.2 ± 4.0	143.8 ± 2.4	145.7 ± 2.4
DAP (mmHg)	TKX	98.0 ± 3.4	101.2 ± 4.7	100.6 ± 5.1	102.0 ± 2.6	101.9 ± 2.6
	TKM	98.7 ± 3.9	93.9 ± 3.9	93.8 ± 4.5	97.2 ± 2.6	99.9 ± 2.7
MAP* (mmHg)	TKX	112.7 ± 3.4	115.5 ± 4.6	114.7 ± 4.9	115.1 ± 2.5	114.6 ± 2.2
	TKM	115.0 ± 3.9	110.5 ± 3.9	110.6 ± 4.2	112.7 ± 2.5	115.1 ± 2.4

SE for measurements of vital signs in an animal immobilised once (N=1) indicates SE of 5 repeated / consecutive measurements during one immobilisation event; T, Rectal temperature; HR, heart rate; f_R, respiration rate; SpO_2, blood oxygen saturation; SAP, non-invasive systolic arterial blood pressure; DAP, non-invasive diastolic blood pressure; MAP, Mean arterial blood pressure; * Calculated

ABID MEHMOOD

RÉSUMÉ

CONTACT	PERSONAL INFO
Mobile: +971506632023 **Email:** abid@wildbiodiversity.org abidmehmoodsabir@gmail.com	Date of Birth: 19-10-84 Religion: Islam Nationality: Pakistan Country of Residence: United Arab Emirates

PROFESSIONAL SUMMARY

I am an Experienced Manager with a demonstrated history of working with wildlife and conservation industry. Skilled in wildlife management, animal restraint, conservation, animal welfare, animal husbandry, operations management, team building and training, developing policies, procedures and SOPs, with 11 international publications, well-developed presentation and reporting skills. I have a strong professional background with a Doctor of Philosophy, PhD (in final term) in wildlife and conservation. I can efficiently plan, organise and prioritise responsibilities to meet schedules/deadlines without close supervision.

HIGHEST EDUCATION DEGREE: PhD (Thesis submission)

PROFESSIONAL EXPERIENCE: 10 Years, 06 Months (+ 1 year 4 months as M.Sc./M.Phil. Researcher)

KEY POSITIONS HELD:

1. Conservation Manager (Present)
2. Conservationist, ZIMS (Present)
3. Member, IUCN-SSC Reintroduction Specialist Group (Present)
4. Assistant Conservation Manager
5. Senior Wildlife Biologist/Sr. Wildlife Group Supervisor
6. PhD Researcher (Present)
7. Research Associate
8. Reptiles' Curator
9. Lead Conservationist (Present)

KEY SKILLS / CORE QUALIFICATIONS:

PROJECT & TEAM MANAGEMENT

1. Project Management
2. Operations Management
3. Team Leadership & Supervision
4. Strategy and Policy Development
5. Staff and Resources' Management
6. Cross-functional Team Management
7. Team Capacity building and Training

FINANCIAL

1. Project Budget Management
2. Procurement
3. Asset inventories
4. Asset Management
5. Cost Analysis
6. Budget allocation
7. Annual financial projections

WRITING SKILLS

1. Project Proposals
2. SOPs, Policies, Procedures
3. Technical Reports
4. Scientific Publications
5. Annual Reports
6. Progress Analysis
7. Presentations

WILDLIFE MANAGEMENT, CONSERVATION, ECOTOURISM & RESEARCH

- Collections' Management (Reptiles, Birds, Ungulates, Carnivores)
- Animal Husbandry (nutrition, camps management, exhibits' management, breeding)
- Animal Population Management (population control, physical and chemical restraints)
- Conservation (breeding loans, maintenance of diverse and healthy genetic pool, species reintroductions)
- Data Management (data management through Zoological Information Monitoring System-ZIMS)
- Collaboration with national / international organisations
- Camera Trapping / remote animal monitoring
- Habitat Assessment & Enrichment
- Biodiversity Monitoring
- Eco-tourism
- Scientific Research
- Inventories of Protected Areas
- Specimen Preservation
- Live Animal Trapping
- Animal behaviour and Ecology
- Animal welfare

PUBLICATIONS:

Published in International Journals = 13
Abstracts Published: 06
Manuals: 02
Standard Operating Procedures (Total = 22) on • Feeding • Research • Medicine • ZIMS • Live Animal Sale • Census • Safety • Monitoring • Wildlife-Human incident • Post mortem • Incineration • Ecological surveys • Animal Restraint • Biosecurity • Animal Exchange and donations

OTHER PROFESSIONAL AFFILIATIONS

S.	ORGANISATION NAME	ROLE
1	Wildbiodiversity Conservation Services	Lead Conservationist
2	IUCN-Species Survival Commission, Reintroduction Specialist Group	Member

PROFESSIONAL EXPERIENCE (MANAGEMENT / OPERATIONS / RESEARCH / LAB)

POSITION: MANAGER, WILDLIFE AND CONSERVATION

COMPANY:	Barari Forest Management
DURATION:	From November, 2014 till present (current position) – 5 Years and 6 months

RESPONSIBILITIES, ACHIEVEMENTS & SKILLS ACQUIRED

- Animal Collection, Safari & Exhibit Management
- Conservation Introductions, Genetic Improvement, Exchange programs & Breeding Loans' Management
- Animal Husbandry and population management
- Veterinary Care Management
- Eco-tourism, Media Events
- Animal exchanges, donations, and sales
- Coordination with National and International Conservation Organisations
- Data Management, ZIMS, Scientific publications, Reports & Presentations
- Procurement of Animal Feed, Medicine, veterinary supplies, materials & equipment
- Asset and team management

Distinctions & Appreciations

- Lead Member of Alpha Team Series in Al Bayan News Magazine
- Certificate of Appreciation for successful Conservation Introduction of Arabian Tahr
- Certificates of Appreciation for Hard Work and Good Performance for 2015, 2016, and 2017
- Certificate of Participation in environment friendly Walk by Abu Dhabi Airport Authority

POSITION: ASSISTANT MANAGER, WILDLIFE AND CONSERVATION

ORGANISATION:	Barari Forest Management
DURATION:	From November, 2013 till November, 2014 (01 Year)

RESPONSIBILITIES, ACHIEVEMENTS & SKILLS ACQUIRED

- Project Mobilisation
- Animal Husbandry, Nutrition & Feeding
- Arabian Wildlife Park, Safari, Animal Camps, Quarantines, Capture Units & Exhibits'
- Habitat Enrichment
- Population Monitoring, Animal Reproduction and Breeding Programs
- Physical Capture, Caging, Crating, Loading & translocation of Reptiles, Birds, Carnivores & Ungulates
- Chemical Capture of Carnivores, Ungulates
- Biosecurity and Carcass Incineration
- Staff and Vehicles Management
- Reptiles' Management and Pest Birds' Control
- Management of Marine Turtles and Avifauna Monitoring

Distinctions & Appreciations:

- Certificate of Appreciation for Hard Work and Good Performance for 2014
- Certificate of Appreciation for Hard Work and Good Performance for 2013

POSITION: SENIOR WILDLIFE SUPERVISOR (WESTERN REGION); REPTILES CURATOR

ORGANISATION:	Barari Forest Management
DURATION:	02 Years (From November, 2011 till November, 2013)

RESPONSIBILITIES, ACHIEVEMENTS & SKILLS ACQUIRED

- Protected Wildlife Reserves' Supervision (28,000 animals in 20 Wildlife forests & reserves)
- Asset & Stores inventories supervision
- Staff and staff residence supervision
- Vehicles and logistics operations
- Reptiles Curator
- Herpetologist/Consultancy to clients
- Bu Arta Hunting Area Supervisor
- Al Na'am Holding Facility supervisor

Distinctions & Appreciations: Certificate of Appreciation for Hard Work & Good Performance, 2012

POSITION: RESEARCH ASSOCIATE

ORGANISATION:	Arid Agriculture University, Rawalpindi, Pakistan
DURATION:	From 2nd November, 2009 till 20th November, 2011 (02 Years)

RESPONSIBILITIES, ACHIEVEMENTS & SKILLS ACQUIRED

- Protected Areas Inventories of National Park (IUCN category II), Wildlife Sanctuary (IUCN category I-a and I-b), and Game Reserve (IUCN category VI)
- Community Awareness
- Lab Establishment & Procurement and Financial management
- Planning Research & data collection
- Staff & Student Management
- Special Assignment on Human Wildlife conflict
- Training students in wildlife research and study techniques
- Successful assistance in research of: Habitat preference, population, ecology, breeding, feeding, distribution, social organisation and threats of Grey partridge, Indian pangolin, Indian peafowl, Little grebe, Red jungle fowl, Punjab Urial, Rhesus monkey, and Desert Hare

POSITION: M PHIL / M SC RESEARCHER

ORGANISATION:	Arid Agriculture University, Rawalpindi, Pakistan
DURATION:	From April, 2008 till July, 2009 (16 months)

RESPONSIBILITIES, ACHIEVEMENTS & SKILLS ACQUIRED

Study Design, field work: Approvals and permits, arrangement of logistics and equipment, Field data Collection & Recording observations, Trapping, capturing, handling small mammals
Lab work: Anaesthesia of small mammals, Blood, faecal and Urine sample collection, Bacterial culturing and Identification of pathogenic bacteria
Data Management: Analysis of data, Technical reports and Theses, Scientific articles and manuscripts
Study and Field experience acquired in: Conservation, wildlife management, Ecology, animal behaviour, Husbandry, Animal nutrition, population monitoring, integrated pest management, Vegetation analysis, lab work

ACADAMIC QUALIFICATIONS

DEGREE	**PhD (Doctor of Philosophy)**; 2015-2020 (Cont.) **Field:** Animal Sciences
UNIVERSITY	Czech University of Life Sciences, Czech Republic
SUBJECTS	**Research:** Chemical Immobilisation of Carnivores & Ungulates **Subjects:** Ecology; Research Methodology & Design; Management of Research; Biochemistry; Special Molecular Genetics; Animal breeding in tropics & subtropics; Fundamentals of Livestock
DEGREE	**MPhil (Master of Philosophy)**; 2011 **Field:** Wildlife Management
UNIVERSITY	PMAS, Arid Agriculture University Rawalpindi, Pakistan
SUBJECTS	**Research:** Population Density and Habitat Preference of Indian Peafowl (*Pavo cristatus*) in Deva Vatala National Park **Subjects:** Protected Areas Management; Endangered Species & their management; Essentials of Wildlife Management; Bio-statistical Analysis; Forestry Recreation & Park Management; Terrestrial Wildlife Management; Wildlife Food and Foraging; Wildlife Policy, Legislation & International Conventions; Forestry & Environment
DEGREE	**MSc (Master of Science)**; 2009 **Field:** Wildlife Management
UNIVERSITY	PMAS, Arid Agriculture University Rawalpindi, Pakistan
SUBJECTS	**Research:** Prevalence of Salmonella in small mammals inhabiting poultry farms **Subjects:** Mammalogy; An Introduction to Wildlife of Pakistan; Principals of Wildlife Management; Wildlife Study; Techniques Biological Aspects; Elements of Statistics & biometry; Wildlife Study Techniques-II: Management Aspects; Reproductive Biology and Breeding; Ornithology; Experimental Statistics; Wildlife Population Ecology; Wildlife Management at Wetlands; Herpetology; Wild Fish Fauna of Pakistan

TRAININGS AND CERTIFICATIONS

Training/Certification	Year	Institution
Agricultural Fence Design	2019	Udemy
Fundamentals of GIS	2019	University of California, Davis (through Coursera)
Data Analysis in Ecology	2017	Oxford University, United Kingdom
Certified Effective Time Manager	2017	Mind Tools Club, United Kingdom

IELTS	2017	IELTS-IDP
Chemical Immobilisation	2014	Barari Forest Management, UAE
Wild Game Capture	2014	Barari Forest Management, UAE
Writing in the Sciences	2014	Stanford University, USA
ZIMS	2014	Barari Forest Management, UAE
Achieving excellence at work	2013	Brain Power Institute, Dubai, UAE
Computer Software	2003	Soft Logix, Pakistan
English Language Course level-III	2002	Federal Institute of Modern Languages, Pakistan

COMPUTER SKILLS

ZIMS; Windows; DOS; M.S Word; M.S PowerPoint; M.S Excel; M.S Access; M.S Front Page; Coral Draw 9.0; Adobe Photoshop; In-Page (Urdu); E-mail/Internet; Networking Essentials; Google Earth & Mapping

SCIENTIFIC MANUSCRIPTS AND RESEARCH PUBLICATIONS

INTERNATIONAL PUBLICATIONS

1	Kabeer B, Bilal S, Abid S, Mehmood A, Asadi MA, Jilani MJ, Hejcmanová P. 2020. Some Aspects of Breeding Ecology and Threats to Saunders's Tern (*Sternula saundersi*) at an Off-Shore Island of United Arab Emirates. Waterbirds. (Accepted)
2	Kabeer B, Bilal S, Abid S, Hejcmanová P, Asadi MA, Jilani MJ, **Abid Mehmood**. **2020**. Breeding of the Osprey, *Pandion haliaetus*, in natural and artificial nesting substrates in the United Arab Emirates (Aves: Accipitriformes). Zoology in the Middle East. 280-282
3	Kabeer B, Bilal S, Abid S, **Abid Mehmood**, Asadi MA, Jilani MJ, Hejcmanová P. **2020**. Determining population trend and breeding biology of Common Kestrel (*Falco tinnunculus*) at Sir Bani Yas Island of Emirates. Journal of Animal and Plant Sciences 30 (5). (Accepted)
4	**Abid Mehmood**, Abid S, Hejcmanova P, Asadi MA, Kabeer B, Jilani MJ, Bilal S, Ashraf MW. **2019**. Comparison of physiological responses of Arabian striped hyaena (*Hyaena hyaena sultana*) to effective immobilisations with ketamine-medetomidine and ketamine-xylazine in (semi-) captive conditions. PeerJ 7:20
5	Goursi UH, **Abid Mehmood**, Sajid M, Kabir M. **2019**. Conservation of Indian Rok Python in Azad Jammu and Kashmir, Pakistan. International Journal of Conservation Science 10:543–554
6	Kabeer, B., Anwar, M., Rais, M., Jilani, M. J., Asadi, M. A., Abid, S., Bilal, S., Saleem, F., Ahmed, B. H., Yunus, A. W., Zahid, S., Anjum, M., Hejcmanova, P., Sheikh, M. K. and **Abid Mehmood**. **2018**. Study of Feed Preference of Endangered Hog Deer Under Captive Conditions in Pakistan. - Int. J. Conserv. Sci. 9: 337–344
7	Al-Dhaheri S, Soorae PS, De-Kock M, **Abid Mehmood**, Gouws A, Burns K, Rapaie M, Al-Nassan I, Cole J, Zoywed H, Al-Zaabi R. **2017**. Conservation introduction of the Arabian Tahr to Sir Bani Yas Island, Abu Dhabi Emirate UAE : challenges and lessons learnt. Journal of Zoo and Aquarium Research: 5, 137–141.
8	Goursi, U.H., Kabir, M., **Abid Mehmood**, **2017**. Occurrence of Russell's Chain Viper *Daboia Russelii Russelii* in Deva Vatala National Park, Azad Jammu and Kashmir. Int. J. Conserv. Sci. 8, 281–288.
9	Anwar, M., **Abid Mehmood,** Rais, M., Hussain, I., Ashraf, N., Khalil, S., Qureshi, B. D. **2015**. Population Density and Habitat Preference of Indian Peafowl (*Pavo cristatus*) in Deva Vatala National Park, Azad Jammu & Kashmir, Pakistan. Pakistan J. Zool., vol. 47(5), pp. 1381-1386.
10	Goursi, U. H., Rapaie, M., **Abid Mehmood**. **2015**. Conserving the Hidden Nature: An Overview on Conservation Efforts in United Arab Emirates (UAE). Journal of Annual Research and Review in Biology. 7(6): 408-414 p.
11	**Abid Mehmood,** M. S. Ansari, S. Akhter, A. A. Khan, I. Hussain, Shams-ul-Hassan, T. Z. Qureshi and B. A. Rakha. **2012**. Occurrence of Pathogenic Bacteria in Small Mammals - Inhabiting Poultry Farms of Rawalpindi/Islamabad, Pakistan. Pakistan J. Zool., vol.44 (4), pp.1185-1187.
12	**Abid Mehmood**, M. S. Ansari, T. Hussain, S. Akhter, S. A. Khan, S. Hassan, A. A. Khan and B. A. Rakha. **2012**. Common shrew (*Suncus murinus*): A potential reservoir of pathogenic bacteria at poultry farms, Rawalpindi/Islamabad. Pakistan J. Zool., vol. 44(3), pp. 879-880.
13	**Abid Mehmood**, M. S. Ansari, T. Hussain, S. Akhter, S. A. Khan, S. Hassan, A. A. Khan and B. A. Rakha. **2011**. Bandicoot Rat (*Bandicota bengalensis*): A Novel Reservoir of Pathogenic Bacteria at Poultry Farms, Rawalpindi / Islamabad, Pakistan. Pakistan J. Zool., vol. 43(1), pp. 201-202.

PUBLISHED MANUALS

1	**Abid Mehmood**, Soorae P, Gouws A, Burns K, and Blaauw S. **2014**. A Conservation Introduction of Arabian tahr on Sir Bani Yas Island: Operational Management. Barari Forest Management, Environment Agency Abu Dhabi, Al Bustan Zoological Centre, Tourism Development and Investment Company, Abu Dhabi United Arab Emirates

2	**Abid Mehmood**, Soorae P, Gouws A, Burns K, Sarwar G, and Blaauw S. **2014**. A Conservation Introduction of Arabian tahr on Sir Bani Yas Island: Site Selection. Barari Forest Management, Environment Agency Abu Dhabi, Al Bustan Zoological Centre, Tourism Development and Investment Company, Abu Dhabi United Arab Emirates
CONFERENCE ABSTRACTS/POSTERS	
1	Abid S, **Abid Mehmood**, Hejcmanová P. **2020**. Prey preference and hunting behavior of Arabian Striped Hyaena (*Hyaena hyaena sultana*) at Sir Bani Yas Island, United Arab Emirates. Page 40th Pakistan Congress of Zoology (International). Abstract in: The Zoological Society of Pakistan, Pakistan.
2	Abid S, Bilal S, **Abid Mehmood**, Kabeer B, Hejcmanová P. **2019**. Individual variation in hunting behaviour of cheetahs in an intensively managed space-limited reserve at the Sir Bani Yas Island (United Arab Emirates). Abstract in: Wildlife Research and Conservation 2019 (WRC2019). Berlin, Germany.
3	Kabeer B, Hejcmanová P, **Abid Mehmood**, Shah JN, Ahmed S, Jilani MJ, Asadi MA, Ashraf W, Al-Nassan I, Al-Balooshi K, Al-Qubaisi B, Abid S, Bilal S. **2017**. Monitoring waders at one of the offshore islands of the United Arab Emirates: a case study in Sir Bani Yas. WSG Annual Conference15-18 September 2017, Prague. Abstracts of posters: 163-164
4	Ashraf, M.W., **Abid Mehmood**, Kabeer, B., Jilani, M.J., Asadi, M.A., Al Nassan, I., Al Qubaisi, B., **2017**. Esophagatomy in Houbara Bustard (*Chlamydotis macqueeni*) at Sir Bani Yas Island, United Arab Emirates, in: Abstracts - 37th Pakistan Congress of Zoology (International). p. 303-304.
5	Kabeer, B., **Abid Mehmood**, Jilani, M.J., Asadi, M.A., Al Nassan, I., Al Qubaisi, B., Hejcmanova, P., Anjum, M., Muhammad, R., Anwar, M., Bilal, S., Bilal, S., **2017**. Effects of Climate Change on Diversity, Distribution, Habitat Utilization and Breeding of Birds at Sir Bani Yas Island, Abu Dhabi, UAE, in: Abstracts - 37th Pakistan Congress of Zoology (International). p. 338-339.
6	**Abid Mehmood**, Ashraf, M.W., Elrasol, M.A.H., Kabeer, B., Jilani, M.J., Asadi, M.A., Al Nassan, I., Al Qubaisi, B., Hejcmanova, P., Bilal, S., Bilal, S., **2017**. Effects of Anesthetic Combination of Meditomidine-Ketamine in Arabian Striped Hyaena (*Hyaena hyaena sultana*) on Various Physiological Parameters at Sir Bani Yas Island, in: Poster Presentation 23 - 37th Pakistan Congress of Zoology (International). pp. 358–359.
STANDARD OPERATING PROCEDURES DEVELOPED / REVISED	
1	Sir Bani Yas Island Animal Feeding Policy
2	Animal feed regime for Sir Bani Yas Island Collection (all species)
3	Protocol for discarding expired medicine on Sir Bani Yas Island
4	Monitoring Marine Mammals
5	Protocol on incidents involving wildlife-vehicle accidents
6	Protocol on incidents involving humans and dangerous animals
7	Protocol on post mortem
8	Bio waste disposal procedures
9	Procedures for animal euthanasia on Sir Bani Yas Island
10	Protocols for research and volunteering at Sir Bani Yas Island
11	Protocols on Ecological Surveys
12	Protocols and procedures of Carnivore monitoring
13	Protocols on Animal Restraint
14	Procedures of animal census in Arabian Wildlife Park
15	Protocols of animal import on the island
16	Protocols of Animal export from the island
17	Protocols of Animal donation and exchange
18	Protocols of ZIMS data entry
19	Protocols on Chemical Immobilisation
20	Animals capture safety protocols
21	Sir Bani Yas prophylactic measures and biosecurity protocols
22	Animals' breeding loan protocols