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Use of ketamine associated with detomidine or xylazine for semen collection with electroejaculation in pampas deer (*Ozotoceros bezoarticus*)

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ABSTRACT

Semen collection by electroejaculation under general anesthesia is a frequent method used in wild animal species. The study aimed to compare the effectiveness of two anesthetic protocols (ketamine/detomidine (KD) or ketamine/xylazine (KX) for semen collection with electroejaculation in pampas deer (*Ozotoceros bezoarticus*) males. Also, compared the physiological and hematological changes with the two anesthetic protocols. Seven pampas deer males were anesthetized with both protocol in an overcorssed design, reverting the anesthesia with atipamezole in both protocols. The induction time, recovery time, and the time of the anesthesia procedures did not differ between both anesthetic protocols. The heart rate was greater when animals were anesthetized with KD than when KX was used (55.33 ± 2.7 bpm vs 45.12 ± 2.6 bpm; $P = 0.05$). Although there was no difference according to the anesthetic protocol, the oximetry values were below those considered normal in other species. When animals were treated with the KD, the red blood cell number, hematocrit, and hemoglobin concentration were greater than with the KX protocol (17.1 ± 1.3 $\times 10^6$/μL vs 15.9 ± 1.3 $\times 10^6$/μL; $P = 0.006$; 47.2 ± 3.4% vs 43.9 ± 3.4%; $P = 0.008$; and 14.8 ± 0.8 g/dL vs 13.8 ± 0.8 g/dL $P = 0.01$, respectively). Less number of electrical pulses were required for ejaculation in animals treated with KX than with KD (39.8 ± 2.6 vs 52.4 ± 2.6, $P = 0.04$). In samples collected from animals treated with KD, the total number of sperm with integral acrosome was lower, but the percentage of sperm with normal morphology was greater than in animals treated with KX (67.8 ± 67.0 × 10^6 sperm vs 243.1 ± 71.6 × 10^6, $P = 0.03$ and 40.0 ± 2.3% vs 30.6 ± 2.7%, $P = 0.02$; respectively). In conclusion, the use of KX induced less physiological changes, appearing also advantageous in semen quality.

1. Introduction

Semen collection and sperm cryopreservation of wild species are essential for applying reproductive biotechnologies for conservation. Semen collection should be adapted to each species, being electroejaculation (EE) under general anesthesia the most widely used technique in wild animals [1–3]. In this context, the application of reproductive biotechnologies is relevant in species such as the pampas deer (*Ozotoceros bezoarticus*, Linnaeus, 1758), as it is considered near threatened species [4]. In Uruguay, there are two wild populations, and a third population in semi-captive conditions in the Estación de Cría de Fauna Autóctona Cerro Pan de Azúcar (ECFA) (Maldonado, Uruguay; 34°3′S, 54°0′W), constituting an excellent basis for developing conservation strategies.

Capture and handling are some of the most stressful events for wild animals, triggering physiological, hematological, and biochemical responses. Besides, EE is also stressful and painful, amplifying those responses (see review: [5]). To select the anesthetic protocol, the availability, efficiency, safety, and cost of the drug, as well as the availability of an antagonist, should be considered [6]. For selecting the anesthetics associated with semen collection, it should be considered if the drugs interfere with the ejaculatory response [5].

In pampas deer, a combination of ketamine and xylazine has been used for immobilization [7,8], and semen collection with EE [9]. Moreover, this combination can be used repeatedly in the same males without accumulated negative effects [10]. However, when electrical pulses are applied during EE, animals vocalize, probably as a response related to pain. In this sense, EE triggered stress responses, even while

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the animals remained anesthetized [11]. Therefore, an alternative to reduce stress response may be the administration of detomidine. This anesthetic drug reduced stress and pain caused by castration in male horses [12], producing good antinociception in camels [13]. Although alpha-adrenergic agonists do not interfere with semen collection [14], to the best of our knowledge there are no previous studies in deer on the use of detomidine on semen collection with EE. Detomidine has been effectively used in several bovid species for semen collection [14,15].

Considering this information, the aim of this study was to compare the effectiveness of ketamine/detomidine (KD) or ketamine/xylazine (KX) combinations for semen collection by EE in pampas deer males.

2. Materials and methods

2.1. Location and general procedures

All the experimental procedures were approved by the Comisión Honoraria de Experimentación Animal (CHEA), from the Universidad de la República, Uruguay. The study was conducted at the ECFA with seven adults [4–7 years old; 24.9 ± 0.9 kg (mean ± SEM)] pampas deer males, during February (breeding season). The animals were maintained in enclosures of 0.5–1 ha, in stable single-male groups since at least one year before beginning the study, with water, natural pasture, trees, and shrubs ad libitum. Animals also received approximately 600 g of supplement/animal from Monday to Saturday.

2.2. Experimental design

Semen was collected from the seven animals with both anesthetic protocols, in an overcrossed design, totalising 14 collections. Initially, four males were anesthetised with KD and three with KX; two weeks later, each male was anesthetised with the other protocol. The animals were captured using darts containing the anesthetic combination, fired from a blowpipe, calculating the doses estimating the individual body weight on the first capture, and according to the body weight recorded at that capture on the second one. The darts contained ketamine 5% (2.2–2.4 mg/kg; Vetanarcol; Laboratorio König, Buenos Aires, Argentina) and detomidine 1% (0.24–0.36 mg/kg; Dormosedan; Laboratorio Zoetis, Montevideo, Uruguay), or xylazine 10% (2.4–3.2 mg/kg; Sedomin; Laboratorio König, Buenos Aires, Argentina). Both anesthetic protocols were combined with atropine 1% im (0.013 mg/kg; Atropine sulfate; Laboratorio Ion, Montevideo, Uruguay). The induction time was considered as the lapse from the moment in which the animal received the dart until lateral or sternal recumbence. The animals were transported to the ECFAs veterinary room on a stretcher and were monitored during all the procedures.

Electroejaculation was performed according to [9,11,16,17]. Briefly, a rectal probe (300 mm length x 19 mm width, with 30 mm electrodes; P-T Electronics, Model 303, Sandy, USA), was inserted in the rectum, and series of 10 pulses (4–5 s per pulse), with rest intervals of 2–3 s were applied. The first serie was applied with 1 V pulses, increasing 1 V in each series (10 pulses per serie), applying a maximum voltage of 6 V. The total duration of the EE, the total voltages used in each animal, the total pulses used during the whole process and voltage at which ejaculation began to occur were recorded.

After the process finished, the animals were transported back to their enclosures, where they received analgesic (20 mg/kg of dipyrone, Laboratorio Ripoll, Montevideo, Uruguay) and anti-inflammatory protocol (1 mg/kg of dexamethasone, Laboratorio Ripoll, Montevideo, Uruguay), both iv. All animals received the same dose of atipamezole (0.16 mg/kg; Antisedan; Laboratorio Zoetis; Montevideo; Uruguay) iv to antagonize the effects of KD or KX. The recovery time was recorded, considering as the lapse from the administration of atipamezole until the animal was able to walk without assistance. In addition, the time of the anesthesia procedure was calculated as the lapse from the moment that the animal adopted the lateral or sternal recumbence until an attempt of incorporation was observed after reversal administration.

2.3. Physiological responses to anesthesia

Heart, pulse and respiratory rates, oxygen saturation, and rectal temperature were recorded with a Multiparametric Patient Monitor (B30, GE Medical Systems Information Technologies, Inc. Milwaukee, USA). These parameters were recorded when the animal arrived at the veterinary room, before beginning the EE (reported as BEE), immediately after each voltage increase during all the EE (reported as DEE), and after the EE ended (reported as AEE). Rectal temperature was recorded only BEE and AEE.

2.4. Blood collection and hematology

Blood samples were collected BEE and AEE from the antebrachial vein. Samples were placed in commercial tubes containing an anticoagulant (EDTA K_3). Red blood cell count (RBC), hematocrit, haemoglobin concentration, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), white blood cell count (WBC), and platelet count (PLT), were measured using an automatic equipment (Mythic™ 18 Vet Operator's, Orphee S.A., Ginebra, Switzerland).

2.5. Semen evaluation

Semen volume, motility score (scale 0–5), and individual sperm motility were evaluated according to [16], and sperm concentration was determined with a Neubauer chamber. Briefly, sperm motility was directly evaluated from the undiluted fresh samples using a phase-contrast microscope at 40X magnification. Sperm morphology and acrosome integrity were evaluated using an optical microscope with phase contrast (40x) from samples fixed with 2% glutaraldehyde in cacodylate buffer. The hypoosmotic swelling test (1.02 g/100 mL; 100 mOsm) was performed to determine the percentage of sperm with functional membrane. The total number of sperm ejaculated was calculated as volume · sperm concentration. The total number of motile sperm, sperm with progressive motility, sperm with functional membrane, sperm with normal morphology, and sperm with normal acrosome ejaculated were calculated by multiplying the total number of sperm ejaculated · the percentage of sperm with each characteristic.

2.6. Statistical analysis

All data were compared with a mixed model (SAS, University Edition). The model included the effect of the anesthetic protocol (KD vs KX) as a main effect, and the day of collection (crossover design) as a random effect. In the variables recorded repeatedly, the effect of the voltage (BEE, a mean value for data collected DEE, AEE) and its interaction with the anesthetic protocol were also included in the model. Normal distribution of sperm variables was tested with the Shapiro-Wilk test, and those variables that were non-normally distributed were normalized with log transformation. Results were considered significant when $P < 0.05$, and are expressed as LSmean ± SEM.

3. Results

3.1. Capture, anesthesia, and recovery

All the animals survived for at least six months after the study. Both protocols allowed the capture and immobilization of the animals, as well as semen collection by EE in most of the animals with both protocols. The induction time, recovery time, and the time of the anesthetic procedures did not differ according to the anesthetic protocol (Table 1). The atipamezole dose used was effective in reversing the effects of anesthesia in all animals treated with both protocols.

Table 1
Induction and recovery times and total time that remained anesthetized in pampas deer males (n = 7) anesthetized with ketamine/detomidine (KD) or ketamine/xylazine (KX) for semen collection with electroejaculation.

Time (min)	KD	KX
Induction time	4.8 ± 0.5	3.8 ± 0.5
Recovery time	2.1 ± 0.3	2.0 ± 0.3
Total time anesthetized	64.4 ± 2.4	66.3 ± 2.4

The data are expressed as LS mean ± SEM.

3.2. Physiological responses

The main effects on heart, pulse, and respiratory rates, oximetry, and rectal temperature are presented in Table 2. The heart rate was greater when animals were anesthetized with KD than when KX was used (51.4 ± 2.4 beats/min vs 45.1 ± 2.7 beats/min, respectively, $P = 0.05$). There was an increase in heart rate values, with greater values DEE (50.7 ± 3.2 beats/min) than BEE and AEE independently of the treatment (35.9 ± 2.7 beats/min and 45.9 ± 2.8 beats/min, respectively $P < 0.001$), without interaction between protocol and voltage. The respiratory rate varied according to the voltage applied (Table 2), without any effect of protocol or interaction between protocol and voltage. Respiratory rate increased during EE, and remained elevated after the process ended (BEE: 22.6 ± 5.4 breaths/min, DEE: 40.0 ± 4.5 breaths/min and AEE: 36.9 ± 5.4 breaths/min, respectively $P < 0.001$).

Pulse rate and oxygen saturation were not affected by any factor included in the model (Table 2). Rectal temperature was not affected by protocol or time alone, but there was an interaction between these factors ($P = 0.008$). It tended to be lower in animals treated with the KD protocol than in those treated with the KX protocol BEE ($P = 0.09$), but was greater in KD than KX treated animals AEE ($P = 0.03$).

3.3. Blood hematology

The procedure for obtaining semen by EE per se did not modify the hematological variables, so it is presented only the mean value. Nevertheless anesthetic treatments affected the RBC, hematocrit, and hemoglobin values, which were greater when animals were anesthetized with the KD than when KX was applied (Table 3). Platelet concentration tended to be greater with the KD than with the KX protocol ($P = 0.09$). None of the hematological variables analyzed showed an interaction between protocol and voltage.

3.4. Ejaculation and semen parameters

The anesthetic protocols did not affect the length of the procedures, voltage at which ejaculation began, and maximum voltage used during EE (Table 4). The KX protocol required a lower number of pulses than KD during EE ($P = 0.04$, Table 4). It was not possible to collect semen in one animal with the KX protocol, so the results of semen variables are presented in Table 5, not including that animal. The quantitative variables related to the total number of sperm collected were also analyzed considering zero the data from that animal, but there were no differences in any variable. Also, one male treated with KD protocol urinated at the end of ejacultaion in a second cup, so this fraction of the sample was discarded. Ejaculates collected with KX tended to have more sperm, total motile sperm, and total sperm with progressive motility ($P = 0.06$, $P = 0.09$ and $P = 0.07$; respectively, Table 5). The total number of sperm with integral acrosome was greater, and the percentage of sperm with normal morphology was lower in samples collected from animals treated with KX than in animals treated with KD ($P = 0.03$ and $P = 0.02$ respectively, Table 5). There were no effects of protocol in any other seminal variable analyzed (Table 5).

Table 2
Heart, pulse and respiratory rates, oxygen saturation, and rectal temperature in pampas deer males (n = 7) anesthetized with ketamine/detomidine (KD) or ketamine/xylazine (KX) for semen collection with electroejaculation.

	KD			KX			P		
	BEE	DEE	AEE	BEE	DEE	AEE	Protocols	Time	Interaction between treatments and time
Heart rate (beats/min)	35.8 ± 3.5[a]	54.9 ± 5.1[b]	45.4 ± 3.5[b]	36.0 ± 3.5[a]	46.5 ± 4.1[b]	46.3 ± 3.7[b]	0.05	< 0.001	ns
Pulse rate (beats/min)	58.9 ± 9.5	71.7 ± 11.1	63.8 ± 9.5	45.9 ± 9.8	59.5 ± 10.1	52.5 ± 9.5	ns	ns	ns
Respiratory rate (breaths/min)	18.3 ± 5.4[a]	38.8 ± 6.3[b]	35.7 ± 5.4[b]	26.9 ± 5.4[a]	40.5 ± 5.9[b]	37.9 ± 5.4[b]	ns	< 0.001	ns
Oxygen saturation (%)	81.3 ± 4.7	83.5 ± 5.3	83.3 ± 4.5	86.6 ± 4.7	83.6 ± 4.8	87.4 ± 4.5	ns	ns	ns
Rectal temperature (°C)	37.7 ± 0.2[a]	UR	38.5 ± 0.2[b]	38.1 ± 0.2[b]	UR	37.9 ± 0.2[a]	ns	ns	0.008

BEE: before electroejaculation; DEE: during electroejaculation; AEE: after electroejaculation; UR: unregistered data; ns: not significant. Values with different letters differed significantly within each protocol. Data are presented as LSmean ± SEM.

Table 3
Blood variables in pampas deer males anesthetized with ketamine/detomidine (KD) or ketamine/xylazine (KX) for semen collection with electroejaculation.

	KD	KX	*P*
RBC (10^6/μL)	17.1 ± 1.3	15.9 ± 1.3	0.006
Hematocrit (%)	47.2 ± 3.4	43.9 ± 3.4	0.008
Hemoglobin (g/dL)	14.8 ± 0.8	13.8 ± 0.8	0.01
MCV (fL)	27.5 ± 1.2	27.8 ± 1.2	ns
MCH (pg)	8.7 ± 0.4	8.8 ± 0.4	ns
MCHC (g/dL)	31.6 ± 0.6	31.6 ± 0.6	ns
WBC (10^3/μL)	6.9 ± 0.7	6.9 ± 0.7	ns
PLT (10^3/ μL)	711.2 ± 111.6	632.4 ± 112.2	0.09

RBC: red blood cells; MCV: mean corpuscular volume; MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration; WBC: white blood cells; PLT: platelets; ns: not significant. Data are presented as LSmean ± SEM.

Table 4
Variables recorded during electroejaculation (EE) in pampas deer males anesthetized with ketamine/detomidine (KD) or ketamine/xylazine (KX).

	KD	KX	*P*
Time of EE (min)	6.1 ± 0.5	6.9 ± 0.5	ns
Total pulses	52.4 ± 2.6	39.8 ± 2.6	0.04
Voltage to ejaculating	2.5 ± 0.4	2.5 ± 0.4	ns
Maximum voltage used	5.7 ± 0.2	4.8 ± 0.2	ns

EE: Electroejaculation. Data are presented as LSmean ± SEM.

4. Discussion

Overall, both protocols, KD and KX, were effective and safe for semen collection and for maintaining pampas deer immobilized for the period required (50–60 min). The induction and recovery times were short with both protocols, minimizing the risks of accidents of the animals or the technicians. Moreover, the recovery time with KX was shorter than that previously reported in this species [10], which is probably explained by the faster reversal effect of atipamezole [18] than yohimbine, which was the reversor used in the previous study [9]. Shortening the recovery time is advantageous in wild animals to avoid accidents due to prolonged decubitus [19], as well as risks of aggression by other members of the group during the postanesthetic period in captive conditions [20], or by predators in wildlife.

The increase in heart rate during EE was previously reported in other studies [21,22], being probably consequence of the greater requirements caused by intensive muscular contractions. However, in general, the heart rate was greater with the KD than with the KX protocol, probably due to a greater number of electrical pulses and a lower depression and sensitivity of the heart, and by a greater increase during the EE process, as was previously reported [5,9]. First, if more pulses are applied, it is expected that greater physiological responses are triggered. Secondly, detomidine is more specific than xylazine to bind the α2-adrenergic receptors (260:1 vs 160:1, α2:α1-receptor binding specificity ratios, respectively [23]), and thus, cause a less depression of the heart activity. Rectal temperature, another main indicator of the physiological status of the animals, changed differently according to the protocol, as BEE tended to be lower in KD than KX, but AEE was greater in KD. This differ from reports in other wild animals such as *Hyaena hyaena sultana*, as their temperature tended to increase with time of anesthesia with xylazine [24]. It is well known that α-2 agonists induce dose-dependent hypo- or hyperthermias, leaving the animal exposed to environmental temperatures [14]. However in this case, it is not easy to explain the differing effects observed with both protocols.

To the best of our knowledge, there are not basal hematological reference data in pampas deer, making difficult to interprete the results. The greater RBC, hematocrit and hemoglobin recorded with the KD protocol is probably an anesthetic effect [25]. In effect, in cervids physical capture is stressful, provoking increases of RBC, hematocrit, and hemoglobin, probably due to the spleen contractions induced by the release of catecholamines [26], suggesting therefore that KD is less effective in controlling stress and/or pain. It should also be considered that unlike xylazine, detomidine increases blood pressure, probably increasing those hematological values (cattle:[27]).

Electroejaculation modified other physiological variables, but changes were similar when animals were anesthetized with KX or KD. In general, oximetry values were lower, causing mild hypoxemia, an effect previously reported when α_2-agonist drugs are used to immobilize cervids [9,19,28]. Hypoxemia can be explained by the decrease in oxygen saturation produced by α_2-agonists [29], but also by lung parenchymal injury and secondary edema, as previously described in sheep [23]. Capture stress, and the prolonged lateral decubitus could have also contributed to the hypoxemic status observed in this study. Therefore, oxygen supplementation is strongly recommended during this type of procedures.

In this study, semen could be successfully collected with both protocols in most animals. However, it is interesting to understand the

Table 5
Sperm variables in fresh semen under two different anesthesia protocols in pampas deer males.

	Raw data		Transformation	Transformed data		*P*
	KD	KX		KD	KX	
Ejaculate general characteristics						
Semen volume (uL)	254.3 ± 79.3	350.8 ± 85.6	Log (x)	2.2 ± 0.2	2.5 ± 0.2	ns
Semen concentration (sperm × 10^6/mL)	782.4 ± 263.0	1043.3 ± 263.0	-	-	-	ns
Total sperm ejaculated (× 10^6)	129.5 ± 85.5	380.4 ± 85.5	-	-	-	0.06
Motility						
Quality of the motility (0–5)	3.54 ± 0.30	3.35 ± 0.35	-	-	-	ns
Motile sperm (%)	70.4 ± 9.8	70.5 ± 10.3	-	-	-	ns
Total motile sperm (× 10^6)	82.3 ± 76.7	277.3 ± 82.8	Log (x)	1.7 ± 0.3	2.3 ± 0.3	0.09
Sperm with progressive motility (%)	59.4 ± 12.2	56.1 ± 12.5	-	-	-	ns
Total sperm with progressive motility (× 10^6)	66.3 ± 64.8	214.4 ± 68.6	Log (x)	1.6 ± 0.3	2.1 ± 0.3	0.07
Membrane, morphology and acrosome						
Sperm with functional membrane (%)	91.2 ± 2.2	90.6 ± 2.3	-	-	-	ns
Total sperm with functional membrane (× 10^6)	136.8 ± 93.5	321.0 ± 93.5	-	-	-	ns
Sperm with normal morphology (%)	40.0 ± 2.3	30.6 ± 2.7	-	-	-	0.02
Total sperm with normal morphology (× 10^6)	53.1 ± 32.0	117.1 ± 35.1	Log (x)	1.4 ± 0.2	1.9 ± 0.3	ns
Sperm with integral acrosome (%)	73.1 ± 3.3	71.5 ± 3.6	-	-	-	ns
Total sperm with integral acrosome (× 10^6)	67.8 ± 67.0	243.1 ± 71.6	Log (x)	1.5 ± 0.4	2.3 ± 0.3	0.03

KD: Ketamine/Detomidine; KX: Ketamine/Xylazine; ns: not significant. Data are presented as LSmean ± SEM.

effect of different α-agonistic drugs on the process of ejaculation. As the emission is primarily an α1 event, and ejaculation an α2 event [30,31], different anesthetic drugs can affect differently the results of semen collection during EE, modifying both, semen volume and quality [5–31]. In this sense, the use of KX collected semen with fewer pulses and tended to allow collecting more sperm and more sperm with motile and progressive motility, and allowed collecting more sperm with intact acrosome. As detomidine has less α1 effects than xylazine [23], the latter probably triggered greater effects on the emission of sperm and stimulated the contraction of the epididymal musculature, accessory sex glands and deferent, as reported in rams [32]. It is also possible that detomidine promoted a retrograde ejaculation, as has been reported to occur with α-2 agonist anesthetic drugs in dogs [33]. On the other hand, the greater percentage of morphological normal sperm collected with KD is probably related to different effects on the accessory glands, as differences can only be related to the ejaculatory process. However, to the best of our knowledge there are no previous studies relating anesthetic protocols with seminal plasma composition, making difficult to interprete these results. Overall, it appears that the inclusion of xylazine instead of detomidine is probably having a greater positive impact on the ejaculatory process, although more research with experimental models in which more animals can be used is still required.

5. Conclusion

Both anesthetic protocols allowed to applied safely the procedures, including capture and semen collection with EE in pampas deer. The use of KX induced less physiological changes, and appears as probably advantageous in semen quality.

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CRediT authorship contribution statement

Fernando Fumagalli:. Conceptualization, Methodology, Formal analysis, Investigation, Writing – original draft. **Florencia Beracochea:** Writing – original draft, Formal analysis. **Rodolfo Ungerfeld:** Conceptualization, Methodology, Investigation, Writing – original draft, Formal analysis, Supervision.

Declaration of Competing Interest

None of the authors has any financial or personal relationships that could inappropriately influence or bias the content of the article.

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