Emerging Animal Species 4 (2022) 100010

Contents lists available at ScienceDirect

Emerging Animal Species

journal homepage: www.elsevier.com/locate/eas

The repetition of semen collection does not affect the physiological and biochemical response to electroejaculation of anesthetized adult and yearling pampas deer (*Ozotoceros bezoarticus*) males

Fernando Fumagalli [a,*,1], Matías Villagrán [b], Rodolfo Ungerfeld [b]

[a] Departamento de Clínicas y Hospital Veterinario, Facultad de Veterinaria, Universidad de la República, Montevideo, Uruguay
[b] Departamento de Biociencias Veterinarias, Facultad de Veterinaria, Universidad de la República, Montevideo, Uruguay

ARTICLE INFO

Keywords:
Conservation
Pain
Stress
Animal welfare

ABSTRACT

The aim of this study was to determine if multiple captures, general anesthesia and electroejaculation (EE) modify the heart rate (HR), pulse rate (PR), respiratory rate (RR), rectal temperature (RT), oxygen saturation, and the cortisol, alkaline phosphatase (AP) and creatine kinase (CK) responses to EE in adult and yearling pampas deer (*Ozotoceros bezoarticus*) males. Individuals were captured, anesthetized and electroejaculated on four occasions, once per season, from June to the following March (first to fourth collection respectively). Rectal temperature, as well as cortisol, AP and CK serum concentrations were determined before and after electroejaculation (BEE and AEE). The HR ($P < 0$ 0.0001), PR ($P < 0.0001$), RR ($P < 0.001$), CK ($P < 0.001$) and cortisol concentration ($P = 0.003$) increased AEE, while RT ($P = 0.004$) decreased AEE. Heart rate increased in the first and third collections ($P < 0.01$), RR increased in the fourth collection ($P < 0.001$), and the highest values of RT were observed in the third and fourth collections ($P < 0.0001$). We concluded that repeated handling to collect semen did not have a sustained negative effect on the response to EE, which supports repeated semen collection from the same individuals.

Introduction

Reproductive biotechnologies, such as sperm cryopreservation, artificial insemination or *in vitro* fertilization are used to preserve endangered species (Holt and Pickard, 1999) including deer (Garde et al., 2006). For this purpose, techniques for semen collection should be adequately adapted to the biology of each species. As an artificial vagina can only be used in tame trained individuals, electroejaculation (EE) is the most widely used technique for semen collection in wild animals, including deer species (Duarte et al., 1993; Duarte et al., 1997; Beracochea et al., 2014). In these species, animals have to be anesthetized to collect semen by EE (Umapathy et al., 2007; Martínez et al., 2008; Santiago-Moreno et al., 2011; Fumagalli et al., 2012). Usually, captive populations of endangered species consist of a small number of animals, so semen has to be collected repeatedly from few individuals.

Several authors have reported that EE causes stress and pain in animals and humans (Abril-Sánchez et al., 2019). Even in cattle, it is considered a painful procedure (Adcock et al., 2018). It also triggers changes in heart, pulse and respiratory rates (HR, PR, RR, respectively), oxygen saturation (SpO_2), rectal temperature (RT), and alkaline phosphatase (AP), creatine kinase (CK) and cortisol concentrations in several ruminant species (e.g. bucks, bulls, deer, camels; see review) (Abril-Sánchez et al., 2019). During EE, most species vocalize repeatedly, even under general anesthesia (Fumagalli et al., 2015), probably indicating a pain response. Other factors, such as the age of the animals, modulate the response to EE in anesthetized pampas deer males (*Ozotoceros bezoarticus*), an endangered species (Fumagalli et al., 2012).

Xylazine is the drug most commonly used for anesthesia and sedation in ruminants (Celly et al., 1997), but at least in sheep, its effectiveness decreases when it is administered repeatedly (Karasu and Gençcelep, 2015). Thus, repeated semen collection can lead to a pharmacological "habituation" to the anesthetic combination used in successive captures, decreasing its effectiveness, as happens with the use of tranquilizers during semen collection in bulls (Wells et al., 1966). Therefore, the aim of the present study was to determine whether the repetition of captures and anesthesia modifies the physi-

* Corresponding author.
E-mail address: fernandofuma1@gmail.com (F. Fumagalli).
[1] ORCID: https://orcid.org/0000-0001-9127-3546.

https://doi.org/10.1016/j.eas.2022.100010
Received 24 February 2022; Revised 26 April 2022; Accepted 8 June 2022

ological and biochemical response of adult and yearling pampas deer (*Ozotoceros bezoarticus*) males to EE, as measured by HR, PR, RT, SpO_2, as well as cortisol, AP and CK serum concentrations.

Materials and methods

General procedures, animals and facilities

The study was approved by the Comisión Honoraria de Experimentación Animal (CHEA), from the Universidad de la República, Uruguay. It was conducted at the Estación de Cría de Fauna Autóctona "Cerro Pan de Azúcar" (ECFA); Maldonado, Uruguay; 34° 3′ S, 55° 1' W) with all the pampas deer males allocated in two single male groups, one consisting of six adult [4–7 years old; 29.6 ± 2.7 kg (mean ± SEM)] and the other of five yearling (1.5–2 years old; 25.0 ± 2.2 kg) males. Semen was collected with EE on four occasions, once during each season: winter (June-July), spring (September), summer (January) and autumn (March), totalizing 44 collections. Each group of males (adults and yearlings) was housed in a paddock of approximately 0.5 ha, with *ad libitum* water, and natural pasture, trees and shrubs. Animals also received approximately 600 g of supplement from Monday to Saturday.

Capture, general anesthesia, and EE

Animals were anesthetized by ECFA staff in a random order, intercalating one adult and one yearling male, with darts containing ketamine 5% (1.75 mg/kg; Vetanarcol; Laboratorio König, Buenos Aires, Argentina), xylazine 10% (2.2 mg/kg; Sedomin; Laboratorio König, Buenos Aires, Argentina) and atropine 1% (0.013 mg/kg; Atropine sulfate; Laboratorio Ion, Montevideo, Uruguay) fired through a blowpipe. At each collection, the body weight was estimated according to the weight at the previous collection. The induction time was considered to be the period from the moment in which the animal received the dart until lateral or sternal recumbence. The animals were transported to the ECFA's veterinary room on a stretcher, and vital signs were monitored during all procedures.

Semen was collected using an electroejaculator with a rectal probe (300 mm length × 19 mm width, with 30 mm electrodes) (Model 303; P-T Electronics, Sandy, OR, USA). Electroejaculation was performed using electrical stimulating periods of 4–5 s and rest intervals of 2–3 s. The first 10 pulses were of 1 V, increasing 1 V every 10 pulses until ejaculation, applying a maximum voltage of 8 V. The voltage at which erection (VER) and ejaculation (VEY) occurred was recorded.

After the procedure 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 treatment (1 mg/kg of dexamethasone, Laboratorio Ripoll, Montevideo, Uruguay), and yohimbine hydrochloride (0.26 mg/kg; Reverze, Laboratorio Vetcross, Montevideo, Uruguay) to revert the anesthesia. Time to recovery (TR) was recorded as the time from the administration of yohimbine hydrochloride to the moment when the animal was able to walk unassisted. The total time of the procedure was considered to be the interval between the application of the dart and the application of the yohimbine.

Physiological recordings

Heart rate, PR, RR, RT, and SpO_2 were recorded while the animal was in the veterinary room, before beginning the EE (BEE), during EE, immediately after each increase in voltage, and after the EE finished (AEE). Blood samples were collected BEE and AEE to measure serum cortisol, CK and AP. Heart rate was recorded continuously with a Polar A3 heart rate monitor (S120™, Polar Electro Oy, Kempele, Finland). Respiratory rate was recorded counting the thoracic movements during 15 s and calculating the rate for 1 min. The SpO_2 was recorded using a pulse oximeter (Pulse 503 Oximeter; CSI, Criticare Systems, Waukesha, USA), with a transmission probe placed on the tongue. Rectal temperature was recorded BEE and AEE with a digital thermometer.

Blood enzymes and serum cortisol determinations

Blood samples were collected BEE and AEE, and placed in tubes without anticoagulants. The samples were immediately centrifuged at 1080 g for 20 min, and the serum was stored at −20 °C. Creatine kinase and AP were measured in the Laboratorio de Análisis Clínicos (Facultad de Veterinaria, Montevideo, Uruguay), using a semi-automatic ultraviolet–visible spectrophotometer (Metrolab 1600 DR; Metrolab, Buenos Aires, Argentina). Serum cortisol concentrations were measured in the Laboratorio de Endocrinología y Metabolismo Animal (Facultad de Veterinaria) by radioimmunoassay using a solid-phase kit (DPC; Siemens, Los Angeles, USA). The analytical sensitivity of the assay for cortisol was 8.1 nmol/L, and the intra-assay coefficient of variation was 8%.

Statistical analysis

The animal was assumed to be the experimental unit, and the treatment was the repetition. As the repetition was the treatment, each animal was its own control. No animal or data points were excluded during the study, so all samplings included 11 animals. All variables were analyzed using a mixed model (SAS, University Edition). The model included the effect of category (adult vs yearling males, referred to as parity from here onwards), and collection number (collections 1, 2, 3 and 4), as well as the interaction between parity and collection number. Of the variables that were recorded along with the during EE, the voltage (BEE, 1 V to 6 V, AEE) and its interaction with parity and collection number were also included in the model. Results were considered significant when $P < 0.05$, and are expressed as least squares means (LS means) ± SEM.

Results

None of the animals showed any type of organic alteration and all animals survived for at least 6 months after the end of the study. Semen was collected in the 44 procedures, but in 3 (6.8%), the semen was contaminated with urine (one adult and two yearling males). No animal required humane endpoints.

Induction, recovery and voltages for ejaculation and erection

The general LS means and main effects are presented in Table 1. The total duration of the procedure was greater in adult than yearling males, irrespective of the collection number. The induction time varied between collections, being shorter in the last procedure than in the others (winter to autumn: 16.7 ± 1.9 vs 4.4 ± 2.4 min; $P = 0.002$). There was no difference in recovery time for parity or collection number.

Yearling males required a higher voltage than adult males to ejaculate ($P = 0.03$). This voltage also varied between collections ($P < 0.001$), requiring the lowest voltage on the third collection, without interaction between parity and collection. Penis erection was observed in 18/44 (40.9%) procedures. Yearling males tended to need higher voltages to reach erection than adults ($P = 0.06$).

Physiological responses

The main effects of the factors included in the model for the physiological variables are summarized in Table 2. There were no signifi-

Table 1
Comparison of parameters recorded during semen collection in adults (n = 6) and yearlings (n = 5) pampas deer (*Ozotoceros bezoarticus*) males.

Variable	Category		P value Cat.	Collection number				P value Coll.
	Adult	Yearling		1st	2nd	3rd	4th	
Total time of the procedures (min)	36.1 ± 1.0 [a]	33.2 ± 1.0[b]	0.05	36.4 ± 1.3	33.6 ± 1.3	34.4 ± 1.4	34.1 ± 1.5	NS
Induction time (min)	10.3 ± 1.5	11.4 ± 1.7	NS	16.7 ± 1.9[a]	13.9 ± 2.7[a]	8.4 ± 2.2[b]	4.4 ± 2.4[b]	0.002
Recovery time (min)	2.4 ± 0.4	2.6 ± 0.4	NS	2.3 ± 0.5	1.4 ± 0.6	3.5 ± 0.6	2.9 ± 0.7	NS
Erection voltage (Volt)	4.0 ± 0.2	4.7 ± 0.2	0.06	3.7 ± 0.3	4.3 ± 0.3	4.6 ± 0.4	4.8 ± 0.3	NS
Ejaculation voltage (Volt)	2.9 ± 0.2[a]	3.8 ± 0.2[b]	0.03	3.3 ± 0.3[a]	5.2 ± 0.3[b]	2.1 ± 0.3[c]	2.9 ± 0.4[ac]	0.001

Abbreviations: NS, not significant; Cat, Category; Coll, Collection.
Values with different letters differed significantly; interaction was not significant for any variable.
Data are presented as LS mean ± SEM.

Table 2
P values of physiological variables recorded in adult (n = 6) and yearlings (n = 5) pampas deer (*Ozotoceros bezoarticus*) males during repeated semen collection with electroejaculation.

Variables	Main factors			Interactions			
	Category	Collection number	Voltage	Cat*Coll	Cat*Vol	Coll*Vol	Cat*Coll*Vol
Heart rate (beats/min)	<0.0001	0.0008	<0.0001	NS	NS	NS	NS
Pulse rate (beats/min)	0.048	0.063	<0.0001	0.073	NS	NS	NS
Respiratory rate (breath/min)	NS	<0.0001	0.0005	NS	NS	NS	NS
Oxygen Saturation (%)	NS	0.046	NS	NS	NS	NS	NS

Abbreviation: NS, no significant; Cat, Category; Coll, Collection; Vol, Voltage.

cant interactions, although the interaction between parity and collection number in pulse rate tended to be significant ($P = 0.07$). Heart rate, PR, RR and SpO_2 are presented in Fig. 1. The HR was higher in adults than in yearlings (124.31 ± 3.1 vs 98.42 beats/min; $P < 0.0001$). It also increased significantly during EE ($P < 0.0001$) (Fig. 1B). The HR varied among collections ($P = 0.0008$), with higher values in winter than in autumn ($P = 0.0003$), in spring than in autumn ($P = 0.024$) and in summer than in autumn ($P = 0.001$) (Fig. 1A).

The PR was higher in adult than in yearling males (97.29 ± 4.0 vs 85.02 ± 4.0 beats/min; $P = 0.04$). It increased significantly during EE ($P < 0.0001$) (Fig. 1D). The PR tended to vary among collections ($P = 0.062$) (Fig. 1C). The RR in adult and yearling males is presented in Fig. 1E and 1F. The RR varied among the collections ($P < 0.0001$), with the greatest value in the fourth collection (autumn) (Fig. 1E). The RR increased during EE ($P = 0.0005$) (Fig. 1F). The SpO_2 is presented in Fig. 1GH. The SpO_2 varied among collections ($P = 0.04$), with the highest value in the first collection (winter), and the lowest in the third collection (summer) (Fig. 1G). The main effects on RT are presented in Table 3, and values are shown in Fig. 2AB. The RT varied among the collections ($P < 0.0001$), with the highest value in the fourth collection (autumn) and the lowest in the first collection (winter) (Fig. 2A). Also, RT decreased AEE ($P = 0.01$) (Fig. 2B).

Blood enzymes and serum cortisol

The main effects on AP, CK and cortisol are presented in Table 3, and AP and CK serum concentrations are presented in Fig. 2C–H. The concentration of CK varied among the collections ($P < 0.0001$), with the highest CK in the first collection, and the lowest in the fourth collection (Fig. 2E). The concentration of CK increased after EE (Fig. 2F). There was a significant interaction between the collection number and the moment of blood extraction ($P = 0.05$): the CK concentration increased after EE only in the first and second collection.

The concentration of AP varied among the collections ($P < 0.0001$): the highest value was recorded in the second, and the lowest in the first collection (Fig. 2C). There was a significant interaction between collection number and parity ($P = 0.03$): in the second collection the AP concentration was higher in yearling than in adult males ($P = 0.03$). The concentration of cortisol is presented in Fig. 2G and 2H. Cortisol concentration tended to vary between collections ($P = 0.08$), and was higher AEE ($P = 0.005$).

Discussion

To the best of our knowledge, this is the first study demonstrating that semen can be collected repeatedly from the same individuals without modifying the general response pattern to EE in deer. Although we observed variations in all studied parameters during the different collections, all were transient changes. As these changes were not sustained during the following collections, they cannot be a consequence of the repetition of the procedure. Considering that the pattern of changes differed among variables, it seems that these variations were more related to environmental or physiological conditions of the animals (e.g. climate, breeding cycle, or antler cycle). DelGiudice et al. (1986) reported that repeated capture and anesthesia (10 times in 5 months) did not affect the reproductive results of pregnant white-tailed female deer. Similarly, Wells et al. (Wells et al., 1966) reported that, in weekly EE, sedated animals showed better physiological responses than animals that did not receive sedation, while semen characteristics were not affected. On the other hand, the repetition of EE in animals without tranquilization was more stressful and produced discomfort. In the present study, each capture took place approximately 3 months after the previous one, ensuring that the animals had enough time to recover from the previous procedure. Overall, the present results allow the conclusion that it is possible to capture and handle the same animals repeatedly, without increasing the negative general effects of the procedure, as long as the resting periods used in this study are respected. Although the study was performed on a single species, this is likely to hold true for similar species.

The pharmacological association and anesthetic doses used in this study were effective, as the animals remained immobilized during the 44 collections, without negative complications related to the anes-

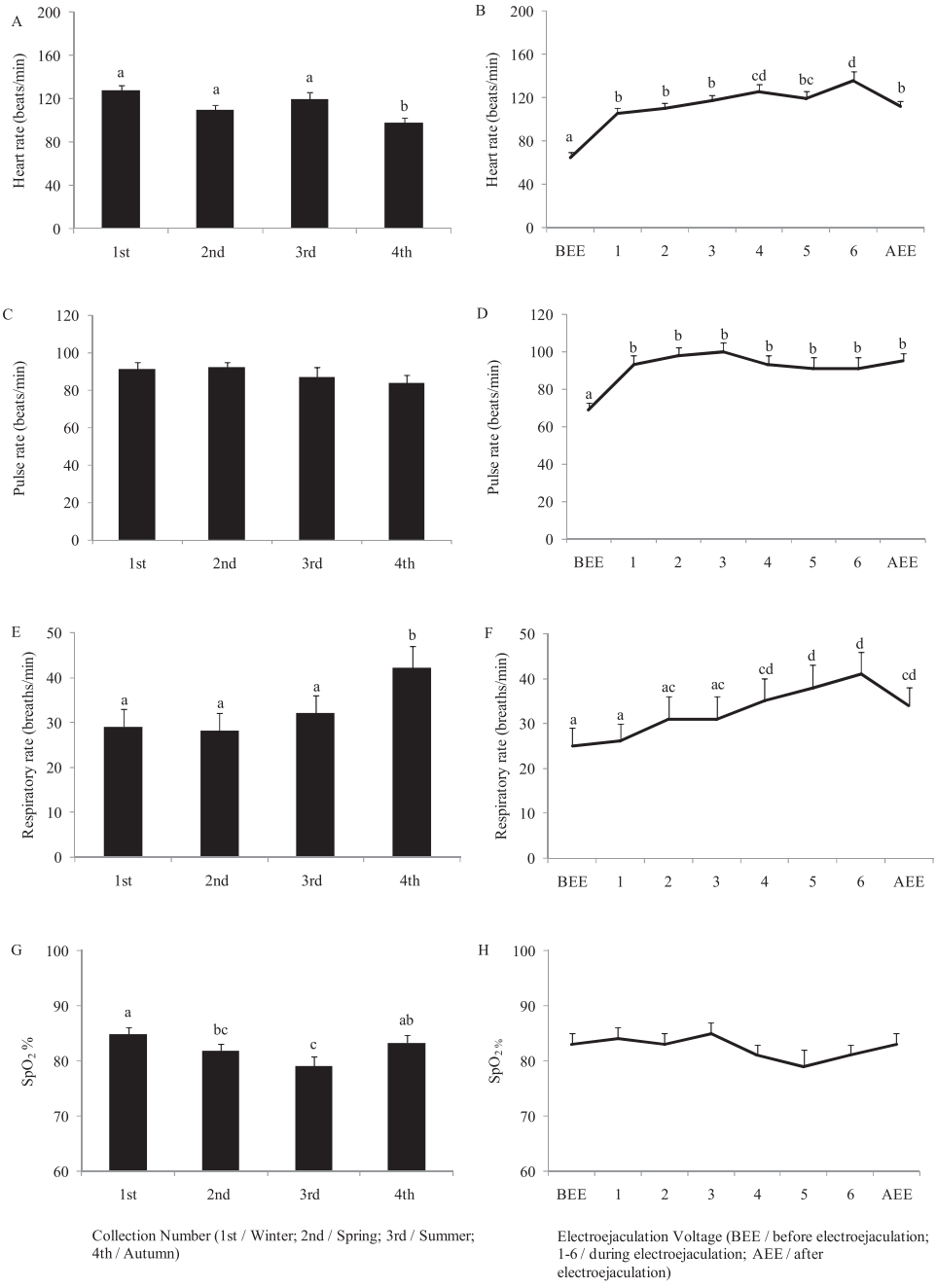

Fig. 1. (A/B) Heart rate, (C/D) Pulse rate, (E/F) Respiratory rate, and (G/H) Oxygen saturation in different semen collections by capture and electroejaculation (left panel, bar graphs), and according to the moment in relation to electroejaculation (right panel): before (BEE), during (voltages 1 to 6) and after (AEE) electroejaculation (line graphs) in adult and yearling anesthetized pampas deer (*Ozotoceros bezoarticus*) males. Data are presented as LS means ± SEM. Different letters indicate significant differences ($p < 0.05$).

Table 3
P values of rectal temperature, enzymes and serum cortisol concentrations recorded in adult (n = 6) and yearlings (n = 5) pampas deer (*Ozotoceros bezoarticus*) males during repeated semen collection with electroejaculation.

Parameters							
	Main factors			Interaction			
	Category	Collection number	B/A	Cat/Coll	Cat/B/A	Coll/B/A	Cat/Coll/B/A
Rectal Temperature (°C)	NS	<0.0001	0.019	NS	NS	NS	NS
Creatine kinase (UI/L)	NS	0.011	0.002	NS	NS	0.05	NS
Alkaline phosphatase (UI/L)	NS	<0.0001	NS	0.038	NS	NS	NS
Cortisol (μg/dl)	NS	0.084	0.005	NS	NS	NS	NS

Abbreviations: B/A, before and after electroejaculation; NS, no significant; Cat, Category; Coll, Collection.

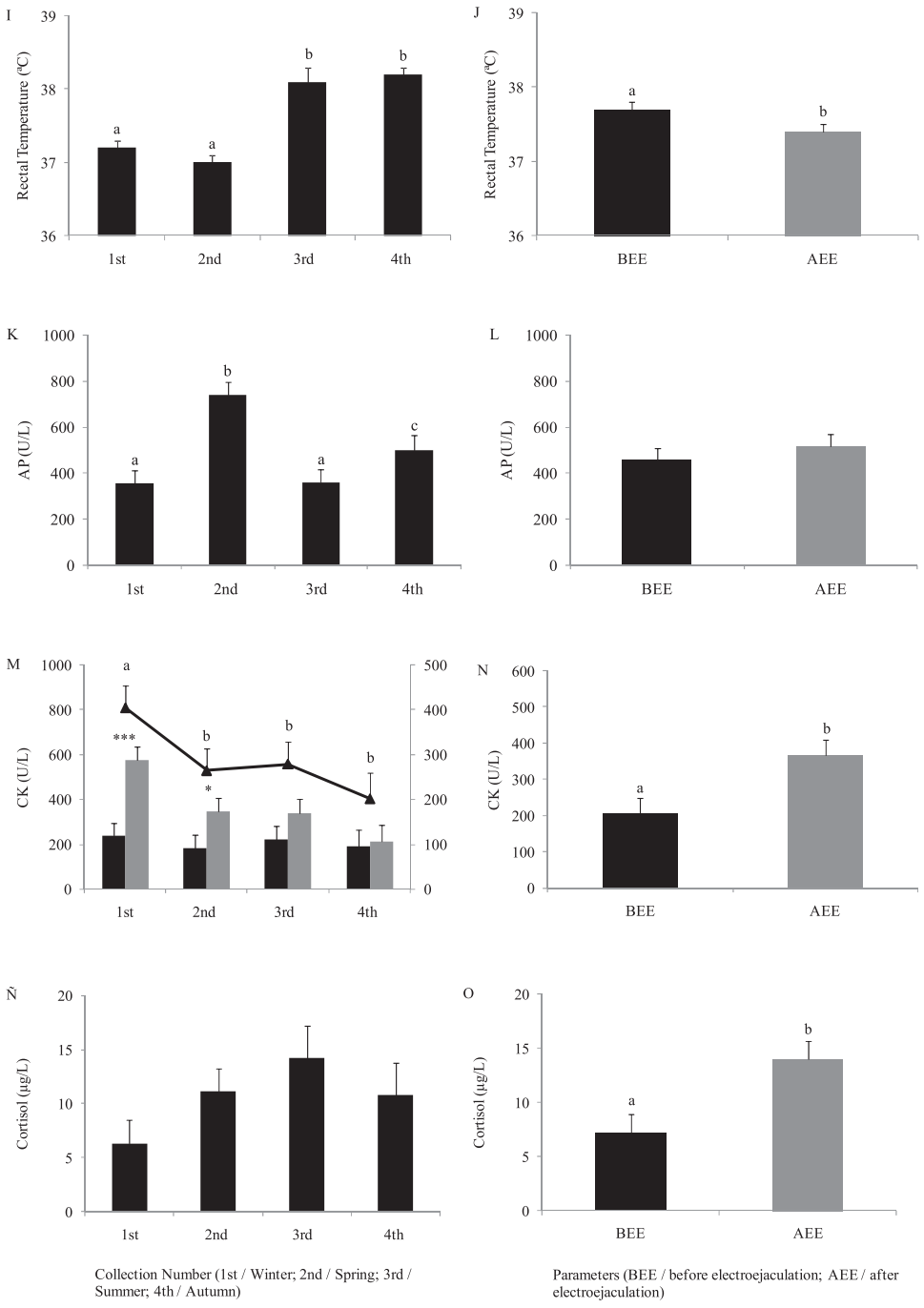

Fig. 2. Rectal Temperature (I/J), Alkalinephosphatase (K/L), Creatine kinase (M/N), Cortisol (Ñ/O), of adults and yearlings pampas deer. The first bar graph shows what happens during 1st, 2nd, 3rd, 4th collection of capture and electroejaculation. In graph M, the bars shows the Creatine kinase before (black) and after (dark gray) electroejaculation during 1st, 2nd, 3rd, 4th collection. Continuous line shows de Creatine kinase in the 1st, 2nd, 3rd, 4th, collection of capture and electroejaculation. The second Bars graph shows Rectal Temperature (J), Creatine kinase (N), Alkalinephosphatase (L), Cortisol (Ñ) before (black) and after (dark gray), electroejaculation in adult and yearling anesthetized pampas deer. Data are presented as the mean ± standard error of the mean. Differences between times are shown with different letters: $p < 0.05$, interaction are showed with * ($p \leq 0.05$), ** ($p \leq 0.01$), *** ($p \leq 0.001$).

thetic treatment. Moreover, in all cases, sperm was effectively collected, and there were no deaths related to the procedure. Both the induction and recovery times reported in this study are similar to those previously reported in the same species (Fumagalli et al., 2012) and other ruminants anesthetized with similar protocols (Santiago-Moreno et al., 2010). In general, the induction time was short, minimizing the risks of possible accidents or injuries of the animals or the staff that participated in the study.

Adult males required fewer electrical pulses to ejaculate and also tended to require fewer pulses to achieve penis erection. As far as we know, no previous studies determined that adult males are more sensitive to stimulation with the electrical pulses used in EE. Although

our design does not allow us to establish whether these differences are the consequence of the development of the genital tract or the sexual maturity of adult animals, these findings should be taken into account to improve procedures according to male parity, knowing that young males may require more pulses to achieve similar results. Irrespective of parity, pampas deer males required fewer electrical pulses when semen was collected in summer or autumn, which might be related to increased semen concentration, testicular volume, or higher testosterone concentration (Ungerfeld et al., 2020), a hormone that promotes the ejaculatory reflex (Fritz et al., 2019).

In agreement with previous reports (Fumagalli et al., 2012), anesthetized pampas deer males' responses were related to the accumulated number of pulses and to the voltage that was applied. Heart rate, PR, and RR increased as pulses were applied, probably due to the stress caused by EE and the greater metabolic requirements provoked by the intense muscle contractions. In effect, the increase in CK concentration indicates that there was intense muscular activity (Kramer et al., 1997), which partially explains the increase in HR. The increases in cortisol, RR and HR upon initiation could be associated with the stress and pain caused by EE, as previously described in the same (Fumagalli et al., 2015) and other species (Abril-Sánchez et al., 2019; Tharwat et al., 2014). In this study, low values of oxygen saturation were found (below 90% throughout the process). Several authors reported these same concentrations in other anesthetized cervids (elk (Read et al., 2001); hog deer (Arnemo et al., 2005); reindeer (Risling et al., 2011). This effect could be partly due to the use of α-2 agonists inducing a decrease in oxygen saturation (Risling et al., 2011). Moreover, stress during capture, the prolonged lateral decubitus and the intense muscular contractions induced by EE could also have contributed to the hypoxemic status observed in this study.

Conclusion

Overall, the procedures used in this study to collect semen repeatedly were effective. Although the procedure affected animal welfare, it did not provoke deaths. The repetition itself did not affect the negative response to EE, allowing the repeated collection of semen from the same males, provided they were handled with the care required for a non-domestic species. Therefore, this protocol might be beneficial for the species' conservation purposes. Despite the methodological difficulties associated with a low number of animals, studies like the present should be performed testing other anesthetic and analgesic combinations in endangered species.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Rodolfo Ungerfeld reports financial support was provided by University of the Republic Sectoral Commission for Scientific Research.

Acknowledgements

Authors acknowledge Tabaré González-Sierra†, Director of the ECFA, and Johnny Brioso, Ricardo Sorello and Edgardo Barrios for help with animal management. We appreciate the assistance of Florencia Beracochea during semen collection and analysis. Financial support was provided by CSIC (Universidad de la República, Montevideo, Uruguay), the Intendencia de Maldonado (Uruguay), and the Agencia Nacional de Investigación e Innovación (ANII).

Author contributions

Fernando Fumagalli recorded the data, made the initial data analysis and drafted the first version of the manuscript. Matias Villagrán participated in data collection and in writing the manuscript. Rodolfo Ungerfeld directed and coordinated the study and data analysis, and the manuscript preparation and writing, including the submitted version.

References

Abril-Sánchez, S., Freitas-de-Melo, A., Giriboni, J., Santiago-Moreno, J., Ungerfeld, R., 2019. Sperm collection by electroejaculation in small ruminants: A review on welfare problems and alternative techniques. Anim. Reprod. Sci. 205, 1–9.

S.J.J. Adcock, C.B. Tucker, Painful procedures: When and what should we be measuring in cattle?, in: C.B. Tucker, (Eds.), Advances in Cattle Welfare, Wood head Publishing Series in Food Science, Technology and Nutrition, Elsevier, UK, 2018, pp. 157-198.

Arnemo, J.M., Storaas, T., Khadka, C.B., Wegge, P., 2005. Use of medetomidine-ketamine and atipamezole for reversible immobilization of free-ranging hog deer *(Axis porcinus)* captured in drive nets. J. Wildl. Dis. 41 (2), 467–470.

Beracochea, F., Gil, J., Sestelo, A., Garde, J.J., Santiago-Moreno, J., Fumagalli, F., Ungerfeld, R., 2014. Sperm characterization and identification of sperm subpopulations in ejaculates from pampas deer (*Ozotoceros bezoarticus*). Anim. Reprod. Sci. 149 (3-4), 224–230.

Celly, C.S., Mcdonell, W., Young, S.S., Black, W.D., 1997. The comparative hypoxaemic effect of four α2 adrenoceptor agonists (xylazine, romifidine, detomidine and medetomidine) in sheep. J. Vet. Pharmacol. Ther. 20, 464–471.

DelGiudice, G.D., Mech, L.D., Paul, W.J., Karns, P.D., 1986. Effects on fawn survival of multiple immobilizations of captive pregnant white-tailed deer. J. Wild. Dis. 22 (2), 245–248.

J.M.B. Duarte, I.G. Arantes, J.M. Garcia, A.A. Nascimento. Captura e avaliacao de uma populacao de *Ozotoceros bezoarticus leucogaster* no Brasil, in: UICN. Population and Viability Assessment for the Pampas Deer. SSC/IUCN, 1993. No pages.

Duarte, J.M.B., Garcia, J.M., 1997. Tecnologia da reproducao para propagacao e conservacao de especies ameacadas de extincao. In: Duarte, J.M. (Ed.), Biologia e Conservacao de Cervıdeos Sul-americanos. FUNEPA Brasil, pp. 228–238.

Fritz, W.F., Sena, L.S., Becker, S.E., Katz, L.S., 2019. Differential effects of androgens, estrogens and socio-sexual context on sexual behaviors in the castrated male goat. Horm, Behav. 109, 10–17.

Fumagalli, F., Villagrán, M., Damián, J.P., Ungerfeld, R., 2012. Physiological and biochemical parameters in response to electroejaculation in adult and yearling anesthetized pampas deer (*Ozotoceros bezoarticus*) males. Reprod. Domest. Anim. 47, 308–312.

Fumagalli, F., Damian, J.P., Ungerfeld, R., 2015. Vocalizations during electroejaculation in anaesthetized adult and young pampas deer (*Ozotoceros bezoarticus*) males. Reprod. Domest. Anim. 50, 321–326.

J.J. Garde, F. Martinez-Pastor, M. Gomendio, A.F. Malo, A.J. Soler, Fernandez-Santos MR, M.C. Esteso, A.J. Garcia, L. Anel, E.R.S, Roldan. The application of reproductive technologies to natural populations of red deer, Reprod. Domes. Anim. 41 (2006) 93-102.

Holt, W.V., Pickard, A.R., 1999. Role of reproductive technologies and genetic resource banks in animal conservation. Reproduction 4, 143–150.

Karasu, A., Gençcelep, M., 2015. The effect of xylazine HCl used in repeated sedations for sheep on biochemical and clinical values. Kafkas Univ. Vet. Fak. Derg. 21, 831–836.

Kramer, J.W., Hoffman, W.E., 1997. Clinical Enzymology. In: Kaneko, J.J., Harvey, J.W., Bruss, M.L. (Eds.), Clinical Biochemistry of Domestic Animals. 5th ed. Academic Press Inc, San Diego, pp. 309–325.

Martínez, A.F., Martínez-Pastor, F., Álvarez, M., Fernández-Santos, M.R., Esteso, M.C., de Paz, P., Garde, J.J., Anel, L., 2008. Sperm parameters on Iberian red deer: electroejaculation and post-mortem collection. Theriogenology 70, 216–226.

Read, M.R., Caulkett, N.A., Symington, A., Shury, T.K., 2001. Treatment of hypoxemia during xylazine-tiletamine-zolazepam immobilization of wapiti. Can. Vet. J. 42, 861–864.

Risling, T.E., Fahlman, Å., Caulkett, N.A., Kutz, S., 2011. Physiological and behavioural effects of hipoxemia in reindeer (*Rangifer tarandus*) immobilised with xylazine-etorphine. Anim. Prod. Sci. 51, 355–358.

J. Santiago-Moreno, M.A. Coloma, A. Toledano-Díaz, A. Gómez-Brunet, A. Pulido-Pastor, A. López-Sebastián, Métodos de estudio de la actividad reproductiva en ungulados de interés cinegético, in; J. Santiago Moreno, A. López-Sebastián, (Eds), Ungulados silvestres de España: biología y tecnologías reproductivas para su conservación y aprovechamiento cinegético, INIA Madrid, 2010, pp. 53–55.

Santiago-Moreno, J., Toledano-Díaz, A., Sookhthezary, A., Gómez-Guillamón, F., de la Vega, R.S., Pulido-Pastor, A., López-Sebastián, A., 2011. Effects of anesthetic protocols on electroejaculation variables of Iberian ibex (*Capra pyrenaica*). Res. Vet. Sci. 90 (1), 150–155.

Tharwat, M., Ali, A., Al-Sobayil, F., Derar, R., Al-Hawas, A., 2014. Influence of stimulation by electroejaculation on myocardial function, acid–base and electrolyte status, and hematobiochemical profiles in male dromedary camels. Theriogenology 82 (6), 800–806.

Umapathy, G., Sontakke, S.D., Reddy, A., Shivaji, S., 2007. Seasonal variations in semen characteristics, semen cryopreservation, estrus synchronization, and successful artificial insemination in the spotted deer (*Axis axis*). Theriogenology 67 (8), 1371–1378.

Ungerfeld, R., Villagrán, M., Gil-Laureiro, J., Sestelo, A., Beracochea, F., Fumagalli, F., Bielli, A., 2020. Adult and yearling pampas deer stags (*Ozotoceros bezoarticus*) display mild reproductive seasonal patterns with maximum values in autumn. Anim. Reprod. 17, 1–12.

Wells, M.E., Philpot, W.N., Musgrave, S.D., Jones, E.W., Brock, W.E., 1966. Effect of method of semen collection and tranquilization on semen quality and bull behavior. J. Dairy Sci. 49 (5), 500–503.