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Comparison of two injectable anaesthetic protocols in Egyptian fruit bats (*Rousettus aegyptiacus*) undergoing gonadectomy

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Egyptian fruit bats have gained increasing interest being a natural reservoir for emerging zoonotic viruses. Anaesthesia is often required to allow safe handling of bats. We aimed to compare the sedative and cardiopulmonary effects of two balanced anaesthetic protocols in bats undergoing gonadectomy. Group DK (n = 10) received intramuscular dexmedetomidine (40 µg/kg) and ketamine (7 mg/kg), whereas group DBM (n = 10) received intramuscular dexmedetomidine (40 µg/kg), butorphanol (0.3 mg/kg) and midazolam (0.3 mg/kg). Induction time and cardiopulmonary parameters were recorded. If anaesthetic plan was inadequate, isoflurane was titrated-to-effect. At the end of surgery venous blood gas analysis was performed and atipamezole or atipamezole-flumazenil was administered for timed and scored recovery. In DBM group heart rate and peripheral oxygen saturation were significantly higher ($p = 0.001$; $p = 0.003$ respectively), while respiratory rate was significantly lower ($p = 0.001$). All bats required isoflurane supplementation with no significant differences between groups. Induction and recovery times showed no significant differences. In group DK a better recovery was scored ($p = 0.034$). Sodium and chloride were significantly higher in DBM group ($p = 0.001$; $p = 0.002$ respectively). Both anaesthetic protocols were comparable and can be recommended for minor procedures in bats.

Pteropid bats have been studied in various research fields as they have been identified as a natural reservoir for various emerging zoonotic viruses, including Marburg virus[1], Hendra virus, Nipah virus[2] and lyssavirus variants[3]. Furthermore, among the family *Pteropodidae*, also the Egyptian fruit bat (*Rousettus aegyptiacus*) showed characteristics of a reservoir host for SARS-CoV-2[4]. Besides, Egyptian fruit bats are commonly housed in zoological environments because they are small, amenable to handling and reproduce readily in captivity[5].

The safe collection of biological samples from pteropid bats, such as blood and swabs from the throat, urethra and rectum, is essential for both the animal and the operator[6]. During sampling, the physical restraint of bats can expose the handler to bite and scratch injuries, resulting in potential zoonoses transmission. Short-term anaesthesia facilitates operator safety and minimizes stress for the bat[7,8]. Furthermore, in zoological settings, short-term anaesthesia is important to apply contraception protocols, to prevent overpopulation and inbreeding in highly fertile bat colonies[9,10].

A total isoflurane inhalation anaesthesia is often the method of choice for bats, having the advantage of wide safety margins, very little metabolization, and quick induction and recovery times[11,12]. Side effects that may occur with extensive inhaled anaesthetic use are dose-dependent and include respiratory and myocardial depression and decrease in sympathetic activity, leading to decreased cardiac output and hypotension[13]. The use of a balanced anaesthesia protocol allowed a reduction in isoflurane concentration compared to a total inhaled anaesthesia, preventing the development of the typical halogenate side effects, and providing analgesia[14,15].

Previously reported injectable anaesthetic protocols used in bats include alpha-2 adrenergic agonists (i.e. xylazine or medetomidine) and ketamine (KET)[6–8,16]. The use of alpha-2 adrenergic agonists results in sedation, analgesia, muscle relaxation, anxiolysis, and reduces the anaesthetic requirements of injectable and inhalant

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agents during induction and maintenance of general anaesthesia[17]. Dexmedetomidine (DEX) is a highly selective alpha-2 agonist and it is the active enantiomer of medetomidine[18].

Ketamine induces anaesthesia and amnesia by functional dissociation of the central nervous system resulting in catalepsy, immobility, amnesia, and marked analgesia[17]. But its use alone is highly discouraged, due to the poor muscle relaxation, with slow and often excitative recoveries[16]. The alpha-2 adrenergic agonists-KET combination provide satisfactory analgesia for minor procedures and muscle relaxation together with some cardiovascular side effects, generally well tolerated in patients without pre-existing cardiac diseases[6,19]; nevertheless, this combination ensured a good cardiovascular stability in anesthetized pigs[20]. Finally, DEX-KET may be associated with prolonged recovery and hypothermia[6,19]. This balanced protocol has not been evaluated for major surgeries in bats before, but it provided a suitable anaesthesia and analgesia in other species[21–25].

Butorphanol (BUT) is a k agonist-μ antagonist opioid with mild sedative and analgesic properties[17]. Opioids are often combined with alpha-2 adrenergic agonists because they potentiate their sedative and analgesic effects with minimal additional cardiovascular effects[26].

Midazolam (MDZ) is a benzodiazepine that has sedative-hypnotic, anxiolytic and muscle relaxant effects and enhances the sedative and antinociceptive effects of alpha-2-adrenergic agonists[27].

A subcutaneous combination of medetomidine, MDZ and opioids has been shown to be safe for Egyptian fruit bat anaesthesia, with no apparent morbidity or mortality[19].

The purpose of the study was to evaluate and compare sedative effects of two different injectable anaesthetic protocols, DEX and KET (group DK) versus DEX, BUT and MDZ (group DBM) in bats undergoing gonadectomy and to record physiological and adverse effects following administration of both protocols. We also evaluated the duration of induction, timing and quality of recovery achieved by both combinations. We hypothesized that both injectable protocols are suitable to maintain a surgical anaesthetic plan in Egyptian fruit bats with few side effects.

Methods

The present study complies with ethical standards and was conducted under the approval of the Institutional Ethical Committee for Animal Care at the University of Milan (OPBA_104_2021). All procedures were carried out in accordance with the relevant guidelines and regulations and the study was carried out in compliance with the ARRIVE guidelines. Private owner informed written consent was obtained.

Twenty healthy male and female Egyptian fruit bats (age unknown, body weight between 100 and 150 g) presented at the Veterinary Teaching Hospital of the University of Milan to perform gonadectomy were included in the study.

All bats were housed together throughout the hospitalization period in a large mesh cage (height 2.5 m, width 1.5 m, length 2.0 m) in a controlled-environment room (22–25 °C and 60% humidity) and exposed to a natural photoperiod (light/dark alternation period of 8/16 h). They received water ad libitum and were fed with a mixture of seasonal fruits and vegetables. All the procedures were performed after an acclimation period of 10 days. During this period, they were considered healthy based on observation of normal behaviour without stereotypic attitudes, normal activity levels and appetite along with normal weight, size and wingspan length, in absence of clinical signs.

The day of the surgery each bat was captured inside the cage using protective leather gloves and was temporarily placed inside a perforated canvas bag on a digital laboratory scale (Precisa BJ610C, Precisa Instrument, Dietikon, Switzerland) to accurately measure its body weight. Patients were then randomly assigned to either group DK or DBM (www.randomizer.org). Bats in group DK received an intramuscular (IM) administration of DEX (40 μg/kg)(Dexdomitor 0.5 mg/ml; Vetoquinol Italia S.r.l., Italy) and KET (7 mg/kg) (Lobotor 100 mg/ml; ACME S.r.l., Italy) and those in group DBM received an IM injection of DEX (40 μg/kg), BUT (0.3 mg/kg) (Nargesic 10 mg/ml; ACME S.r.l., Italy) and MDZ (0.3 mg/kg)(Midazolam Hameln 5 mg/ml; Hameln pharma gmbh, Germany). All syringes were prepared, labelled in a way that did not reveal their content and injected in the bats' thigh muscles by an experienced anaesthetist not involved in the study. All anaesthetic procedures were performed by another experienced anaesthetist, who was unaware of the treatment administered.

Immediately after drugs' administration, the bat was placed in a transparent plexiglass cage and observed continuously to monitor the induction phase and record the induction time. The induction time was defined as the interval from administration of the drugs to the absence of movement following a gentle foot palpation.

Upon the loss of response following palpation, the bat was positioned in dorsal recumbency on a warm air blanket (Bair Hugger 505 Warming Unit; 3M, Germany), which was covered with an adsorbent pet sheet. In addition, a monopolar electrosurgical plate was placed under the bat. A complete physical examination was performed, and wingspan and the body length (head to tail) were measured. Based on the size of the animal and development of the reproductive system, the age of each bat was estimated and classified into "juvenile" or "adult". Adult males were distinguished on the basis of fully developed penis and testes, a body size ≥ 15 cm and a wingspan ≥ 48 cm; adult females were distinguished from juvenile on the basis of worn or enlarged nipples or if palpably pregnant, a body size ≥ 14 cm and a wingspan ≥ 48 cm. Juveniles (< 12 months old) were classified on their smaller size and rudimentary development of sexual characteristics[28].

A 22-gauge venous catheter (Jelco IV Catheter Radiopaque; Smiths Medical Italia S.r.l., Italy) was inserted in the left cephalic vein. A multiparameter monitor (S5 Compact Anesthesia Monitor; Datex-Ohmeda, USA) was used throughout the anaesthetic period. Oxygen 100% flow-by at 1 L/min was administered via a facemask, which was attached to a side-stream spirometer. A pulse oximeter was connected on the right hind limb and disposable foam pad electrodes for electrocardiographic measurements were positioned as in Fig. 1.

Heart rate (HR), respiratory rate (RR) and peripheral oxygen saturation (SpO_2) were continuously monitored and recorded every 5 min during surgery. Rectal temperature (RT) was measured at the beginning and at the end of the surgical procedure using a digital thermometer (Pic VedoFamily; Pikdare S.p.A., Italy).

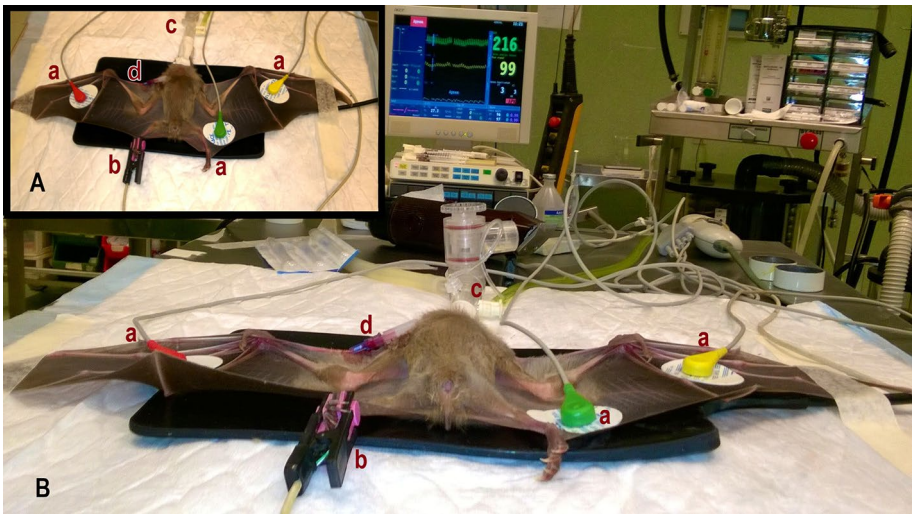

Figure 1. Egyptian fruit bat in dorsal recumbency, instrumented with monitoring devices (**A**) and overview in operating room (**B**). Disposable foam pad electrodes (**a**) positioned on the ventral aspect of wings for electrocardiographic measurements; pulse oximetry probe (**b**) placed on the right hind limb for haemoglobin saturation measurement; side-stream spirometer (**c**) attached to the facemask for multi gas analysis, and 22-gauge catheter (**d**) inserted in the left cephalic vein for collection of samples for blood gas analysis.

Score	Description
1	Poor recovery: compulsive movements, biting, wing-chewing, wing flapping
2	Weak recovery: tremors, twitching, reduced responsiveness to environmental stimuli
3	Excellent recovery: uneventful, good response to stimuli, rapid ability to fly

Table 1. Scoring system used to assess recovery from anaesthesia.

Depth of anaesthesia was assessed every 10 min by easy extension and flexion of the wing without any voluntary movement[29] or presence of muscle tone by opening the jaw[30]. In case of spontaneous movement or presence of muscle tone, the anaesthesia depth was considered inadequate, and the fraction of inspired isoflurane (FI-ISO) was titrated-to-effect to the minimum concentration to achieve immobility and loss of muscle tone and this value was recorded and then adjusted over time as needed.

All gonadectomy surgeries were performed by the same experienced surgeon and total surgery time was recorded. Females found to be pregnant underwent ovariohysterectomy, while non-pregnant females were performed ovariectomy.

During the entire procedure, side effects including arrhythmia, irregular breathing pattern, twitching and tremors were recorded as existing or not, regardless of severity or duration.

At the end of the surgery, venous blood gas analysis (Stat Profile pHOx Ultra; Nova biomedical Italia S.r.l., Italy) was performed. Analysis included venous pH, venous partial pressure of oxygen (PvO_2) and carbon dioxide ($PvCO_2$), base excess (BE) and electrolytes (Na^+, K^+, Cl^-) as well as bicarbonate (HCO^{3-}) and total haemoglobin (Hb). Then, bats in group DK received IM atipamezole (200 µg/kg) (Antisedan 5 mg/ml; Vetoquinol Italia S.r.l., Italy), while bats in group DBM received IM atipamezole (200 µg/kg) and IM flumazenil (0.03 mg/kg) (Flumazenil Kabi 0.1 mg/ml; Fresenius Kabi Italia S.r.l., Italy). Following the IM injection of reversal drugs in the thigh muscles, each bat was returned to the plexiglass cage.

Recovery time, namely the time from the injection of the antagonists to flying, was recorded and recovery quality scored on a scale of 1–3 (Table 1). All the recoveries were observed continuously and evaluated by the same anaesthetist.

After recovery, all bats were administered meloxicam (0.2 mg/kg) (Metacam 5 mg/ml; Boehringer Ingelheim Vetmedica GmbH; Germany) subcutaneously and were replaced in the mesh cage. All bats were monitored every hour for 12 h, and then were observed daily for a week to evaluate any side effects.

A power analysis was performed and determined that a minimum of 18 bats would be required to detect a clinically relevant difference in induction time of 4 min or more between the two groups with a power of 85% and $\alpha = 0.05$ (two-tailed).

Statistical analysis was performed using IBM SPSS Statistics 26.0 (SPSS Inc, Chicago, USA). The normality of data distribution was assessed by a Shapiro–Wilk test at the $\alpha = 0.05$ level. Descriptive statistics were reported as mean ± standard deviation (SD) or median (range) for continuous and ordinal variables, respectively. Pearson's chi-squared test was used to evaluate significant differences in nominal data. Analysis of variance (ANOVAs), followed by Bonferroni's post hoc test, and Mann–Whitney U test or Wilcoxon's test was applied for normal

	DK	DBM
Induction time (seconds)	149 ± 170	169 ± 48
Surgery time (minutes)	53 ± 16	44 ± 12
Recovery time (seconds)	345 ± 150	424 ± 210

Table 2. Induction time, surgery time and recovery time in 20 Egyptian fruit bats anaesthetized for gonadectomy. Bats in group DK (n = 10) received dexmedetomidine (DEX) and ketamine (KET) combination and bats in group DBM (n = 10) received DEX, butorphanol (BUT) and midazolam (MDZ) administration. Atipamezole (group DK) or atipamezole and flumazenil combination (group DBM) was administered intramuscularly at the end of the surgery. Results are presented as mean ± standard deviation.

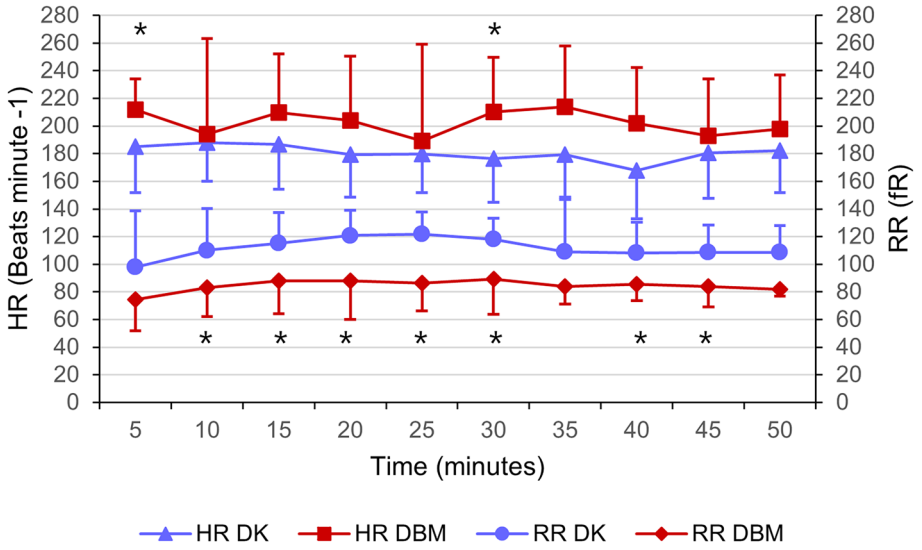

Figure 2. Heart rate (HR) and respiratory rate (RR) in 20 Egyptian fruit bats during general anaesthesia for gonadectomy. Bats in group DK (n = 10) received DEX and KET combination and bats in group DBM (n = 10) received DEX, BUT and MDZ administration. Results are presented as mean ± standard deviation. Significant differences ($p < 0.05$) at specific time points in HR and RR were found (represented as asterisk).

and non-normal data, respectively, to assess significant differences between and within groups. The influence of total surgery time on recovery time was evaluated by Pearson's correlation. Differences with $p < 0.05$ were considered significant.

Results

Twenty Egyptian fruit bats were included in the study: ten bats (5 males, 5 females) received DK treatment and ten bats (4 males, 6 females) received DBM treatment. No significant differences in gender, age (DK 8 adults, 2 juveniles; DBM 7 adults, 3 juveniles), female reproductive status (DK 5 pregnant; DBM 5 pregnant) and body weight (DK 111.4 ± 9.38 g; DBM 111.6 ± 6.91 g) were recorded. There were no significant differences in mean induction time and in total surgery time between the two treatment groups (Table 2).

Heart rate, RR and SpO_2 were compared between groups for the first 50 min following induction (on further time-points some of the bats had already recovered). A significantly higher heart rate was recorded in DBM group (DK 181 ± 31 bpm; DBM 203 ± 47 bpm) ($p = 0.001$), while respiratory rate was significantly lower than DK group (DK 112 ± 26 rpm; DBM 85 ± 21 rpm) ($p = 0.001$). A significant difference was observed in peripheral oxygen saturation, where in the DBM group it was higher than in the DK group (DK 98.1 ± 1.9%; DBM 99.1 ± 0.9%) ($p = 0.003$). All bats required isoflurane supplementation during surgery and no significant difference in FI-ISO was observed between groups (DK 0.71 ± 0.06%; DBM 0.70 ± 0.08%). No statistically significant differences were observed within groups over time in HR, RR, SpO_2 and FI-ISO parameters. Results and time points significances are summarized in Figs. 2 and 3.

There were no significant differences in initial (DK 37.5 °C ± 0.7; DBM 37.7 °C ± 0.7) or final (DK 36.8 °C ± 1.3; DBM 37.2 °C ± 1.1) rectal temperature between treatments and RT at the end of the surgery did not decrease significantly compared to the beginning of the surgical procedures in either group.

There was no significant difference between groups in venous blood gas analysis except for Na^+ (mmol/L) ($p = 0.001$) and Cl^- (mmol/L) ($p = 0.002$) that where significantly higher in DBM group (Table 3).

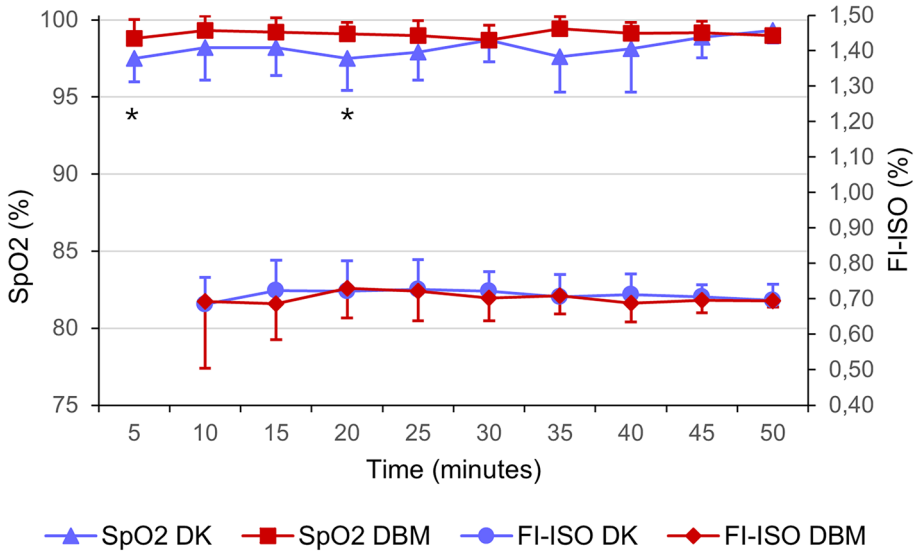

Figure 3. Peripheral oxygen saturation (SpO_2) and fraction of inspired isoflurane (FI-ISO) in 20 Egyptian fruit bats during general anaesthesia for gonadectomy. Bats in group DK (n = 10) received DEX and KET combination and bats in group DBM (n = 10) received DEX, BUT and MDZ administration. Results are presented as mean ± standard deviation. Significant differences at specific time points ($p < 0.05$) in SpO_2 were found (represented as asterisk).

Parameter	Group	Mean ± SD
$PvCO_2$ (mmHg)	DK	39.10 ± 6.4
	DBM	40.90 ± 5.0
PvO_2 (mmHg)	DK	237.80 ± 137.0
	DBM	277.00 ± 117.1
pH	DK	7.35 ± 0.04
	DBM	7.35 ± 0.02
Hb (g/dL)	DK	13.70 ± 0.88
	DBM	13.48 ± 1.33
BE (mmol/L)	DK	− 4.10 ± 1.47
	DBM	− 3.06 ± 2.05
HCO^{3-} (mmol/L)	DK	20.73 ± 1.00
	DBM	21.81 ± 1.59
Na^+ (mmol/L)	DK	134.8 ± 1.75*
	DBM	139.5 ± 1.58*
K^+ (mmol/L)	DK	3.67 ± 0.52
	DBM	3.65 ± 0.66
Cl^- (mmol/L)	DK	103.40 ± 1.78*
	DBM	107.50 ± 2.42*

Table 3. Venous blood-gas values, venous pH, total haemoglobin, venous base excess, venous bicarbonate, and electrolytes in 20 Egyptian fruit bats anaesthetized for gonadectomy. Bats in group DK (n = 10) received DEX and KET combination and bats in group DBM (n = 10) received DEX, BUT and MDZ administration. Results are presented as mean ± standard deviation (SD). * Significant differences ($p < 0.05$) between groups. $PvCO_2$, PvO_2, venous oxygen and carbon dioxide partial pressures; Hb, total haemoglobin; BE, venous base excess; HCO^{3-} venous bicarbonate; Na^+ ionized sodium; K^+ ionized potassium; Cl^- ionized chlorine.

Recovery times did not significantly differ between groups and no correlation was observed between total surgery time and recovery duration (Table 2). A significantly lower recovery quality was observed in the DBM group (DK median 3, range 3–3 and DBM median 3, range 2–3) ($p = 0.034$) as shown in Fig. 4.

No side effects, such as arrhythmia or irregular breathing pattern, twitching and tremors, were observed in any bat following intramuscular administration and during the surgery. After recovery, all bats returned to normal behavior, good activity levels and appetite; no side effects were observed during the follow-up period.

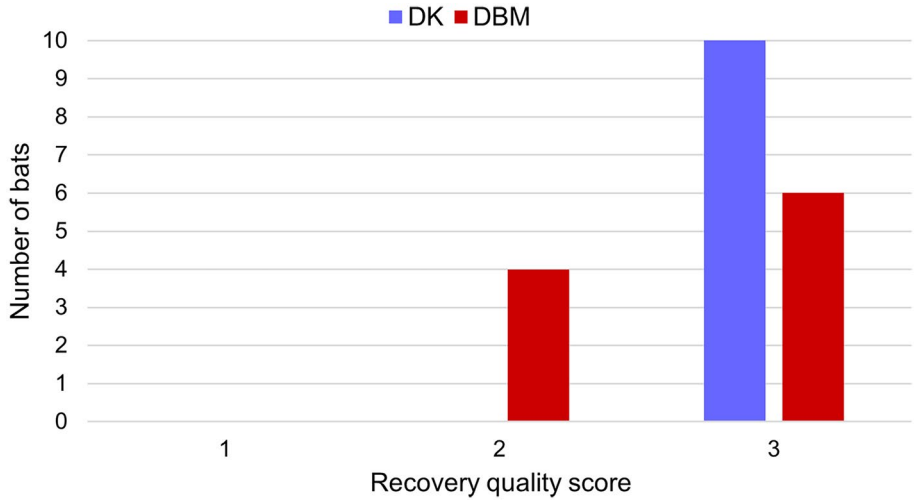

Figure 4. Recovery quality scores in 20 Egyptian fruit bats anaesthetized for gonadectomy. Bats in group DK (n = 10) received DEX and KET combination and bats in group DBM (n = 10) received DEX, BUT and MDZ administration. Significant differences ($p < 0.05$) between groups were found.

Discussion

The present study aimed to evaluate sedative effects of two different injectable anaesthetic protocols, DEX and KET (DK) versus DEX, BUT and MDZ (DBM) in bats undergoing gonadectomy.

Different studies report the use of alpha-2 adrenergic agonists in association with KET in bats[6–8,16]. Dexmedetomidine-ketamine combinations have been used in a variety of mammalian species[31–33]. To the authors' knowledge, no studies have been carried out in Egyptian fruit bats, or any other Chiroptera species, using DEX as a part of a balanced anaesthetic protocol. In the present study, this association produced high-quality immobilization with rapid and smooth induction.

Anaesthesiologic protocols including the association of alpha-2 adrenergic agonists, opioids and benzodiazepines have already been described in veterinary medicine in several species[34,35]. Regarding bats, only Tuval et al.[19] have compared different subcutaneous combinations of medetomidine, MDZ and opioids in *R. aegyptiacus* with no apparent morbidity and mortality. Compared with the work of Tuval et al.[19], the dosages and total volumes of drugs used in this study were considerably lower, probably also due to the different route of administration. In our study, IM administration was performed in the thigh muscles; no behaviour referable to muscle soreness or pain was observed following injection or during recovery phase, and no bats had difficulty in flying following the procedure.

A rapid and gentle induction was observed in all bats in the DBM group, and they reached the desired level of sedation in slightly longer time than the DK group, but with less intragroup variability. It has been shown that the association of these three drug classes, at lower doses than would be made if only one agent were used, results in a synergistic central nervous system depressant response while minimizing the undesirable side effects of each drug[19,36,37]. The combination of several not purely anaesthetic drugs has successfully induced anaesthesia with surgical tolerance in various species[38–40], including bats[41,42]. Further advantage of this association is that each drug can be completely antagonized enabling precise timing of sedative effects and in the case of emergency conditions, allowing for safer patient management.

In the present study, butorphanol reversal was not performed to preserve post-operative pain management in all bats included in DBM group.

All bats required isoflurane supplementation without differences in FI-ISO between groups and it only became necessary during the surgical phase, while injectable anaesthetics were satisfactory for the patient preparation. The use of 2–2.5% isoflurane via facemask for the maintenance of general anaesthesia in Chiroptera species has been described in several works[9,11,12,43]. In the present study, the FI-ISO necessary to obtain an adequate surgical plane of anaesthesia was much lower than that reported in literature, suggesting that both protocols may have had a sparing effect on isoflurane. The minimal alveolar concentration reduction of inhaled anaesthetics after administration of DEX, KET, BUT and MDZ is reported in various species[18,44,45]. The results of this study suggested that to obtain an adequate plane for surgical anaesthesia during gonadectomy, isoflurane had to be administered, even if at lower concentrations than those reported in the literature. However, in the authors' opinion, for minor procedures (physical examination, manipulation, blood and swab sampling or skin biopsy) both these injectable anaesthesia protocols could be sufficient to achieve immobility in bats without stress and were used safely by the operator during the entire anaesthetic period. Nevertheless, the authors suggest supplementing analgesia, for example through locoregional techniques (intratesticular block, lavages with local anaesthetics on ovarian pedicles or intraperitoneal) allowing these balanced anaesthesia protocols to be more suitable for major surgeries and field anaesthesia.

Noll et al.[46] reported a resting HR of 248 ± 3 bpm in telemetrically monitored adults of *R. aegyptiacus*. In the present study, baseline values in manually restrained bats before drugs administration were not measured. However, physical restraint is stressful and would have altered values themselves, as reported in previous studies[30]. Therefore, considering the resting parameters in the literature, it is possible to assume that there was a decrease in HR following the administration of both injectable protocols and this finding is probably imputable to the effect of DEX. The cardiac effects of DEX observed in this study are the same as those described for other mammalian species and other alpha-2 agonists[18,19,32]. Dose-dependent bradycardia following DEX administration results primarily from a baroreceptor reflex to hypertension, caused by peripheral vasoconstriction and enhanced vagal activity; then it is prolonged by a decrease in sympathetic tone[18]. Unfortunately, in the present study, arterial blood pressure could not be measured, and there are no studies on bats regarding changes in these parameters following administration of alpha-2 adrenergic agonists. Further studies would be helpful to clarify this aspect. Opioids decrease HR by increasing parasympathetic tone[17], while KET should partly balance the effects on the cardiovascular system induced by alpha-2 adrenergic agonists[32]. Nevertheless, the use of adjunctive drugs, such as alpha-2 adrenergic agonists, tends to blunt the sympathomimetic effect of KET and to decrease cardiac output and arterial blood pressure[17]. Indeed, in this study, HR was significantly lower in DK group than DBM group.

Comparing baseline values of RR reported by Tuval and colleagues (2018), in the present study both protocols showed a slight reduction in the values of this physiological parameter if compared to other studies[19,30]. Bats in group DBM showed the greatest reduction in RR during anaesthesia, being significantly lower than which occurred in group DK. However, SpO_2 was significantly higher in the DBM group, although in both groups the measured values were within the normal ranges. Oxygen saturation values have always remained within normal ranges probably because 100% oxygen was administered throughout the anaesthesia period, as oxygen supply was reported to improve arterial oxygenation during anaesthesia in other species[47]. In the present study, arterial blood gas analysis could not be performed, but peripheral venous blood drawn anaerobically is known to correlate reasonably well with arterial values, at least for pH, bicarbonate and carbon dioxide tension values (CO_2)[48]. Kelly et al.[49] showed that a $PvCO_2$ of less than 45 mmHg has a 100% negative predictive value to rule out arterial partial pressure of CO_2 ($PaCO_2$) greater than 50 mmHg in humans. Therefore, normal peripheral $PvCO_2$ can be used as a screen to exclude hypercapnic respiratory disease[50] and in the present study, hypoventilation induced by inhaled and injectable anaesthetics was neither reflected in changes in $PvCO_2$, pH, or PvO_2 outside physiological ranges in other mammalian species[51] nor in significant differences between the two groups.

Blood gas analysis did not show significant differences in the other values, except for electrolytes, where Na^+ and Cl^- were significantly higher in DBM group. However, no references have been found in the literature to explain a correlation between the plasma concentration of these ions and the effects induced by anaesthetic drugs. So, in healthy animals this difference could be reasonably attributed to animal feeding and/or to water intake[52]. Furthermore, it has been shown that food intake increases during the first week of pregnancy in rats, resulting in increased renal excretion of sodium and chloride, which decreases during the last weeks of pregnancy, leading to higher plasma concentrations[53]. The different stage of pregnancy at which females were at the time of the present study may have biased the results.

Due to the anatomical conformation of the wings and the small size with an high ratio between surface and body mass, Chiroptera are particularly susceptible to heat loss[19,43]. Rectal temperature was measured at the beginning and at the end of the surgical procedure and all bats were warmed with heating pad during general anaesthesia. No significant differences between initial and final temperatures within and between groups were found, suggesting that active warming counteracted the hypothermia induced by general anaesthesia.

In both groups, the simultaneous use of several drugs, with their synergistic effects, may have contributed in achieving an excellent and rapid anaesthetic plan, to reduce the single drug dosages and to avoid the appearance of side effects, as reported by other authors[37,54]. Indeed, no complications associated with the balanced anaesthesia have been observed in the two groups during induction, maintenance, and recovery from general anaesthesia.

Recovery time never exceeded 11 min, without significant differences between groups, which suggests that the use of antagonists (atipamezole and atipamezole/flumazenil) at the end of the surgery is advisable to ensure a rapid recovery. In 4 out of 10 bats of DBM group tremors and twitching were observed during recovery along with a greater difficulty in recovering normal wakefulness and responsiveness to environmental stimuli, probably due to the residual sedative effect of BUT. This result is similar to that observed by Tuval et al.[19], where the recovery of bats administered a combination of medetomidine, MDZ and BUT were significantly longer than other groups. Therefore, they concluded that the administration of atipamezole following the use of a protocol containing an alpha-2 adrenergic agonist and BUT in Egyptian fruit bats is recommended.

A limitation of the present study is that the depth of anaesthesia was assessed without evaluating reflexes and probably prevented us from detecting small differences in isoflurane requirements between groups. In addition, although FI-ISO was accurately recorded, no bat was intubated, and it was not possible to determine intraoperative end-tidal concentrations of isoflurane. Further studies are justified to evaluate minimal alveolar concentration reduction induced by both protocols. Finally, the nature of the clinical study could not consider the variability in gender and reproductive status, which, despite being similar in the two groups, resulted in surgical procedures with different invasiveness and possible different pain magnitude. We did not evaluate and compare the quality of analgesia induced by both protocols, although changes in cardiorespiratory parameters possibly related to nociception were never recorded in any bat during surgery. In addition, no bats showed signs of postoperative pain such as paying attention or licking the wound; they resumed regular feeding within two hours post-operatively, normal behaviour, and activity level until the end of the observation period, with no differences between groups.

Conclusions

In conclusion, DK and DBM protocols produce a smooth anaesthesia induction without side effects in Egyptian fruit bats with similar sedative and cardiorespiratory results. As Egyptian fruit bats are of increasing interest as experimental animals due to their role as virus reservoirs, chemical restraint of this species is becoming increasingly important to improve research in this field. These injectable drug combinations may be useful for minor procedures in Egyptian fruit bats. For surgical procedures, the authors advise to combine isoflurane in order to maintain an adequate hypnotic status.

Data availability

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

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Author contributions

M.A., F.A.B., V.R. and G.R. designed and conceptualized the study. M.A., F.A.B., A.G., P.M. and F.B. analyzed and interpreted the data. M.A., F.D.C., A.P., P.M., W.M. and G.R. collected the data. G.R. supported organizational and coordinative tasks. M.A., F.A.B. and V.R. wrote the manuscript. All authors revised the manuscript. All authors approved the final version of the manuscript.

Competing interests

The authors declare no competing interests.

Additional information

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