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# Immobilization of leopards (*Panthera pardus*)

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## Table of contents

## Summary

Chemical immobilization and anaesthesia are often necessary to enable veterinary procedures in wild animals. Numerous protocols can be composed with available drugs in different combinations and ratios. The aim of this literature study was to identify advantages and disadvantages with available, and the most common, immobilization protocols used in leopards and to compare immobilization of wild leopards with immobilization of captive leopards.

In this thesis, effects of drugs from the substance classes NMDA-receptor antagonists, α2-receptor agonists, benzodiazepines, opioids and dopamine-receptor antagonists were studied in different protocols. All drugs have their advances and drawbacks. Desirable effects can be potentiated and unwanted effects can be reduced, or eliminated, by combining different drugs. With the right ratios, the immobilization can be optimized for both the individual and the situation.

Leopards are classified as vulnerable which strengthens the importance of preserving each individual by learning how to immobilize them safely. This is important not only for the leopard but also for the team working with the animal. Reliable and predictable sedation or anaesthesia eases the work and enhances safety during the treatment procedure. Some of the mentioned sedatives or anaesthetic agents can be reversed by using an antidot. Protocols with accessible reversal drugs have increased in popularity since it enables a quick recovery, which could be beneficial in field situations.

Working with free-ranging and captive animals is different in many aspects, for example capturing method, choice of protocol and dosages. A captive animal is more relaxed which is beneficial due to the negative effect of stress on immobilization. Free-ranging animals often require higher doses because of the higher stress levels. Stress also makes the effect of drugs more unpredictable. Most challenging in field situations is that underlaying health conditions are unknown, body weight is uncertain and working conditions can be tough. Therefore, close monitoring and knowledge of how to treat an overdose or underdose is of outer importance.

When working with wild leopards a quick effect, wide safety margin, reliability and availability of reversal drugs are valuable factors. However, which protocol to recommend seems to be built mainly upon personal preferences, experience, and availability. Unquestionably most important is to possess good knowledge of the drugs used to facilitate a safe sedation or anaesthesia.

## Sammanfattning

Kemisk immobilisering och anestesi är ofta en nödvändighet för att möjliggöra veterinärt arbete med vilda djur. Med de substanser som finns tillgängliga kan många immobiliseringsprotokoll kombineras i olika kombinationer och med olika proportioner. Målet med den här litteraturstudien var att identifiera fördelar och nackdelar med de tillgängliga protokoll som används för leoparder samt att jämföra immobilisering av vilda leoparder med immobilisering av leoparder i fångenskap.

I denna uppsats studerades protokoll innehållandes läkemedel som tillhör substansklasserna NMDA-receptorantagonister, α2-receptorantagonister, bensodiazepiner, opioider och dopamin-receptorantagonister. Alla substanser har sina för- och nackdelar. Önskvärda effekter kan potentieras och oönskade effekter kan reduceras, eller helt elimineras, genom att kombinera olika substanser tack vare synergistiska effekter mellan läkemedlen. Med rätt proportioner kan immobiliseringen optimeras, både för individ och situation.

Leoparder klassas som en sårbar art vilket ökar vikten av lära sig söva dem på ett säkert sätt för öka chansen för artens överlevnad. Detta är viktigt både för leoparden och för teamet som arbetar med djuret. Tillförlitlig och förutsägbar sedering eller anestesi underlättar arbetet och ökar säkerheten för alla inblandade. En del substanser kan reverseras med antidot. Den typen av protokoll har ökat i popularitet eftersom det möjliggör ett snabbt uppvak vilket kan vara en stor fördel i fältsituationer.

Att arbeta med vilda djur jämfört med djur i fångenskap skiljer sig i många avseenden, exempelvis val av protokoll, fångstmetod och läkemedelsdoser. Ett hållet djur är oftast lugnare vilket är en fördel eftersom stress har en negativ inverkan på läkemedlens effekt. Vilda djur kräver oftast högre läkemedelsdoser på grund av de högre stressnivåerna och läkemedlens effekter blir mer oförutsägbara. Det mest utmanande vid immobilisering av vilda djur är att djurens underliggande hälsotillstånd och exakta vikt är okänd samt att omständigheterna i fält vara tuffa. Det är därför ytterst viktigt att noggrant kontrollera djurets kroppsliga parametrar för att i tid upptäcka tecken på överdos respektive underdos samt att veta hur denna ska hanteras.

Vid immobilisering av vilda leoparder är en snabb effekt, bred säkerhetsmarginal, pålitlighet och tillgänglighet av reverserande läkemedel önskvärda egenskaper. Vilket protokoll som rekommenderas bygger främst på personliga preferenser, erfarenhet och tillgänglighet. Det allra viktigaste är att besitta goda kunskaper om substanserna som används för att främja en säker sedering eller anestesi.

## Introduction

The leopard (*Panthera pardus*) is the smallest of four large felids in the genus *Panthera* that also includes lion (*Panthera leo*), tiger (*Panthera tigris*) and jaguar (*Panthera onca*). Leopards are solitary, highly adaptable and have accomplished to thrive in various habitats (Stein & Hayssen, 2013). The colour of the coat and its pattern vary greatly geographically. Phylogenetic analysis has resulted in further division of *Panthera pardus* into nine subspecies widely distributed throughout southern Asia and sub-Saharan Africa (Uphyrkina *et al.*, 2001). This thesis will mainly focus on leopards (*Panthera pardus*) without further division into subspecies. The International Union for Conservation of Nature (IUCN, 2015) classifies leopards as vulnerable with declining populations. An increasing human population, habitat loss and poaching are examples of factors causing a steady decline (Uphyrkina *et al.*, 2001). In 2016, only around 25% of the species' historical range remained (Jacobson *et al.*, 2016).

Immobilization is required to enable several veterinary procedures in wild animals. Chemical immobilization aims to disable mobility and can result in anything from light sedation to unconsciousness depending on the method, drugs used and doses (Fahlman, 2005). For short and simple procedures chemical immobilization can be used, but more complicated and prolonged operations require general anaesthesia, resulting in loss of consciousness and sensation (Morris, 2001; Fahlman, 2005).

Before chemical immobilization, the leopard must first be localised and captured. Traditional capture methods for wild felids are box traps (de Araujo *et al*., 2020). The use of foot snares for felids has increased in popularity for safer, simpler and more cost-efficient captures (Johansson *et al*. 2022). After capture, the approach is made quietly and not closer to the animal than ten meters. From distance, the bodyweight is estimated to calculate drug dose required for immobilization. Post injection, ten minutes is often adequate for drugs to give effect (de Araujo *et al*., 2020). Signs of immobilization are salivation, lip licking, staggering movements, and dilation of pupils. Tactile responses can be examined to test if the leopard is fully sedated. When the leopard is fully sedated and safe to work with, the tongue should be kept outside the mouth and the head straight (Deka *et al.*, 2012). Correct positioning is important to facilitate breathing. Blindfolds can be used to limit external stimuli (Johansson *et al*., 2022). Body temperature, heart and respiratory rate should be monitored continuously during the procedure (Deka *et al.*, 2012).

After examination or treatment is completed, a leopard caught with a cage, can be put back in the cage with the rear end facing the veterinarian to enable continuous monitoring of body temperature. The cage should be covered to block the sight of the team which helps the leopard to stay calm. Ear-movements and eye-blinking are initial signs of recovery. When fully awaken the animal can be released (Deka *et al.*, 2012).

To restrict this thesis the following questions were addressed:

- What protocols are used to immobilize leopards and what are their pros and cons?
- Is there a difference between immobilization of free-ranging versus captive leopards?

## Method & material

The method chosen for this article was a literature study, and the literature was collected from the databases *Google Scholar*, *Web of Science*, *PubMed* and the library catalogue *Primo* at the Swedish University of Agricultural Sciences. Words that have been used for search consisted of: *"immobilization", "Panthera pardus", "anaesthesia", "α2-receptor agonist", "benzodiazepine", "NMDA-receptor antagonist", "opioid", "dopamine receptor antagonist "wildlife", "wild felids"* and *"reversal".* All search words were used in different combinations. Relevant articles were also found in references of studied articles. Since the number of relevant articles was limited, no restriction was used based on the year of publication.

Very few studies were found based solely on leopards, therefore literature covering other wild felids was used. Two experienced veterinarians in South Africa were consulted who forwarded useful articles containing protocols they themselves use. The veterinarians were also questioned which anaesthetic agents they used and if they had experienced any side effects.

## Literature review

### *NMDA-receptor antagonists*

Glutamate, the main excitatory neurotransmitter, interacts primarily with the N-methyl-D-aspartic acid (NMDA)-receptor (Olney, 1997). By binding the ion-coupled NMDA-receptor, antagonism results in disturbed neural activity (Chen & Lipton, 2006). Two commonly used NMDA-receptor antagonists and dissociative agents in veterinary medicine are ketamine and tiletamine. Exact effect of dissociative anaesthesia is unknown (Kohrs & Durieux, 1998). NMDA-receptor antagonists put the animal in a cataleptic state, with eyes kept open, a slow nystagmic gaze and intact corneal reflex (Lin *et al.*, 1993).

Ketamine and tiletamine can provide deep anaesthesia and analgesia depending on dose (Cohen *et al.*, 2018). Blood pressure may increase but without clinically significant respiratory depression (Idvall *et al.*, 1979). Tiletamine has a prolonged duration and better analgetic effect than ketamine, effective for general anaesthesia in cats (Lin *et al*.,1993). Both drugs increase muscle tonus and are therefore often combined with a centrally acting muscle relaxant (Carter & Story, 2013). Eventual seizures can be mastered by a slow intravenous administration of diazepam (Deem, 2004). Provided analgesia is proved insufficient for surgery but can be increased with supplemental substances (Lin *et al*., 1993).

Iteration of ketamine results in prolonged induction time, increased stress, and weak immobilization, but is considered unproblematic for adjusting the depth and duration (Belsare & Athreya, 2010). Tiletamine overdoses in cats are characterized by hypoventilation and apnoea. Apnoea can be reduced or completely avoided with a slow intravenous injection of benzodiazepines (Lin *et al*., 1993). To reduce side effects of tiletamine, the substance is only available in combination with the benzodiazepine zolazepam (Klein & Klide, 1989).

Direct antagonists of ketamine and tiletamine are to my knowledge absent (Kreeger & Arnemo, 2007 see Fahlman, 2008).

### *α2-receptor agonists*

Alpha2-receptor agonists (α2-agonists) provide sedation, muscle relaxation and analgetic effects (Flaherty, 2013). The four primarily used α2-agonists are xylazine, medetomidine, romfidine and detomidine (Carter & Story, 2013).

The sedating and analgetic effect, intensity and duration of medetomidine is dose-dependent in cats (Ansah *et al.*, 2002; Jalanka & Roeken, 1990). After injection of xylazine the analgetic effect lasts for 20 minutes and does not depend on the dose (Knight, 1980 see Williams *et al*., 2002). Compared to other drugs in the substance group, xylazine has a relatively short duration and low alpha2:alpha1-receptor specificity (Carter & Story, 2013).

Several of the side effects of α2-agonists are also dose-dependent (Flaherty, 2013). Vasoconstriction and reduced cardiac output secondary to bradycardia have been described and administration should therefore be avoided in animals with cardiovascular or hemodynamic impairment (Carter & Story, 2013). In felids, α2-agonists may stimulate emesis during induction and recovery. Emesis can be reduced by adding the anti-emetic, metoclopramide, in

the induction dose (Morris, 2001). α2-agonists inhibit the release of norepinephrine, which controls thermoregulation. Administration may therefore result in hypothermia or hyperthermia depending on the ambient temperature (Kurz, 2008).

Two antidotes able to reverse α2-receptor agonism are yohimbine and, the more selective atipamezole. Atipamezole may, if administered intravenously, cause immediate recovery, hyperexcitation, tachycardia and significant hypotension. Antidote combinations containing atipamezole should therefore be administered intramuscularly or at low pace intravenously (Paddleford & Harvey, 1999).

### *Benzodiazepines*

Benzodiazepines are agonists on the γ-aminobutyric acid-A ($GABA_A$)-receptor, causing increased affinity for the inhibitory neurotransmitter GABA. Benzodiazepines cause amnesia, muscle relaxation and anxiolysis but lack analgesic effects. Depressive influence on cardiorespiratory function is minimal and the drug is overall safe to use, even in compromised animals (Klein & Klide, 1989). Effects are quick and duration short, tolerance development is rare, and the substances are relatively safe to overdose. Benzodiazepines can also be used as anti-convulsant (Lin *et al*., 1993).

Diazepam and midazolam are frequently used sedatives in wild cats. Midazolam has almost replaced diazepam because of its more predictable intramuscular absorption. Another popular benzodiazepine is zolazepam (Ramsay, 2014). CNS depression by zolazepam is limited and the drug is therefore combined with tiletamine (Telazol®/Zoletil®) resulting in an anaesthetic agent without sedative-hypnotic-respiratory depressant effects. Zolazepam has a longer duration than tiletamine and recovery may therefore be prolonged when using the combination (Lin *et al*., 1993).

One available reversal agent is flumazenil. The high cost is a major factor to consider when using flumazenil in larger animals (Walzer & Huber, 2002).

### *Opioids*

Butorphanol is a mixed agonist-antagonist opioid analgesic with a potency greater than morphine. Butorphanol is widely used for its sedative and analgesic effects (Bush *et al*., 2012).

Use of butorphanol results in minimal cardiopulmonary depression compared to other opioids. Respiratory depression may occur, although it is dose-dependent and eventually reaches a ceiling after which no further depression occurs. Common CNS effects are nausea and regurgitation. Arousal might occur abruptly if butorphanol is used alone and the animal is stimulated, possessing potential personnel danger. When butorphanol is used in combination with other drugs, anaesthesia is considered safer because of synergistic effects and lower doses also reduce adverse effects (Bush *et al*., 2012).

Available antidotes for complete reversal of opioids are naloxone, nalmefene, and naltrexone. Partial reversal can be achieved using diprenorphine, which revers the undesirable μ-opioid receptor effects while useful, sedative, effects remain (Bush et al., 2012).

### *Dopamine-receptor antagonists*

One dopamine-receptor antagonist with increasing popularity is the tranquilizer azaperone. Antagonism of the dopamine-receptor is associated with potential side effects, for example muscle tremors and restlessness (Potter & Hollister, 2001). Azaperone is also an antagonist on α1-adrenoreceptors, causing relaxation in smooth muscle which results in vasodilation, decreased blood pressure and eventual increase of heart rate (Gregorio *et al.*, 2010; Lees & Serrano, 1976).

## Discussion

Capture is a stressful process for the animal and may result in injuries (Deem, 2004). In studies where dogs have been used to catch jaguars, the cats have fled up in trees and later fallen when drugs start to give effect. Minimizing the stressful elements of capture is important due to major impact of stress on physiological parameters which interfere with, and may compromise the effect of anaesthesia (Deem, 2004).

Since administration of immobilization agents takes place from a distance, remote drug delivery systems (RDDS) are used. For immobilization of felids in cages or snares, blowpipes or pole syringes can be used while in free-ranging situations, rifles loaded with lightweight darts are preferred (Deem, 2004). Because of the different aspects to consider, deciding on a capture method is undoubtedly difficult since all methods have drawbacks. If using snares or cages, the leopard will experience high stress levels since the animal is physically caught but still fully conscious. Darting a free-ranging animal probably shortens the period of acute stress but increases the risk of darting injuries.

It is difficult to decide which immobilization protocol to use for free-ranging animals. Immobilization should always take place in areas where the risk of a sedated animal obtaining injuries or drowning is minimized. Anaesthetic or sedative agents with available reversal drugs are preferred to avoid leaving the animal in a semi-sedated state, exposed to predators (Carter & Story, 2013). A leopard is a big carnivore that, in a normal state, can defend itself but predators may take advantage of a leopard in a weakened state. However, it is not recommended to leave an unrecovered animal due to various reasons.

I have found only few articles about immobilization of leopards. According to Morris (2001), the pharmacokinetics of most wild felids are similar to domestic cats. This must be interpreted with caution since effects of certain drugs may be species specific. Most sedative and anaesthetic drugs are considered relatively safe in wild felids, but anyone working with an immobilized animal should know how to react if emergencies arise (Deem, 2004).

When going through the articles, I found that most drug protocols are built upon combinations. By combining different drugs, wanted effects can be potentiated and side effects counteracted.

Combinations often result in a low total volume which is desirable when using RDDS but also to reduce many side effects, especially dose-dependent effects.

Every veterinarian has personal preferences for which protocol to use. Knowledge of different drugs and experience with protocols simplify the procedure and signs of under- or overdosing will be noticed easier and sooner.

### *α2-receptor agonist + NMDA-receptor antagonist*

Ketamine is the most common dissociative agent for immobilization of wild carnivores and is often combined with α2-agonists (Morris, 2001). Medetomidine's potentiating effect on ketamine is greater than xylazine's (Jalanda & Roeken, 1990). A low dose is cost-efficient, saves valuable atipamezole and reduces side effects (Morris, 2001).

Ketamine+xylazine combinations are often used in wild felids and studies have shown the combination successfully used in snow leopards, jaguars, lions, different leopard subspecies (*Panthera pardus* spp.), pumas (*Puma concolor*), and clouded leopards (*Neofelis nebulosa*) (Morris, 2001; Deem, 2004; Fahlman, 2008). If the initial dose is considered too low, supplemental doses of ketamine can be given intramuscularly. Iteration of xylazine should be avoided (Belsare & Athreya, 2010). Lions require higher doses of ketamine and supplemental doses may be inevitable. Recoveries may occur very abruptly (Fahlman, 2008). Combinations that may cause abrupt arousals should, in my opinion, be avoided considered large carnivores since quick recoveries possess a great danger for the team.

In a study, using the ketamine+medetomidine combination, three out of five lions vomited after atipamezole reversal (Tomizawa *et al.*, 1997). In snow leopards atipamezole has provided smooth recoveries and in leopards no vomiting was observed after use of yohimbine (Jalanka, 1989; Deka *et al.*, 2012).

In a study, 55 wild Indian leopards (*Panthera pardus fusca*), were immobilized with a ketamine+xylazine combination. Regurgitation was noted in six leopards and seizures in three (Belsare & Athreya, 2010). Important to note is that drug availability in India is very limited which restricts the possibility to compare the combination with other drug protocols. In the study, doses were adjusted subjectively according to estimated weight and physiological parameters and therefore, the risk of under- or overdosing is large. I am unsure if the side effects are due to inaccurate doses, or the effect of the actual drugs used, but seizure may indicate a ketamine-overdose. Another study from India included four Indian leopards, immobilized with the same combination. Three out of four cats had elevated body temperatures, but other physiological parameters remained normal (Deka *et al.*, 2012). α2-agonists might be a factor contributing to hyperthermia, but stress is probably also an important circumstance in this study.

In domestic cats, the ketamine+medetomidine combination has, after reversal with atipamezole, resulted in significant tachycardia. Medetomidine has a suppressive effect on heart rate which may result in bradycardia (Verstegen *et al.*, 1991). When medetomidine is reversed, the cardiovascular stimulating effect by ketamine remains, and tachycardia arise (Tomizawa *et al.*, 1997). All cats in the study also showed moderate ataxia during the recovery period (Verstegen *et al.*, 1991). Tachycardia and ataxia arose after reversal which indicate that ketamine had not

been metabolised. A possible solution to avoid these side effects is reduction of the ketamine dose or postponing the administration of reversal.

The most common side effects for wild felids immobilized with ketamine+medetomidine are regurgitation, short-term apnoea and reduced respiratory rates if ratios of medetomidine are high (Ramsay, 2014). Hypoventilation and apnoea are possible side effects of ketamine, and α2-agonists are known to have a slight suppressive effect on respiration. I have not yet found evidence on how often this occurs in leopards during immobilization. Besides regurgitation, it is to me unknown if other side effects have been observed in leopards whilst using this combination.

Finally, detomidine used in combination with ketamine has been used in African leopards and pumas resulting in an immobilization almost identical to the ketamine+medetomidine combination (Morris, 2001).

Time requiring procedures with α2-agonists may result in hyperkalaemia, observed in studies involving tigers, cheetahs (*Acinonyx jubatus*), pumas and Persian leopards (*Panthera pardus saxicolor*) (Jimenez *et al.*, 2020; Reilly *et al.*, 2014; Bernal *et al.*, 2019). I strongly recommend continuous monitoring of electrolytes during longer procedures to ensure that potassium is not reaching dangerously high concentrations. A recently published study, where a one-year-old black leopard was anaesthetised with alfaxalone+ketamine+isoflurane proved that alfaxalone, may be a profitable alternative to α2-agonists to avoid hyperkalaemia. The combination is solely recommended for captive animals, able to hand-inject, due to the large volume needed (Jimenez *et al.*, 2020). In future, alfaxalone might be available in higher concentration and thus become an alternative also in free-ranging animals. If this combination is generally successful, should be interpreted with caution since the study only included one, very young, leopard.

### *NMDA-receptor antagonist + benzodiazepine*

Tiletamine+zolazepam (Telazol®) alone, provides a cataleptic deep anaesthesia with maintenance of normal pharyngeal-laryngeal reflexes. A common side effect is hypersalivation (Lukasik & Gillies, 2003). Hypersalivation in combination with retained gag reflexes may result in vomiting and refluxes. According to Deem (2004) hypersalivation can be reduced with atropine. If atropine is used, eventual side effects must be taken in consideration since atropine affects various systems in the body.

Telazol® provides a shorter induction time, longer duration, and usually better muscle relaxation than ketamine but the recovery may be prolonged (Ramsay, 2014). A prolonged recovery may be beneficial when working with large carnivores. Both due to personnel safety but also to give the animal time to metabolize the drugs. On the contrary I would not recommend leaving a semi-sedated animal without observation.

Deem (2004) recommends a ketamine+Telazol® combination for wild jaguars. Ketamine can be added directly to the initial dose, or as a supplement to adjust depth of anaesthesia. Flumazenil enables quicker recoveries by reversing the effect of zolazepam. Since tiletamine cannot be reversed, one must wait until tiletamine has been metabolised before administering flumazenil.

According to Morris (2001), cheetahs and tigers respond differently to ketamine compared to other felids. They have an enhanced risk of seizures and a few tigers have been reported dead after use of Telazol®. According to Kreeger & Armstrong (2010) literature has claimed Telazol® contraindicated in tigers. However, their search for confirmation of this statement was unsuccessful. Searching for validation resulted in an assumption that the contraindication had become an unsubstantiated dogma since the mortality rate of Telazol® was not significantly, or at all, higher in tigers than other animals or by other anaesthetic agents. I cannot see why tigers, also related to the *Panthera* genus, would respond differently compared to leopards. A study of Lewis *et al.* (2014) could not identify any statistically significant differences in tigers' versus leopards' pharmacokinetics of Telazol® and leopards seemed unaffected of Telazol®.

Tiletamine and zolazepam are only available at the market as a pre-set combination. A possible area of future research could be to investigate the perfect ratio between the two drugs. It could be possible that some side effects can be avoided with an increased ratio of zolazepam. In addition, different species almost certainly benefit from different ratios.

### *α2-agonist + NMDA-receptor antagonist + benzodiazepine*

Medetomidine+Telazol® has been reported safe and effective for many different wildlife species. In wild snow leopards in Mongolia the combination provided normal muscle relaxation without vomiting nor hypersalivation. Body temperature, heart and respiration rates decreased significantly but were kept within clinical acceptable range. It was noted that higher doses of medetomidine resulted in prolonged duration (Johansson *et al.*, 2013). The ambient temperatures were very low and medetomidines interference with thermoregulation supposedly caused the hypothermia. The α2-agonists´suppressive cardiac effect is also a possible background to the bradycardia.

In a study including 17 free-ranging lions, it was noticed that the dose of Telazol®, in combination with medetomidine, can be kept very low. Only one tenth of a standardized dose of Telazol® for wild lions is sufficient for a successful anaesthesia. Because of the low doses, prolonged recoveries were avoided. 11 out of 21 lions developed hyperthermia (Fahlman, 2008). A possible explanation is that the hyperthermia was caused by medetomidine, like in the study by Johansson *et al.* (2013). In contrast to the study of Johansson *et al.* the ambient temperature during Fahlman´s study ranged from 30-33°C, resulting in hyperthermia rather than hypothermia. Hyperthermia developed, despite use of different capture methods, which indicates that type of drugs is a strongly contributing factor.

Medetomidine+Telazol® combinations have been successfully used in, seemingly, healthy African leopards (A. Fraser, personal communication). In a published article on two old, very compromised captured Arabian leopards (*Panthera pardus nimr*), medetomidine+Telazol® was used in combination with 100% oxygen gas intubation. Due to renal failure, lower doses were administered but the anaesthesia was, despite the health conditions, adequate and safe. Time for induction and recovery were shorter than in previous experiences with healthy leopards using medetomidine+ketamine (Golachowski *et al.*, 2018). Overall, this study

suggests that Medetomidine+Telazol® combinations may be advantageous compared to the medetomidine+ketamine combination in compromised leopards although the results must be interpreted with caution since the study only contained two leopards.

The results of these studies show that the medetomidine+Telazol® combination is safe to use even in compromised animals. Most important to monitor is body temperature which seems to fluctuate individually and depends greatly on ambient temperatures.

### *Opioid + benzodiazepine + α2-agonist*

The new BMM-combination (butorphanol+midazolam+medetomidine) is completely reversible and affirmed ideal for field procedures where a quick recovery and release are desirable (Alves, 2022; Lafortune *et al.,* 2005). Ketamine can be added in stressful situations when adrenaline competes with medetomidine. Alves (2022) has immobilized extremely compromised and stressed spotted hyenas (*Crocuta crocuta*) safely with the combination. Safe and reliable immobilization with no vomiting, apnoea or sudden arousals have been noted in studies of black-footed cats (*Felis nigripes*) even when doses for wild versus captured cats differentiated a lot (Eggers *et al.,* 2020). In cheetahs, abrupt arousals have been observed (Lafortune *et al.,* 2005). Low doses of medetomidine and/or butorphanol can result in sudden arousal when used without a dissociative agent (Bush *et al.,* 2012). With evidence of previous studies, higher doses and addition of a dissociative agent might be inevitable, especially in free-ranging animals who are stimulated easier.

According to Bush *et al.* (2012) the BMM combination has been successfully used in many cheetahs, including very compromised ones, and also in free-ranging lions, African wild dogs (*Lycaon pictus*) and spotted hyenas. Physiological parameters have remained good but bradycardia, accentuated sinus arrhythmia and hypertension have arisen in cheetahs (Lafortune *et al.,* 2005). Colburn (2017) reported that butorphanol+dexmedetomidine+midazolam combinations provided a quick induction, good muscle relaxation and rapid recoveries, but did cause severe hypertension in all cheetahs in the study. With evidence of the last two described studies, hypertension appears to be an important side effect in cheetahs. Although, since cheetahs may respond differently to immobilization agents, the results cannot be extrapolated directly to leopards.

To my knowledge no articles have been published, showcasing the combination has been used in leopards, although other felids have been successfully immobilized. According to field veterinarians in South Africa, the BMM-combination has been used safely in leopards. In stressful situations, for example cage traps, or for personnel safety ketamine has been added unproblematically (A. Fraser, personal communication; Z. Glyphis, personal communication).

Major advantages are the availability of reversal agents and a safe and reliable immobilization. Addition of ketamine appears to be unproblematic and is a good supplement for adjusting depth and duration. The biggest disadvantage is, in my opinion, the development of hypertension seen in cheetahs. Possibly the hypertension can be avoided by adjusting the ratio of the different drugs used. The cheetahs who experienced hypertension were all captive and since captivity is

usually an indication for a lower dose, the hypertension might therefore be a symptom of an overdose.

### *Opioid + dopamine-antagonist + α2-agonist*

Recently the fully reversible BAM-combination (butorphanol+azaperone+medetomidine) has become very popular for its use in hoofstock and large carnivores. Total volume can be kept very low and eventual addition of ketamine, useful in stressful situations, provides an even more effective immobilization (Bush *et al.*, 2012).

In a study made of Semjonov *et al.* (2017) evaluating the BAM combination in 20 lions, induction times were similar to the BMM combination, slightly longer than medetomidine+Telazol® but much shorter than ketamine+xylazine according to compared studies. Increased ratio of medetomidine reduces induction time (Semjonov, 2020).

Studies have shown that BAM provides a reliable and reversible immobilization in various species, for example lion, cheetah, blesbok (*Damaliscus pygargus phillipsi*), leopard, and wild dogs (Semjonov, 2020; Alves, 2022). A slight bradycardia has been noticed in lions, but observations of cheetahs have shown indication of slight increases of heart rates (Semjonov, 2020). A possible approach to tackle the cardiovascular issues could be increased doses of azaperone which may counteract cardiovascular effects of medetomidine, like bradycardia. For the cheetahs with tachycardia, the reversed might apply. With a decreased ratio of azaperone, medetomidine´s suppressive cardiac effect might decelerate the heart rate.

According to Bush *et al.* (2012), immobilization with BAM is characterised by excellent respiration, good muscle relaxation and lack of hyperthermia. BAM provides a smoother and more rapid recovery than BMM and other combinations. In lions, reversal with naltrexone+atipamezole provided a shortened time for recovery compared to naltrexone+yohimbine. During initial recovery, slight signs of ataxia were observed (Semjonov, 2020).

I have not yet found any published articles where BAM has been used in leopards but through personal communication, I have received confirmation that the combination has been safely used in African leopards (A. Fraser, personal communication; Z. Glyphis, personal communication). As a side effect during induction, temporary apnoea is quite common (A. Fraser, personal communication).

Major advantages for the BAM-combination are, in my opinion, the very low volume, possibility to add ketamine and the fully reversibility. Disadvantages are cardiovascular effects and ataxia during recovery after reversal. It could be possible to manage the cardiovascular effects by decreasing the ratio of medetomidine which will not agonise the α2-receptors to the same extent. The lower ratio would also reduce the reversal dose needed or even avoid it. Another possibility might be to increase the ratio of azaperone resulting in vasodilation which may increase the heart rate.

### *Other combinations worth mentioning used in wild felids*

Ketamine+butorphanol+medetomidine has been used successfully in free ranging servals (*Leptailurus serval*) providing a predictable and reliable immobilization similar to the BMM combination but with increased ataxia during recovery (Blignaut, 2019). A likely explanation to ataxia is remains of ketamine in the body. I have not yet found any published articles where the combination ketamine+butorphanol+medetomidine has been used in leopards but based on experience by a field veterinarian the combination has been used in African leopards, without any noticed side effects (A. Fraser, personal communication).

The drug combination dexmedetomidine+ketamine+isoflurane has resulted in severe hyperkalaemia in two jaguars. The author presents earlier studies where hyperkalaemia has occurred in tigers, cheetahs, and lions (Romano *et al.*, 2018). Again, this study emphasises the relevance of monitoring during anaesthesia. Electrolyte levels should be monitored frequently. Even after a seemingly successful immobilization, potassium levels can be dangerous elevated and result in death days afterwards.

### *Free-ranging versus captive animals*

Few published studies compare immobilization under field conditions versus captive conditions. Doses needed for free-ranging animals are usually higher than for captive animals (Eggers *et al.*, 2020). Standard doses without having to estimate bodyweight is useful since estimation is subjective and often very difficult. If dosing per kilogram bodyweight, one must balance the risk of the animal escaping versus the risk of overdosing. In field situations, drugs safe to overdose are therefore useful since an exact estimation is impossible but the team must know how to deal with both under- and overdosing.

It is more difficult to manage immobilization of sick animals than healthy ones. An important problem to bear in mind is that health status of a free-ranging animal is difficult to predict. In captive felids it is easier since the animal most probably has been monitored, at least a few days in advance.

Choice of drug might differentiate between captive versus field circumstances because of total volume. Drugs must be available in very high concentrations to enable use in field. Capture method may also be a factor of impact on the choice of protocol. Wildlife veterinarian Zoe Glyphis prefers to use the medetomidine+Telazol® combination in wild leopards caught with a cage to minimize the risk of spontaneous arousals (Z. Glyphis, personal communication). For free-ranging leopards the BMM or BAM combination is preferred due to its reversibility which avoids leaving the animal in a semi-sedated state. The medetomidine+ketamine combination is preferred due to its cost efficiency and predictive anaesthesia when working with relaxed, captive leopards. Although, very ataxic awakenings might occur if medetomidine is reversed to soon. According to the veterinarian, complications with anaesthesia are rarely seen in leopards, except for the above-mentioned ataxia.

The method of capture has a great impact on physiological parameters. Physical exertion and stress increase the risk of capture-associated morbidity and mortality (Arnemo *et al.*, 2006). An exhausted animal has elevated levels of lactic acid which can predispose to muscle fatigue,

cardiac arrythmias and organ failure (Spraker, 1993 see Fahlman, 2008). To prevent capture myopathy azaperone can be used since azaperone exerts protective effects by increasing blood flow through muscles due to vasodilation (Mentaberre *et al.*, 2010).

Stressful situations cause release of catecholamines in the body, such as epinephrine, which compete with certain immobilization agents and tend to suppress their effect (Morris, 2001). In stressful situations supplementary drugs or doses may be inevitable and reversal cannot be administered to early if rough recoveries are to be avoided (Alves, 2022). Total time for immobilization might therefore be prolonged in wild animals compared to captive animals due to the higher levels of catecholamines in wild animals resulting in need of increased and/or supplementary doses.

Hyperthermia and acidosis can cause death during, or after the procedure (Fahlman, 2005). An initial increase of body temperature is to be expected in a stressed animal but may later decrease due to effect of drugs (Johansson *et al*., 2013). Ambient temperatures cannot be controlled and individuals respond differently to drugs. It is therefore of outer necessity to bring equipment enabling both an increase and decrease of body temperature, for example blankets, water, and fans.

Conditions while working with captive animals can be controlled to an extent that is impossible in the field. Captive leopards are also more used to the sight of humans compared to wild leopards. Merely the sight of a human can terrify a wild leopard. Deka *et al.* (2012) state that avoidance of auditory and visual stimuli of humans ease the work with wild leopards who are unaccustomed to messy surroundings.

In captive circumstances, where leopards can be kept safe, reversal may not be necessary. Leopards can then metabolise the drugs in a natural way while monitoring can be continued (Belsare & Athreya, 2010). I would never recommend leaving a wild animal in a semi-sedated state, unobserved.

I believe that the most important when immobilizing animals is to possess good knowledge of the drugs you decide to use. Knowledge, in combination with close monitoring is of outer importance to react and treat the animal correctly. In Fahlman´s (2008) study the same protocol was used, capturing methods varied and so did ambient temperatures. Despite this, animals anesthetised in +3°C developed hyperthermia while hypothermia developed in +33°C. This highlights the individual differences and strengthens the importance of close monitoring.

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### *Personal communication*

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