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Immobilization of lions (*Panthera leo)*

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SAMMANFATTNING

Det finns flertalet situationer då det kan vara nödvändigt att immobilisera ett lejon. Ett kritiskt steg i immobiliseringsprocessen är att välja det mest fördelaktiga läkemedelsprotokollet och därefter administrera det på ett korrekt sätt. Om hela proceduren inte är noggrant planerad och utförd, kan det medföra situationer som är farliga för både djuret och de människor som arbetar med det.

Idag används ett flertal olika läkemedelsprotokoll och syftet med den här litteraturstudien är att utvärdera fyra läkemedelskombinationer och deras respektive effekter för att se om någon av dem är att föredra. Genom att använda kombinationer av läkemedel, i stället för monoterapier, kan man minska mängden läkemedel som används på grund av de synergistiska effekter som uppstår mellan de aktiva substanserna. Detta är både mer ekonomiskt fördelaktigt och bidrar till en minskning av aversiva bieffekter hos det immobiliserade djuret.

De läkemedelskombinationer som utvärderades i den här studien är butorfanol-azaperon-medetomidin, butorfanol-midazolam-medetomidin, medetomidin-zolazepam-tiletamin samt xylazinhydroklorid-ketaminhydroklorid. Studien ämnar också undersöka om effektiviteten av immobiliseringen påverkas av att lejonet lever i fångenskap eller är frilevande, samt om det observerats någon skillnad hos ensamlevande kontra flocklevande lejon.

Enligt litteraturen så påverkas immobiliseringen av huruvida ett lejon lever i fångenskap eller inte, både gällande effektiviteten av läkemedlen samt hur man ska planera proceduren. Frilevande lejon kräver en högre läkemedelsdos medan det är enklare att övervaka och motverka eventuella komplikationer hos hållna lejon. Alla läkemedelsprotokoll som undersöktes i den här studien hade dock en adekvat effekt hos både fria lejon och lejon i fångenskap, förutsatt att de administrerades på ett korrekt sätt.

Sammanfattningsvis så är alla protokoll som diskuteras i den här uppsatsen bra alternativ för att immobilisera lejon. Xylazinhydroklorid-ketaminhydroklorid-kombinationen hade emellertid en längre induktionstid och inducerade kräkning hos två av lejonen. En förlängd induktionstid kan potentiellt göra att det blir svårare att lokalisera det immobiliserade djuret och kräkningar kan leda till inandning av regurgiterat material, vilket i sin tur kan orsaka aspirationspneumonier. Därför bör det sistnämnda protokollet användas med försiktighet.

ABSTRACT

There are several situations in which it might be necessary to immobilize a lion. In the immobilization process, a critical step is to select the most advantageous drug protocol and then administer it correctly. If the procedure is not carefully planned and executed, it could prove dangerous to both the animal itself and the people working with it.

Various protocols are being used today, and the aim of this literature study was to evaluate four drug combinations and their effects to see if any of them is preferable. Using combinations of drugs, instead of monotherapy, decreases the amount of drugs needed due to synergetic effects. This is desirable as it is more cost effective and contributes to reduce adverse effects in the immobilized animals.

The combinations reviewed in this thesis were butorphanol-azaperone-medetomidine, butorphanol-midazolam-medetomidine, medetomidine-zolazepam-tiletamine and xylazine hydrochloride-ketamine hydrochloride. The study discusses if the effectiveness of the immobilization differs between captive and free-ranging lions, as well as between solitary and pack living lions.

According to the literature, whether a lion is free ranging or confined has an impact on the effectiveness of the drugs and the planning of the procedure. Free ranging lions require a higher drug dose than captive lions and it is easier to monitor and counteract complications in captive lions. However, all the reviewed drug protocols were shown to have adequate effect in both cases if administered properly.

In conclusion, all protocols mentioned in this thesis are good alternatives for immobilizing lions. However, the xylazine hydrochloride-ketamine hydrochloride combination has a longer induction time and induced vomiting in two lions. A prolonged induction time could potentially make it more difficult to locate the immobilized animal and the vomiting could cause aspiration of the regurgitated matter, which in turn could lead to aspiration pneumonia. Therefore, this protocol should be used with caution.

INTRODUCTION

Throughout history, lions have captured the hearts of people from all around the world. Despite this, the lion population is declining, and the species is currently classified as vulnerable on IUCNs Red List (Bauer *et al.* 2016). Some of the reasons behind the decline is poaching, habitat loss and a decreased number of available prey (Fahlman 2008). Because of this, it is more important than ever to take measures to ensure the longevity of the species.

Working with wildlife is always a challenge concerning both human safety and the safety of the animal. Immobilizing lions is not an exception, and there is always a risk of severe side effects and death (Arnemo et al. 2014). There are several possible reasons to immobilize a lion. GPS collaring, gathering samples, controlling problematic individuals and testing for diseases are just a few examples (Fahlman *et al.* 2005; Semjonov 2020). Immobilization is the process of making something unable to move and there are two types – chemical and physical (Fahlman 2008). Many questions arise when dealing with chemical immobilization of wild felids, and few studies have been published on the subject. Hence, veterinarians often apply knowledge gathered from studies made on domestic cats (Ramsay 2014).

The questions I focus on in this thesis are:

- What pros and cons are there with different drug protocols for lion immobilization?
- What differences are there when immobilizing free ranging lions versus captive lions?
- Is the immobilization affected by whether the lion is solitary or lives in a pride?

MATERIALS AND METHODS

I have used several search engines and databases such as Google Scholar, SLU Primo, Epsilon and Science Direct to search for articles related to the subject in English and Swedish. Some of the words I searched for was "*lion*", "*lejon*", "*panthera leo*", "*immobilization*", "*immobilisation*", "*immobili**" and "*anaesthesia*". In some of the articles, there were references listed that proved to be useful to this thesis, which helped me move forward. I have also found information on certain websites, for example IUCN. When choosing what articles to include, I focused on the most recently published ones as the field of medicine is constantly evolving.

LITERATURE REVIEW

Preparations

To immobilize a lion, you must first locate it. This can be done with radio or GPS tracking if the individual is previously collared, by playing attracting sounds or by using bait (Fahlman *et al.* 2005). Food baits might also attract non-target species, so it can be beneficial to use other olfactory attractants such as urine (Laubscher *et al.* 2015) or certain perfumes (Riley *et al.* 2017).

It is important to consider the safety of both the animal and the people around it when deciding what immobilizing method to use. When chemically immobilizing an animal, it is crucial to evaluate all aspects of the protocol beforehand, as different species and terrains may require different protocols (Chinnadurai *et al.* 2016) Assessing the weight of the animal can be done based on visual parameters and is important for calculating the correct doses (Semjonov *et al.* 2017). If the animal is already immobilized you can use a heart-girth measurement to estimate the weight for further drug administration and backtracking (Bertram 1975).

It is best to starve the lion before immobilizing it to avoid regurgitation, which is easily achieved with captive lions. With free ranging animals it is recommended to schedule the procedure to a time when it is unlikely that the animal has been feeding (Chinnadurai *et al.* 2016). To avoid hyperthermia, it is best to avoid immobilizing during the hottest hours of the day (Semjonov *et al.* 2017). Furthermore, it is also recommended to avoid immobilizing animals during the periods of time where the females normally are carrying, birthing or caring for their offspring (Chinnadurai *et al.* 2016).

Drugs

Alpha 2 adrenoreceptor agonists – medetomidine, xylazine

Alpha 2 adrenoreceptor agonists (α2 agonists) exert their action on presynaptic adrenoreceptors in cholinergic and noradrenergic nerve terminals. The activation of these auto receptors inhibits the release of noradrenaline, which causes sedation, analgesia and muscle relaxation (Ritter *et al*. 2019). Some of the observed adverse effects are vomiting, induced labour, cardiovascular depression and ataxia, but the effects can be reversed with α2 antagonists. (Papich 2015).

Antagonists

Two commonly used α2 antagonists associated with reversing the immobilizing effects of medetomidine are atipamezole and yohimbine (Semjonov *et al*. 2017). Atipamezole is a selective and specific a2 antagonist that rapidly counteracts the effects of a2 agonists (Karhuvaara *et al.* 1991).

Benzodiazepines – zolazepam, diazepam, midazolam

Benzodiazepines act by binding allosterically to synaptic and extrasynaptic $GABA_A$ receptors and thus inhibiting the synaptic transmission in the central nervous system. This enhances the effect of the neurotransmitter γ-aminobutyric acid (GABA) by increasing the affinity of the receptor and opening chloride channels, which has a sedative, muscle-relaxant and anticonvulsive effect (Sigel & Ernst 2018).

Benzodiazepines generally do not cause adverse effects even in higher does (Sigel & Ernst 2018). The primary adverse effects observed are numbness, confusion, and a negative impact

on coordination. There is also a risk of tolerance development and addiction if the drug is administered repeatedly (Ritter *et al.* 2019).

Antagonists

Null modulators are the most preferable type of antagonist and act by binding to the GABA receptor without affecting it in any way other than blocking the access for the benzodiazepines (Sigel & Ernst 2018). The antagonists can also act as negative or positive allosteric modulators, depending on the drug concentration. Flumazenil is for example a weak partial negative allosteric modulator in lower concentrations, but a weak positive allosteric modulator in higher concentrations (Sigel & Baur 1988).

Opioids - butorphanol

Opioids act as both anaesthetics and analgesics by inhibiting the transmission of nociceptive information in the dorsal horn and the release of neurotransmitters in the spinal cord. They also activate descending inhibitory pathways and inhibit supraspinal afferent pathways (Schnellbacher 2010). There are four types of G protein-coupled receptors that are affected by opioids – μ, κ, δ and NOP. All of them have similar cellular effects when activated, but their uneven distribution in the central nervous system makes it possible to achieve different physiological outcomes by using selective drugs (Ritter et al. 2019). Some of the observed adverse effects are respiratory depression, vomiting, nausea and constipation (Ritter et al. 2019).

Antagonist

Naltrexone is an example of a long-acting, pure opioid antagonist, without any agonistic effects (O'Dell *et al.* 2017). It is nonselective, which means that it binds to all four of the different opioid receptors. By doing so, it prevents other opioid peptides from binding to the receptor and exerting their effect (Papich 2015).

Dopamine 2 receptor antagonists - azaperone

Dopamine acts as a neurotransmitter and a precursor for noradrenaline and is essential to most cognitive functions (Costa & Schoenbaum 2022). When D2 receptors are stimulated by their ligand it causes hyperpolarization, which inhibits the action potential and the release of dopamine stored in vesicles in dopaminergic axons. The antagonists cause sedation, increased non-REM sleep (Monti & Monti 2007), antiemetic effects and are also somewhat muscle-relaxant (Ritter *et al.* 2019).

N-methyl-D-aspartate receptor antagonists – ketamine, tiletamine

Ketamine and tiletamine are the most common dissociative anaesthetics used in wild carnivores (Fahlman 2008). Their mechanisms of action are not fully understood, but they act as non-competitive antagonists to N-methyl-D-aspartate (NMDA) receptors, which is thought to reduce the activity in mesocortical dopaminergic neurons and thus decreasing the amount of released dopamine. However, the inhibition of NMDA receptors also affects GABAergic neurons which instead can lead to enhanced dopamine release. Ketamine contributes to analgesia, but the animal will still have muscle tonus and be somewhat conscious. Therefore, it must be combined with muscle-relaxant and sedating drugs (Ritter *et al.* 2019).

There is no current specific antagonist for ketamine and tiletamine (Caulkett & Arnemo 2007 see Chinnadurai *et al.* 2016; Kreeger & Arnemo 2007 see Fahlman 2008)

Immobilization

Physical restraint can be used for lions that are afraid of vehicles and people, as they are more difficult to approach and dart. The current method for physical restraint with the least drawbacks is foot snares (Frank *et al.* 2003). However, this thesis will be focusing on chemical immobilization.

One way to deliver drugs from a distance is by using a darting rifle. It is for example possible to use a gas powered dart gun (Fahlman *et al.* 2005) or a cartridge fired projector (Semjonov *et al.* 2017). It is crucial that the person handling the weapon has proper knowledge and practical training as a faulty use increases the risk of injuring the animal (Chinnadurai *et al.* 2016). A poorly placed dart can lead to serious consequences, especially if it hits the abdomen, thorax or vital structures of the neck (Arnemo *et al.* 2014).

Monitoring and maintenance

When a lion is immobilized, there are several things to monitor regularly to avoid critical complications. This is done with a physical examination of the cardiovascular, pulmonary and central nervous system (Chinnadurai *et al.* 2016).

Vital signs such as heart rate, respiratory rate, and rectal temperature should be kept under close inspection (Jacquier *et al.* 2006). You should monitor the capillary refill time (Chinnadurai *et al.* 2016), oxygen saturation, muscular tonus in the extremities and jaw and the presence of reflexes (Fahlman *et al.* 2005). If the lion shows signs of hypoxemia you should administer supplemental oxygen (Jacquier *et al.* 2006; Semjonov *et al.* 2017)

The lion should be placed in a lateral recumbency (Fahlman *et al.* 2005) with the head in such an angle that the risk of inhaling possible regurgitated gastric content is decreased (Arnemo *et al.* 2014). To avoid hyperthermia, it is best to keep the immobilized lion in shade. If signs of hyperthermia develops it has been proven effective to spray the animal with water (Jacquier *et al.* 2006) or to use fans to cool it down (Fahlman *et al.* 2005). It is also advised to minimize the amount of visual and auditory stimuli. A blindfold works well to reduce visual stimulation and potential eye damage, while earplugs decrease auditory stimulation. An isotonic ophthalmic solution or gel should be used to lubricate the eyes. (Arnemo *et al*. 2014).

Depending on the procedure it might be necessary to administer postoperative analgesia. Two commonly used analgesics are opioids and NSAIDs (Whiteside 2014). It is also important to treat the dart wounds. Long-acting antibiotics can be administered intramuscularly as a prophylactic measure (Jacquier *et al.* 2006).

Intubation

Intubating an animal for a short procedure is normally not necessary. However, if the haemoglobin saturation is lower than 85% or the respiratory rate drops below six breaths per minute an endotracheal intubation might be considered (Ramsay 2014). When intubating a lion a 16-24 mm (Semjonov *et al.* 2017) or 18-23 mm endotracheal tube might be used. As felids have relatively small muzzles and the larynx is easily accessed, intubation is often uncomplicated. However, the intubation must be performed cautiously as the endotracheal tubes made for large animals often are too long, which can lead to endobronchial intubation. Numbing of the larynx is normally not necessary for intubating felids, but can be done with lidocaine (Ramsay 2014).

Reversal

The antagonist can be administered intramuscularly in the shoulder (Fahlman *et al.* 2005) or thigh muscle (Jacquier *et al.* 2006). It is possible to administer the antagonist intravenously for a quicker reversal, but it can lead to changes in heart rate, blood pressure and cardiac output. Intravenous administration has also caused over-alertness and excitation in some animals. When the antagonist is administered intramuscularly, the absorption is prolonged, allowing for a more gradual reversal (Fahlman *et al.* 2005).

The first signs of awakening in a lion are palpebral movement, head movement and movement of the extremities. The lion will try to get up sternally and later attempt to sit and stand upright. Some lions show signs of hind leg ataxia for a couple of minutes after getting up (Fahlman *et al.* 2005; Jacquier *et al.* 2006).

DISCUSSION

Pros and cons with different drug protocols

There are several things to consider when choosing drug protocol. The nature and duration of the procedure, drug interactions, adverse effects and the size and health status of the animal are a few of the important aspects. Drugs with a wide safety margin are therefore preferred (Fahlman 2008).

Some drugs should not be administered alone. For procedures involving visceral pain, ketamine should never be the only drug administered for immobilization. Even though it contributes with good somatic analgesia, the visceral analgesia is deficient. The number of adverse effects showed in the animal, such as increased muscle tonus and excessive salivation, are also higher when ketamine is used alone (Chinnadurai *et al.* 2016).

The most common practice when immobilizing wild animals is to combine drugs. This allows for lower doses thanks to synergetic effects and reduces the risk of adverse effects (Fahlman 2008). Some of the combinations used on lions are butorphanol-azaperone-medetomidine (Semjonov *et al.* 2017; Semjonov 2020), butorphanol-midazolam-medetomidine (Wenger *et al.* 2010), medetomidine-zolazepam-tiletamine (Jacquier *et al.* 2006) and xylazine hydrochloride-ketamine hydrochloride (Herbst *et al.* 1985).

Butorphanol-azaperone-medetomidine (BAM)

The drug combination BAM consists of the opioid butorphanol, the D2 antagonist azaperone and the a2 agonist medetomidine. There are few published articles about immobilizing lions with BAM, and the two articles mentioned in this thesis are both written by the same author. This does not necessarily mean that the conclusions are affected by the authors subjective views, but I would like additional research done for the results to be more reliable.

For immobilizing lions, BAM is satisfactory but can also cause adverse effects. All lions in Semjonov *et al.*'s (2017) study developed a slight bradycardia during immobilization. The authors speculate that this may be because of the effects of medetomidine on peripheral a2 receptors. Some lions also had an elevated rectal temperature and a mild metabolic acidosis. However, all lions had a good capillary refill time and none of them showed signs of apnoea. According to Semjonov *et al*. (2017) a single dose of BAM (butorphanol, 0.18 ± 0.03 mg kg^{-1}; azaperone, 0.07 ± 0.01 mg kg^{-1}; medetomidine, 0.07 ± 0.01 mg kg^{-1}) provided adequate muscle relaxation and analgesia for about an hour, and the mean induction times observed were 4-10 minutes.

There is no available reversal drug for azaparone (Granholm *et al.* 2006) but an intramuscular injection of naltrexone-yohimbine or naltrexone-atipamezole can reverse the effects of BAM (Semjonov *et al.* 2017). Unfortunately, atipamezole also reverses the analgetic effects of a2 agonists (Granholm *et al.* 2006). BAM is a combination of multiple drugs with analgetic effects, but it is still important to assess the level of pain the animal is experiencing or will be experiencing later. Granholm *et al.* (2006) also states that the body temperature of cats rises when atipamezole is administered. If the lion is hypothermic, an elevation in body temperature can be positive, but if it is hyperthermic, it could lead to complications.

Butorphanol-midazolam-medetomidine (BMM)

The BMM combination contains the opioid butorphanol, the a2 agonist medetomidine and the benzodiazepine midazolam, which provides a rapid induction and immobilization. The 33 lions in the study of Wenger *et al.* (2010) reached lateral recumbency within 2.2-11.3 minutes and a single dose (medetomidine, 0.05 mg kg^{-1}; butorphanol, 0.3 mg kg^{-1}; midazolam, 0.2 mg kg^{-1}; hyaluronidase 1250 IU) provided immobilization for about 45 minutes. The physical parameters were measured every 10 minutes and several adverse effects were reported. Five lions developed bradyarrhythmia and two had elevated body temperatures. The arterial blood gas analyses indicated a mild metabolic acidosis. A combination of naltrexone, atipamezole and flumazenil was used for reversal, which proved effective as the lions were up and walking in 1.7-22 minutes. However, one lion vomited, and eight lions lay back down, only to get up again a few minutes later.

The time between measurements in the study of Wenger *et al.* (2010) was 10 minutes, which is twice as long as in other studies mentioned. The monitoring of immobilized animals should be continuous, and physical parameters should be recorded every 5-10 minutes (Ozeki & Caulkett 2014). Therefore, the 10 minutes intervals should be enough, but I would prefer a more continuous measuring.

Wenger *et al.* (2010) added hyaluronidase to the BMM combination. Hyaluronidase is an enzyme that breaks down hyaluronan to increase drug absorption into tissue. It has been observed to significantly shorten the induction time of BMM in black rhinos (Kock 1992) but Wagner *et al.* (2010) did not see the same effect in lions. On the other hand, the reversal drugs worked very rapidly in some of the individuals. Some studies argue that the intramuscular half-life of hyaluronidase is 7.5 minutes and the subcutaneous half-life is 5.1 minutes (Muckenschnabel *et al.* 1998), while others say that hyaluronidase still has effect in subcutaneous tissue an hour after injection (Kim *et al*. 2018). If the half-life of hyaluronidase is longer than anticipated, it might have contributed to the rapid effect of the reversal drugs. However, the plasma half-life of hyaluronidase is only two minutes so it will quickly degrade in the systemic circulation (Murray *et al.* 2021). Because the hyaluronidase did not seem to influence the induction times in lion, it would be interesting to study the BMM combination without hyaluronidase to see if it affects the reversal. A reversal that happens too quickly poses a threat to both the animal and the people around it, as they will not have enough time to withdraw.

Medetomidine-zolazepam-tiletamine (MZT)

The MZT combination is a mixture of the a2 agonist medetomidine, the benzodiazepine zolazepam and the NMDA-agonist tiletamine. A combination of tiletamine-zolazepam is commonly used for immobilization as it has a wide safety margin and does not significantly affect cardiopulmonary functions or the temperature regulation mechanism of the animal.

Unfortunately, the elimination of tiletamine takes long and there is no effective antagonist, which leads to a prolonged recovery time (Jacquier *et al*. 2006). However, if you combine tiletamine and zolazepam with medetomidine you can reduce the tiletamine dose with up to 75%. This is advantageous as the recovery time shortens significantly and the small volumes required for a successful immobilization potentially cause less tissue damage at the injection site (Cattet *et al*. 1999).

The MZT combination contributes with a smooth induction and recovery. It induces an adequate muscle relaxation and analgesia for minor surgical procedures, but the mean induction time varied from 5.9-14.1 minutes between studies (Fahlman *et al.* 2005; Jacquier *et al.* 2006). The sedative duration of a single dose (medetomidine, 0.06 mg/kg; zolazepam-tiletamine, 1.45 mg/kg) was at least one hour, but the anaesthesia lasted for over two hours in four lions (Fahlman *et al.* 2005). The physiological data of the animals were collected every 5 minutes and the reversal was induced with an injection of atipamezole (Jacquier *et al.* 2006).

This drug combination appears to be good regarding adverse effects. The rectal temperatures were not affected, and a slight hypoxemia was seen only in two of the animals in the study. However, the lion administered with the largest dose of MZT developed bradypnea, bradycardia and its recovery time was prolonged (Jacquier *et al*. 2006).

Xylazine hydrochloride and ketamine hydrochloride

This is a commonly used drug combination with a wide safety margin (Logan *et al.* 1986), consisting of the a2 agonist xylazine and the NMDA agonist ketamine. In the study of Herbst *et al.* (1985), the induction time ranged between 22 and 92 minutes in free ranging lions, but in all cases except five, an additional injection of ketamine hydrochloride was needed. The doses ranged from 3.8-16.7mg/kg ketamine hydrochloride (KHCL) and 0.46-1.17mg/kg xylazine hydrochloride (XHCL). The duration of the anaesthesia was not clearly stated in this study, but out of 13 lions, five lifted their heads within 21-80 minutes after their last injection. In a study made on wild mountain lions (*Felis concolor*), the combination (KHCL, 4.7-15.2mg/kg; XHCL, 0.8-2.5mg/kg) offered a duration of 36-82 minutes (Logan *et al.* 1986), which is similar to the time it took for the lions to lift their head in the previously mentioned study. Few adverse effects were reported. In Herbst *et al.* (1985) one lioness became excited, and six others retched or vomited during induction. To avoid emesis, the authors recommend administrating diazepam.

Even though there is no reversal agent for ketamine, the reversal progressed smoothly without complications (Herbst *et al.* 1985). According to Logan *et al.* (1986) the drug response varied greatly between individuals. Therefore, it is safer to approach the lions based on what signs of consciousness they show, rather than the amount of time passed since they were darted.

Conclusions

All immobilization protocols mentioned have relatively short induction times, except for the xylazine hydrochloride-ketamine hydrochloride combination. Because of the longer induction, I would argue that it would be better to use another protocol or a higher dose of ketamine than mentioned, which should not be a problem thanks to the supposedly wide safety margin. MZT offered the longest duration, which could be beneficial for longer procedures. For shorter procedures it might be better to use BMM, as it had both the shortest induction and duration times.

Regarding adverse effects, all the protocols were adequate. No deaths were reported in any of the articles, and under normal circumstances, no particularly severe adverse effects occurred. However, both the xylazine hydrochloride-ketamine hydrochloride protocol and the reversal of the BMM protocol induced vomiting, which is a common side effect from both a2 agonists and opioids. This should be taken into consideration, as aspiration of vomit could potentially cause aspiration pneumonia (Arnemo *et al*. 2014). All the immobilization drugs are administered intramuscularly. Therefore it might be disadvantageous from an animal welfare point of view to use ketamine, as its low pH-level makes intramuscular injections painful (Papich 2015).

To summarize, I would refrain from using the xylazine hydrochloride-ketamine hydrochloride protocol, but the rest of the protocols are good alternatives for immobilizing lions.

Free ranging lions versus captive lions

Immobilization

A rapid induction is always desired but is particularly important in free ranging lions. If the induction time is prolonged, the localisation of the animal can become problematic, especially if the darting is done during the night or in an area with dense vegetation (Wenger *et al.* 2010). The lion can potentially run long distances or be obscured by vegetation when becoming recumbent, which can prevent it from being found. This is crucial since a lion affected by immobilizing drugs is more vulnerable to its surroundings. Predators, large herbivores, complications from the anaesthesia and harsh environmental factors could all prove fatal. A lion in a confined environment will still benefit from a quick induction, but the consequences of a prolonged one is oftentimes not as severe.

The doses required for a proper induction are often higher for free-ranging animals than for captive ones (Kreeger & Arnemo 2012 see Arnemo *et al.* 2014). When an animal is stressed, an endogenous release of catecholamines can make the induction phase longer or completely prevent sedation (Hernandes-Divers & Foerster 2001). In a study on wild grizzly bears, pursued bears had longer induction times and needed higher drug doses than bears physically restrained with foot snares (Cattet *et al.* 2003). It is currently believed safer to overdose, than underdose immobilization drugs as the delayed induction times can cause further stress and other complications (Arnemo *et al.* 2014). In the study by Herbst *et al.* (1985) one of the lionesses was very agitated and required a much higher dose than the rest of the lions. The induction time was also considerably longer.

With captive animals, it is possible to control surrounding variables such as environment and status of the animal and to more precisely calculate and deal with possible complications. As previously mentioned, it is easier to make sure that a captive lion has not been feeding prior to immobilization and it is also easier to follow up and monitor a captive lion after the procedure.

Capture myopathy

Capture myopathy occurs when an animal is intensely pursued or confined and the fight-or-flight response causes a surge of epinephrine that affects skeletal and heart muscle cell membranes, which leads to ventricular fibrillation (Montané *et al.* 2002). An animal with capture myopathy may present with metabolic acidosis, weakness, incoordination, muscle stiffness, paresis and myoglobinuria (Chalmers and Barret 1977 see Breed *et al.* 2019).

Though capture myopathy is often mentioned regarding wild animals, it also occurs in zoo animals that are chronically stressed by their confinement. Unnatural social environments and

lack of stimulation, can cause a chronic stress that former free-ranging animals are especially vulnerable to (Dickens *et al.* 2010). A study involving cheetahs (*Acinonyx jubatus*) reported that captive cheetahs had much higher concentrations of corticoids in their blood and significantly larger adrenal cortices than free-ranging cheetahs, which indicate chronic stress (Terio *et al.* 2004). However, different species react very differently to captivity (Mason 2010). Therefore, lions could potentially be less stressed in captivity than cheetahs. A study on faecal glucocorticoid metabolites (FGM) reported that captive lions had a good welfare from a physiological point of view (Schildkraut 2016). However, there is no study made on FGMs in free-ranging lions, so we cannot compare the results.

When an animal starts showing signs of capture myopathy, the prognosis is dire. The treatment is long, non-specific and often unsuccessful (Breed *et al.* 2019). Therefore, it is seldom possible to successfully treat animals in the wild. If it occurs in captive animals, it is easier to administer supportive measures as the animal can be constantly supervised. The best treatment for capture myopathy is proactive measures where external stressors are kept to a minimum. As previously mentioned, this could be providing the captive lions with adequate housing and stimulation and having a carefully planned immobilization protocol for wild lions (Breed *et al. 2019)*.

Conclusions

Whether the lion is captive or free-ranging does have an influence on how the immobilization should be executed. In many ways, it is easier to immobilize and monitor a captive animal, but the immobilization of free-ranging animals should also run smoothly if the procedure is planned correctly.

Solitary lions versus lions in a pride

In the reviewed literature there are both positive and negative aspects mentioned regarding the immobilization of solitary animals and animals in a pride. There are various observed behaviours connected to the pride, and the social environment is an important factor to consider when planning the immobilization procedure.

Positive effects of a pride

When a lioness showed signs of being darted, members of the pride approached her and rubbed their heads against hers, which seemed to have a calming effect (Herbst *et al.* 1985). As previously mentioned, stress could prolong induction and cause complications, and as the pride seemed to have a relaxing effect, I would argue that it is beneficial for a smooth and uneventful anaesthesia. On the other hand, the presence of a pride could potentially be a threat to people working with the immobilized animal. However, when Herbs *et al.* (1985) approached a darted lioness, other pride members moved away and did not pay further attention to the team. This could be a unique behaviour for this pride, so I would still advocate for extra alertness.

Negative effects of a pride

Loud noises can increase the risk of sudden arousal, especially cries of distress from younger pride members (Arnemo *et al.* 2014). When animals with social bonds are separated from each other they oftentimes use vocal signalling to regain contact (Panksepp 1998 see Newberry & Swanson 2001), which could potentially disturb the induction and anaesthesia. In Herbst *et al.* (1985) one of the lionesses became excited during the induction. When other pride members approached her, she jumped and attempted to get away. I would argue that this poses a risk since an excited lion could hurt both itself and other pride members.

Another factor to take into consideration is the impact on the pride if the procedure is fatal. Anecdotal reports in dogs describe behaviour resembling mourning, where they exhibit symptoms such as apathy, anorexia, reduced playful behaviour and continuous searching for the late companion. Other animals, such as primates and elephants, have been observed staying with the dead even after the rest of the pride has left, and returning later to resume searching (Newberry & Swanson 2001). I have not found any studies made on mourning behaviour in lions, but if they are affected in a similar way, the loss of a pride member could have dire consequences. A lion that stops eating, pays less attention to its surroundings and gets separated from its pride is at greater risk of falling victim to predators or harsh environmental factors.

Conclusions

From the studies I have read, the immobilization seems to be affected by whether the lion is solitary or member of a pride. However, it would require more research to determine exactly how big impact the social structure has on the procedure. As I mentioned in the introduction, the lion species is declining and every preventive action we can take is necessary. Therefore, I would like to see more research done on the subject.

Pros and cons with the reviewed literature

Most of the articles mentioned in this thesis seemed to be of good quality. However, some of them were written by the same authors and some not peer reviewed, which could mean that the information is highly subjective. To avoid using articles with subjective or faulty information, I have tried to find the common census from multiple sources.

Two additional negative aspects with the studies are that the sample sizes often are quite small and that few studies have been made on the subjects. In Jacquier *et al.* (2006) the authors mention that they would like further studies to get reliable results, which in my opinion is the case in several of the studies.

On a more positive note, I feel that the included literature managed to help me answer the questions stated in this thesis.

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