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Semen collection using electroejaculation and sperm parameters in pacas (*Cuniculus paca, Linnaeus, 1766*)

[*Coleta de sêmen com uso da eletroejaculação e parâmetros espermático em pacas* (Cuniculus paca, *Linnaeus, 1766*)]

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ABSTRACT

Pacas (*Cuniculus paca*) are highly hunted animals because of the flavor of their meat, and commercial breeding is recommended. However, this species has a relatively low reproductive rate. This study aimed to collect semen from pacas through electroejaculation and obtain the sperm parameters of this species for the first time. Seven male pacas were used, submitted to an anesthetic protocol before stimulation by an electroejaculator appropriate for the species. The stimulus protocol was performed in three series: series I, 10 stimuli with 1 and 2 V; series II, 10 stimuli with 3 and 4 V; and series III, 10 stimuli with 5 V and interval between series of 2 s. The collected material was evaluated for color, volume, motility, vigor, and concentration. The sperm parameters collected showed a mean volume of 0.43±0.33 mL, concentration of 45.5±42.44×10^6 sperm/mL, motility of 33.33±32.14%, and mean vigor of 2.6±1.15. In this study, the anesthetic protocol did not seem to favor semen collection by electroejaculation in the pacas. The electrical stimulation protocol was able to stimulate all animals in the study; however, there were few samples with sperm cells and a low rate of motility and vigor in most ejaculates.

Keywords: biotechnology, animal reproduction, wild rodents

RESUMO

*Pacas (*Cuniculus paca*) são animais altamente caçados devido ao sabor de sua carne, sendo a criação comercial delas, recomendada. Entretanto, é uma espécie com baixa taxa reprodutiva. O objetivo deste estudo foi efetuar coleta de sêmen em pacas por meio da eletroejaculação e da obtenção, pela primeira vez, dos parâmetros espermáticos dessa espécie. Foram utilizadas sete pacas machos, as quais, antes dos estímulos por eletroejaculador apropriado para a espécie, foram submetidas a protocolo anestésico. O protocolo de estímulo foi realizado em três séries: série I, 10 estímulos com 1 e 2V; série II, 10 estímulos com 3 e 4V; série III, 10 estímulos com 5V, e intervalo entre as séries de dois segundos. O material coletado foi avaliado quanto à cor, ao volume, à motilidade, ao vigor e à concentração. Os parâmetros espermáticos coletados demonstraram volume médio de 0,43±0,33mL, concentração de 45,5±42,44x10^6 espermatozoides/mL, motilidade de 33,33±32,14% e vigor médio de 2,6±1,15. Neste estudo, o protocolo anestésico parece não ter favorecido a coleta de sêmen por eletroejaculação em pacas. Entretanto, o protocolo de estímulos elétricos foi capaz de estimular todos os animais do estudo. Assim, chegou-se ao resultado de poucas amostras com a presença de células espermáticas, bem como baixo índice de motilidade e vigor na maioria dos ejaculados.*

Palavras-chave: biotecnologia, reprodução animal, roedores silvestres

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INTRODUCTION

The paca (*Cuniculus paca*) is among the most hunted species in Latin America, especially in the western Amazon, mainly because of its meat, which is the most appreciated among all game meats (Deutsch and Puglia, 1988; Ribeiro *et al*., 2016).

Conservationist trends point to the rearing of wild animals for commercial purposes to preserve some species of the Brazilian fauna (Hosken, 1999). However, one of the biggest limitations of the commercial use of paca is its low productivity index in captivity.

The gestation period of this rodent is approximately 150 days (Ribeiro *et al*., 2017), where in the end, they give birth to one calf at a time, rarely giving birth to two specimens, with the female being able to generate only two young per child year (Guimarães, 2008; Bonilla-Morales *et al*., 2013).

The use of reproductive biotechniques in wild species in captivity has potential as a tool for conservation and allows for the implementation of programs for artificial insemination and dissemination of germplasm from zootechnically superior wild animals.

Castelo and Silva (2014) reported that in the electroejaculation technique, the anesthetic combinations used, electrical stimulation protocols, and individual variations, such as the placement and size of the probe, in addition to the presence of feces, should be considered if the collection was successful. These authors also mentioned that the anesthetic protocols should promote good analgesia, have low cost, and enable collection without contamination by urine.

The anesthetic protocol most commonly used in mammalian species is xylazine associated with ketamine and lidocaine administered epidurally (Silva, 2017).

MATERIAL AND METHODS

Although widespread in domestic animals, in the reproductive management of wild animals, the electroejaculation technique was approved by the Ethics Committee on Animal Use, registered with the case number 23107.000125/2017-01, protocol number 14/2017, and Biodiversity Authorization and Information System - SISBIO, with the number of request 41459-1.

Seven male pacas, with a mean age of 3 years and weighing between 6 and 7 kg, were used and duly identified by a chip (subcutaneous). These belonged to the breeding of the Wild Animal Breeding and Research Program—Caboclinho da Mata (registration at Brazilian Institute for the Environment and Renewable Natural Resources - IBAMA, number 509309), located at BR364, km 30, in the municipality of Senador Guiomard, Acre (10°03'22.2"S, 67°36'03.1"W).

Seven animals had proven reproductive efficiency by fertile copulations before the beginning of the study. The selected animals were wormed, evaluated for physical health, and isolated from pens with dirt floors, with an area measuring 12 m². The daily diet consisted of fruits, leaves, roots, and grains, in addition to mineral salt and water ad libitum.

Initially, the animals were contained using a net. After physical evaluation and weighing, the anesthetic protocol was initiated, which consisted of pre-anesthesia with 1% acepromazine at a dose of 0.1 mg/kg Intramuscularly - IM. After 5 min, induction was performed with ketamine at a dose of 20 mg/kg IM and xylazine at a dose of 1.5 mg/kg IM.

The electroejaculatory stimulation protocol was based on the method described by Stradiotti *et al*. (2015). The device used was manufactured according to the specifications of the authors: electroejaculator SA-200 (Eletrogen SA 200, Champion Co.) with a voltage capacity from 0 to 10 V, manual regulation and longitudinal probe of 13 cm in length, and two 6-cm longitudinal electrodes.

After complete sedation, the animals were transported to the surgical center of the farm. The identification chip for the animals was read in a previously prepared room. They then underwent cleaning of the inguinal region, including washing the penis, with saline solution when all impurities in the region were removed. If necessary, urinary bladder emptying and feces removal directly from the rectum were performed manually to reduce the risk of

contamination of samples and facilitate the procedure. Lubricants were used for the insertion of a stimulation probe.

At the end of the initial procedure for preparing the animals, they were subjected to the stimulus protocol, with three series: series I, 10 stimuli with 1 and 2 V; series II, 10 stimuli with 3 and 4 V; and series III, 10 stimuli with 5 V. An interval of 2 s was used between sets. While the stimulus procedure was performed, their responses were verified, such as shivering of the corneal papillae, opening of the diverticulum, change in the color of the glans, and crossing of the hind legs.

The response to the anesthetic protocol was evaluated using the observed qualitative parameters. Therefore, for better understanding, the responses were classified into five degrees of sedation as follows:

Grade 1: preserved alertness, ocular and motor reflexes, and present sound emission
Grade 2: drowsiness, ocular and motor reflexes, and present sound emission
Grade 3: sleeping, ocular and motor reflexes, and present sound emission
Grade 4: sleeping, absent ocular reflexes, motor reflexes, and emission of sounds present only with strong stimuli
Grade 5: sleeping, absent ocular and motor reflexes, and absent sound emission

During the entire procedure, the animals were physically evaluated in terms of physiological parameters (heartbeat, respiratory rate, reflexes, and sedation status) for safety purposes in the maintenance of anesthesia, which were measured with the aid of a stethoscope and observation.

For a better assessment of the response to electrical stimuli, four levels were defined, as follows:
N0: no response to stimuli
N1: slight shivering of the corneal papillae and slight opening of the diverticulum
N2: moderate shivering of the corneal papillae, opening of the diverticulum, slight color change of the glans, and slight motor response at the crossing of the (hind) paws
N3: complete shivering of the corneal papillae, complete opening of the diverticulum, change in the color of the glans, and high motor response at the crossing of the (posterior) paws

The ejaculated material was recovered with the aid of a sterile insulin syringe (without a needle), directly from the urethral opening, and later stored in Eppendorf Tubes. The characteristics were observed immediately using an optical microscope. Macroscopic (color and volume) and microscopic observations of motility (%) and vigor (0–5) were performed. The values and characteristics were noted and tabulated. The concentration was determined with a Neubauer camera using a 1:100 dilution in saline solution. Subjective assessments were performed by two or more researchers, and the average of the observations was considered.

After the procedures, the animals were followed up during the return from sedation and returned to their original pens.

Each animal was subjected to the procedure described twice, with an interval of 7 days between the procedures.

RESULTS

The sperm parameters found showed a mean volume of 0.43±0.33 mL, concentration of $45.5±42.44×10^6$ sperm/mL, motility of 33.33±32.14%, and mean vigor of 2.6±1.15. The results observed during the first and second weeks of the stimuli are shown in Table 1.

Table 1. Tabulation of volume, color, motility, vigor, and concentration of ejaculates obtained in the first week of electrostimulation of male pacas in captivity

Animal	First collection					
No.	Volume (mL)	Color	Sperm motility (%)	Vigor (0–5)	Concentration (millions/mL)	Result
1	0.5	Egg white	-	-	-	Seminal plasma
2	0.1	Egg white	-	-	-	Seminal plasma
3	0.1	Yellowish	10	2	48.75×10^6	Semen
4	0.2	Egg white	0	0	18.75×10^6	Semen
5	0.1	Egg white	0	0	26.25×10^6	Semen
6	1.1	Egg white	-	-	-	Seminal plasma
7	0.5	Egg white	-	-	-	Seminal plasma
	Second collection					
1	0.5	Egg white	-	-	-	Seminal plasma
2	0.8	Egg white	-	-	-	Seminal plasma
3	0.1	Yellowish	70	4	127×10^6	Semen
6	0.8	Egg white	-	-	-	Seminal plasma
7	0.4	Yellowish	20	2	10×10^6	Semen

Table 2. shows the levels of physical response to electrical stimuli according to the series applied

First collection			
No.	Series I (1–2 V)	Series II (3–4 V)	Series III (5 V)
1	N0	N1	N3
2	N0	N1	N3
3	N0	N1	N3
4	N1	N2	N3
5	N0	N1	N3
6	N0	N1	N3
7	N0	N1	N3
Second collection			
1	N0	N1	N3
2	N0	N1	N3
3	N0	N1	N3
6	N0	N2	N3
7	N0	N1	N3

*N0, no response to stimuli; N1, slight prickling of the corneal papillae and slight opening of the diverticulum; N2, moderate prickling of the corneal papillae, opening of the diverticulum, slight color change of the glans, and slight motor response at the crossing of the (hind) paws; N3, complete prickling of the horny papillae, complete opening of the diverticulum, change in the color of the glans, and high motor response at the crossing of the (posterior) paws.

Table 3. Sedation status in relation to procedure time

No.	Start	5 min	10 min	15 min
1	Degree 5	Degree 3	Degree 3	Degree 2
2	Degree 4	Degree 3	Degree 2	Degree 2
3	Degree 5	Degree 4	Degree 4	Degree 3
4	Degree 5	Degree 4	Degree 4	Degree 3
5	Degree 5	Degree 4	Degree 3	Degree 3
6	Degree 5	Degree 3	Degree 2	Degree 2
7	Degree 4	Degree 3	Degree 3	Degree 2
Second collection				
1	Degree 4	Degree 3	Degree 3	Degree 2
2	Degree 5	Degree 3	Degree 2	Degree 2
3	Degree 5	Degree 4	Degree 3	Degree 2
6	Degree 4	Degree 3	Degree 2	Degree 2
7	Degree 5	Degree 3	Degree 3	Degree 3

Degree 1, preserved alertness, ocular and motor reflexes without alteration, and emission of sounds present; degree 2, drowsiness, ocular and motor reflexes present, and emission of sounds present; degree 3, sleeping, ocular and motor reflexes present, and emission of sounds present; degree 4, sleeping, ocular and motor reflexes with alteration, and absent sound emission; degree 5, sleeping, absent ocular and motor reflexes, and absent sound emission.

Table 4. Protocol and procedure time

Nº	Animal (microchip)	Age (months)	Weight (kg)	Acepromazine 1% (mL)	ketamine 10% (mL)	Xilazina 2% (mL)	Total procedure time
1	15258	24	6,0	0,06	1,2	0,45	25 min
2	15259	24	6,15	0,0615	1,2	0,46	24 min
3	79906	26	6,8	0,068	1,36	0,51	29 min
4	79911	23	6,42	0,064	1,28	0,48	30 min
5	27093	24	6,25	0,062	1,25	0,46	27 min
6	152405	25	6,9	0,069	1,38	0,51	23 min
7	88455	24	6,74	0,067	1,34	0,50	33 min
Second collection							
1	15258	24	6,1	0,061	1,22	0,45	30 min
2	15259	24	6,1	0,061	1,22	0,45	26 min
3	79906	26	6,7	0,067	1,34	0,50	27 min
6	152405	25	6,9	0,069	1,38	0,51	25 min
7	88455	24	6,8	0,068	1,36	0,51	32 min

Table 5. Time evaluation, Voltage, and number of series necessary to obtain ejaculates.

Nº	Vol (ml)	Color	Effective stimulus time	effective voltage	Nº de series (serie: 1V – 5 V)	Mot. (%)	Mot. progressive (%)	Concentration (millions/ml)	Result
1	0,5	Egg white	11 min	4V	4	-	-	-	Seminal plasma
2	0,1	Egg white	8:30 min	5V	3	-	-	-	Seminal plasma
3	0,1	Yellowish	10:15 min	5V	4	10	7	$48,75x10^6$	Semen
4	0,2	Egg white	9 min	4V	3	0	0	$18,75x10^6$	Semen
5	0,1	Egg white	10:40 min	5V	4	0	0	$26,25x10^6$	Semen
6	1,1	Egg white	12 min	5V	5	-	-	-	Seminal plasma
7	0,5	Egg white	11:20min	5V	4	-	-	-	Seminal plasma
Second collection									
1	0,5	Egg white	9:25 min	4V	3	-		-	Seminal plasma
2	0,8	Egg white	10:20 min	4V	3	-		-	Seminal plasma
3	0,1	Yellowish	13 min	5V	4	-		$127x10^6$	Semen
6	0,8	Egg white	12:30 min	5V	4	70	50	-	Seminal plasma
7	0,4	Yellowish	11 min	4V	4	20	5	$10\ x10^6$	Semen

*Nº: Number of the animal in the experiment; Vol: Volume of material collected; Color: Observed coloring of the ejaculate; Effective stimulation time - Time until the start of ejaculate release; Effective voltage: Voltage of the device that obtained the best stimulus response in the animal; Number of series: Number of complete series necessary for the release of the ejaculate; Mot: Total motility of the material obtained; Progressive Mot: Progressive ejaculate motility; Concentration: Concentration count of ejaculates with the presence of sperm; Result: Material collected after stimuli.

DISCUSSION

In this study, the anesthetic protocol did not demonstrate good sedation and myorelaxation, with satisfactory results in 57% of the animals used, because these animals, within 5 min of the beginning of the procedures, began to vocalize and became restless, demonstrating a regression in the degree of sedation, as shown in Table 3. Therefore, it differed from the results obtained by Stradiotti *et al*. (2015) in electroejaculation in pacas, obtaining these authors 100% success with the animals worked on using the same anesthetic protocol.

Castelo *et al*. (2015) reported that anesthetic protocols are one of the main factors responsible for the success of semen collection in wild animals. In the present study, a combination of ketamine and xylazine was used as a means of induction, with acepromazine administered previously as a preanesthetic.

The combination of ketamine and xylazine has been widely used in dogs and cats (Minter and Deliberto, 2005). Wild species were also subjected to anesthetic protocols with the following associations: Indian leopards (Jayaprakash *et al*., 2001), llamas (Giuliano *et al*., 2008), Iberian red deer (Martinez *et al*., 2008), coatis (Barros *et al*., 2009), and agoutis (Castelo *et al*., 2015).

Mollineau *et al*. (2008) reported the isolated use of xylazine in agoutis, obtaining ejaculate from 30% of the animals worked on. Later, Mollineau

and Mollineau (2017) observed that with the association of xylazine and ketamine, the success of collection increased to 66.6%. However, the best success rate in agoutis (100%) was reported by Martinez *et al*. (2013), where they used azaperone (4 mg/kg) associated with meperidine (4 mg) as a preanesthetic and, after 10 min, a combination of xylazine hydrochloride (0.4 mg/kg) and ketamine hydrochloride (20 mg/kg) IM. At 5 min, lidocaine (5 mg/kg) was applied at the lumbosacral area.

Tecirlioglu *et al*. (2002), working with rats, and Busso *et al*. (2005), working with chinchillas, performed experiments to observe the influence of anesthesia on the results of electroejaculation and found that the animals presented better results when conscious than when subjected to anesthesia, which was not observed in this experiment because when the animals began to vocalize, arousal diminished, and ejaculation did not occur.

In this experiment, all animals showed excitation characteristics during the collection procedure, such as shivering of the corneal papillae, opening of the diverticulum, change in the color of the glans, and crossing of the hind legs, which were also observed by Stradiotti *et al*. (2015). However, in disagreement with these authors, where the animals showed the first characteristics of excitation still at 1 or 2 V, as shown in Table 2, the animals studied here only manifested the complete opening of the diverticulum, change in the color of the glans, and high motor response (N3) between 4 and 5 V For the same stimulus range, ejaculation occurred. Mollineau *et al*. (2008) reported that in agoutis, the ejaculatory response occurred at approximately 9 V. However, Martinez *et al*. (2013) obtained ejaculates from the agoutis when reaching 6 V, a result that is close to that found in the pacas in this study.

After the beginning of the responses, it was notable that at the end of the last series, the animals remained excited, continuing with the characteristic responses, now also being able to be noticed from the stimuli of the new series with 1V. On average, the animals had seminal plasma 10 min after the start of the stimuli.

Despite the same conditions of anesthesia and stimuli, the animals showed great variation in the response to electroejaculation, that is, of the seven animals stimulated, only four presented ejaculates with the presence of sperm cells during the procedure. These results are superior to those of Mollineau *et al*. (2008), who performed the first electroejaculation protocol in agoutis, achieving success in only 30% of the animals, and Costa *et al*. (2014), where they performed the stimuli in agoutis and observed that only 3 of the 10 stimulated animals had ejaculates with sperm cells. However, these values were lower than those reported by Stradiotti *et al*. (2015).

In the first moment of collection, among those that presented positive results, one animal presented ejaculate with a volume of 0.1 mL with a motility of 10% and vigor of grade 2, and the concentration was 48.75×10^6 spermatozoa/mL. Two animals, despite releasing ejaculates with the presence of sperm, did not show any motility or vigor, and the concentrations were 18.75×10^6 and 26.25×10^6 sperm/mL, respectively. The other animals released only a seminal fraction without the presence of sperm, even with all the excitatory characteristics.

In the second collection, two animals were withdrawn from the experiment because of health problems unrelated to the research. The remaining five were evaluated again, and among them, only two had ejaculates with the presence of sperm. The first animal (No. 3) presented positive results in the first week, although with low motility and vigor. However, in the second week, the ejaculate presented a motility of 70% and vigor of grade 4, with a volume of 0.1 mL and concentration of 127×10^6 spermatozoa/mL. The other animal (No. 7) released a good amount of plasma in the first week, without the presence of sperm. In the second week, it presented sperm cells in the ejaculate, although at a low concentration of 10×10^6 spermatozoa/mL, with 20% motility and grade 2 vigor. The other animals presented ejaculates without the presence of sperm.

The collections carried out in this experiment in the presence of spermatozoa showed a mean volume of 0.43±0.3 mL. Castelo *et al*. (2015) reported that the electroejaculation technique can result in a small volume of ejaculate in wild rodents. The results obtained here were superior

to those of Stradiotti *et al*. (2015), where they observed a mean volume of 0.2±0.17 mL in pacas, and Costa *et al*. (2014), where the mean volume was 0.37±0.1 mL in agoutis.

All animals showed responses to stimuli, and even in cases where there was no ejaculate with the presence of sperm, the animals released seminal plasma, which was translucent and viscous. Seminal plasma was gradually released after the complete opening of the urethral diverticulum and pricking of the corneal papillae. Moreover, a change in the color of the glans to darker tones was observed when the stimulus was activated. In a study on different stimuli for electroejaculation in agoutis, Castelo *et al*. (2015) described that 68.8% of the collections resulted in ejaculates without the presence of sperm; they justified that in the literature, there is a wide variation in results regarding the use of electrostimulation in wild rodents.

Contrasting the results found by Stradiotti *et al*. (2015), who obtained ejaculates with the presence of sperm in 100% of the animals worked on, in the present study, 57% (4/7) of the animals with sperm in the ejaculates were obtained. Stradiotti *et al*. (2015), creators of the probe and electrostimulation protocol used in this experiment, did not describe the motility, vigor, and concentration of the findings. To the best of our knowledge, the present study is the first to report the semen parameters in pacas.

The difference observed between the two studies, despite the same stimulus protocol, can be explained by an individual variation in the animals, anesthetic protocol, or even a better adaptation of the animals used in the study by Stradiotti *et al*. (2015) to the protocol used.

CONCLUSIONS

The anesthetic protocol used in this study did not favor semen collection by electroejaculation in pacas, demonstrating little efficiency in maintaining the state of sedation throughout the procedure. The electrical stimulation protocol was able to promote a positive response in all animals in the study but resulted in few samples with sperm cells and a low rate of motility and vigor. Electroejaculation in wild rodents requires further studies to develop efficient and safe protocols that allow maximum use of this tool in the reproduction of these animals.

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