Theriogenology 126 (2019) 286–294

Contents lists available at ScienceDirect

Theriogenology

journal homepage: www.theriojournal.com

Environmental effects on collared peccaries (*Pecari tajacu*) serum testosterone, testicular morphology, and semen quality in the Caatinga biome

K.M. Maia [a], A.L.P. Souza [b], A.M. Silva [a], J.B.F. Souza-Jr [c], L.L.M. Costa [c], F.Z. Brandão [d], M.F. Oliveira [a], P. Comizzoli [e], A.R. Silva [a, *]

[a] *Laboratory of Animal Germplasm Conservation (LCGA), Departamento de Ciências Animais, Universidade Federal Rural do Semi-Árido (UFERSA), BR 110, Km 47, Costa e Silva, CEP: 59625-900, Mossoró, Brazil*

[b] *Programa de Pós-Graduação em Biotecnologia, Universidade Federal do Ceará, Sobral, Brazil*

[c] *Laboratório de Biometeorologia, Biofísica Ambiental e Bem-Estar Animal (LABBEA), Departamento de Ciências Animais, Universidade Federal Rural do Semi-Árido (UFERSA), Mossoró, RN, Brazil*

[d] *Departamento de Patologia e Clínica Veterinária, Faculdade de Veterinária, Universidade Federal Fluminense (UFF), Rua Vital Brasil Filho, 64, 24320-340, Niteroi, RJ, Brazil*

[e] *Smithsonian Conservation Biology Institute, National Zoological Park, P.O. Box 37012, MRC 5502, Washington, DC, 20008, USA*

A R T I C L E I N F O

Article history:
Received 19 July 2018
Received in revised form
14 December 2018
Accepted 20 December 2018
Available online 21 December 2018

Keywords:
Tayassu tajacu
Wild pig
Temperature
Rainfall
Semiarid weather
Wildlife

A B S T R A C T

The objective of the study was to understand the influence of climatic variations in a semiarid environment on serum testosterone, testicular morphology and semen quality in collared peccaries (*Pecari tajacu*). Reproductive metrics (semen quality, testicular morphometry and testosterone serum profiles) of 10 mature males were measured monthly for 18 months. Meteorological data (rainfall, air temperature, relative humidity, wind speed and radiant heat load) also were recorded during the same period. Rainfall regimes were classified in different classes (Class 1: months with no rain; Class 2: months with up to 50 mm of rain; and Class 3: months with >50 mm of rain). Among rainfall classes, average air temperature (°C) and relative humidity (%) were different. Climatic changes between rainfall classes did not lead to overall variations of testicular size, testosterone production, and semen metrics. However, relative humidity recorded before semen collection (one day, one week, or over 51–55 days) was positively correlated ($P < 0.05$) with semen motility metrics (total motility, beat cross frequency and straightness) and sperm subpopulations (medium and static sperm), as well as with volume. Negative correlations ($P < 0.05$) were revealed between air temperature and the same semen motility patterns and volume. Additionally, radiant head load measured on the day of semen collection negatively influenced ($P < 0.05$) sperm straightness. This study demonstrates for the first time that no seasonal changes could be detected overt the 18-month period on the serum testosterone, testicular morphology and semen quality of collared peccaries raised in the Caatinga biome; however, it is expected that long term environmental changes will influence the reproductive physiology of species leaving in that habitat.

© 2018 Elsevier Inc. All rights reserved.

1. Introduction

Currently, the world population of collared peccaries (*Pecari tajacu* Linnaeus, 1758), a native species of the American continent, has been classified as stable by the International Union for the Conservation of the Nature - IUCN [1]. However, their status in all biomes requires monitoring because various factors like habitat destruction and excessive poaching have considerably reduced wild populations [1], making it vulnerable or even extinct in some areas [2]. Collared peccaries play an important ecological role as seed dispersers and as a prey for large carnivores [1,2]. Moreover, they can serve as a model for the development of conservation strategies for closely related species that are vulnerable, such as the white-lipped peccary (*Tayassu pecari*) and the Chacoan peccary (*Catagonus wagneri*).

* Corresponding author. Laboratory of Animal Germplasm Conservation (LCGA), Department of Animal Sciences, Universidade Federal Rural do Semi-Árido (UFERSA), Francisco Mota Avenue, 572, Mossoró, Rio Grande do Norte, 59625-900, Brazil.

E-mail addresses: legio2000@yahoo.com, alexrs@ufersa.edu.br (A.R. Silva).

https://doi.org/10.1016/j.theriogenology.2018.12.032
0093-691X/© 2018 Elsevier Inc. All rights reserved.

Male reproductive fitness has a substantial effect on population dynamics [3]. Various studies have been focusing at elucidating physiological mechanisms of peccary's male reproduction to establish optimal strategies for its conservation. Peccaries can breed all year round [4] but rainfall can influence their reproductive patterns [5,6]. Males usually reach puberty at the age of 11 months [4] and spermatogenesis lasts 55.1 ± 0.7 days [7]. Ejaculates are composed of three fractions: the first being clear and secreted by the accessory glands, the second is rich in spermatozoa, and the third one ia a jelly fraction. However, semen fractions tend to mix during collection by electroejaculation - the method of choice for collecting semen in that species [5]. Regarding semen characteristics, individuals provide 3.5 mL of ejaculate containing an average of 0.8×10^9 sperm/mL [8]. All this information, although, were derived from punctual studies, and the long-term variation in reproductive performance of peccaries, especially in Caatinga, a biome constituted by a dry and seasonal Neotropical forest, is unknown.

Climate changes, especially the increase in average temperature, has a major impact on ecosystems [9] and impacts the survival of several species [10]. Over the next three decades, temperature will rise between 0.5 and 1.0 °C and rainfall index will drop to 10–20% in Caatinga [11]. Such changes will certainly affect the vegetation of the region [12], thus reducing the food resources for the native animals. Under the influence of climatic conditions, native animals, such as collared peccary, need to regulate their physiological functions to adapt to certain periods of stress.

Recent studies have evidenced interactions between meteorological characteristics of semiarid weather of the Caatinga biome and semen quality in ovine [13] and caprine species [14]. However, there is no information on widely distributed species like the peccary, especially about the climate influence on reproductive metrics as sperm characteristics, testicular biometry and testosterone production. The objective of the study therefore was to understand the short and medium-term influence of meteorological conditions on serum testosterone, testicular morphology and semen quality in collared peccaries (*Pecari tajacu*).

2. Methodology

All the experimental procedures were approved by the UFERSA Ethics Committee (CEUA/UFERSA, n °. 23091,008820/2016-03). The experiment was conducted at the Center for Multiplication of Wild Animals (CEMAS, UFERSA, Mossoró, RN, Brazil) (latitude: 5° 10 ′S, longitude: 37° 10′ W, altitude: 16 m, typical semiarid climate). Semen was evaluated in the Laboratory of Conservation of Animal Germplasm of the same institution. The testosterone analysis was performed at the Universidade Federal Fluminense, Rio de Janeiro, RJ, Brazil. The experiment was conducted from September 2015 to February 2017, with monthly collections of reproductive, physiological and meteorological data.

2.1. Meteorological values

Due to irregularity of rainfall that characterizes the Caatinga, we conducted the experiment for 18 months to increase the amount of data derived from rainy months. The 18 months were grouped according to the rainfall regimes as follows: Class 1 –consisting in 7 rainless months characterizing the typical dry season; Class 2 –constituted by 8 months with a rainfall regime up to 50 mm characterizing an intermediary season; and Class 3 – constituted by 3 months presenting a rainfall regime >50 mm characterizing the rainy season. The classification of rainfall regimes was adapted from Aubréville, [15].

The rainfall regime (mm) was obtained daily from the automatic station of the National Institute of Meteorology - INMET, located in Mossoró, RN, Brazil. During the experiment, a total rainfall of 650 mm was observed, presenting irregular and below expected rains for the rainy season (March, April and May) in the region. Rain peaks were observed in the months of January 2015 and February 2017 (Fig. 1).

Since the weather is a reflex of cumulative meteorological conditions, we verified the influence of rainfall index, air temperature, and relative humidity determined on the day before semen collection, during previous week and over previous 51–55 days (corresponding to the time for spermatogenesis in the species) on various reproductive metrics (semen quality, testicular biometry, testosterone levels).

Data related to air temperature (Ta, °C; Fig. 1) and relative humidity (RH, %; Fig. 1) were obtained from an automatic weather station (Campbell Scientific Brasil, São Paulo, Brazil) located 50 m from the experiment site (latitude: 5° 12′48 ″ S, longitude: 37° 18′44″W and altitude: 37 m above sea level), Mossoró, RN, Brazil. Based on these data, it was possible to trace the monthly profile of these meteorological parameters during the 18 months of the experimental period. Average relative humidity ranged from peak values of 53% during dry season to 71% in rainy season, while average peak values for air temperature ranged from 28 °C in rainy season to 31 °C during dry season. The air temperature remained high throughout the year, regardless of the rainfall index. Among rainfall classes, however, average air temperature (TA, °C; Fig. 1) and relative humidity (RH, %; Fig. 1) were different.

To determine the radiant heat load (RHL, W/m2) on the day of the semen collection, we monitored wind speed, a dry bulb temperature, and a black globe temperature. Wind speed was monitored every hour (from 6 a.m. to 6 p.m.) by means of a thermo-hygrometer-anemometer (Instruterm-HT300, São Paulo, Brazil) [16]. The humid (Tu, °C) and dry (Ta, °C) bulb temperatures were measured using a psychrometer (Bacharach, New Kensington, Pennsylvania, USA). Additionally, temperatures of the black globes (Tg, ºC), one exposed to the sun and another to shade, measured by means of a mercury thermometer, were registered in the center of a black copper globe with 15 cm of diameter, connected to a digital thermometer (MINIPA, MT-600, São Paulo, Brazil). The equipment was positioned in the picket where the animals were located, at an approximate height of the animals' torso, according to the methodology described by Silva [16]. RHL was calculated from the equations proposed by Silva et al. [17].

2.2. Animal management and handling

Ten male adult collared peccaries (3.1 ± 1.5-year-old) and weighting 21.9 ± 1.3 kg at the beginning of the experiment. They reached an average age of 4.6 ± 1.5 years and weight of 23.2 ± 1.2 kg at the end of the study. The peccaries were separated into groups of 2–3 animals, kept in pickets (20 m × 3 m), with a covered area (3 m × 3 m) under natural 12 h photoperiod. They were fed with commercial ration for pigs, and water at will. The diet was supplemented with seasonal fruits as melon, water melon, papaya and coconut, mainly during dry season. During rainy season, the diet was added of corn straw, sorghum and Jitirana (Ipomoea cairica), a typical seasonal vine plant.

On the days of collection, the animals were submitted to food fasting of 12 h before the beginning of the procedures. The animals were initially restrained with a hand net. Subsequently, they were anesthetized with propofol (Propovan®, Cristália, Fortaleza, Brazil) at an intravenous dose of 5 mg/kg in their bolus. Throughout the experimental procedure, the animals were submitted to fluid therapy (sterile saline solution 0.9%) and their heart and respiratory rate were monitored [18].

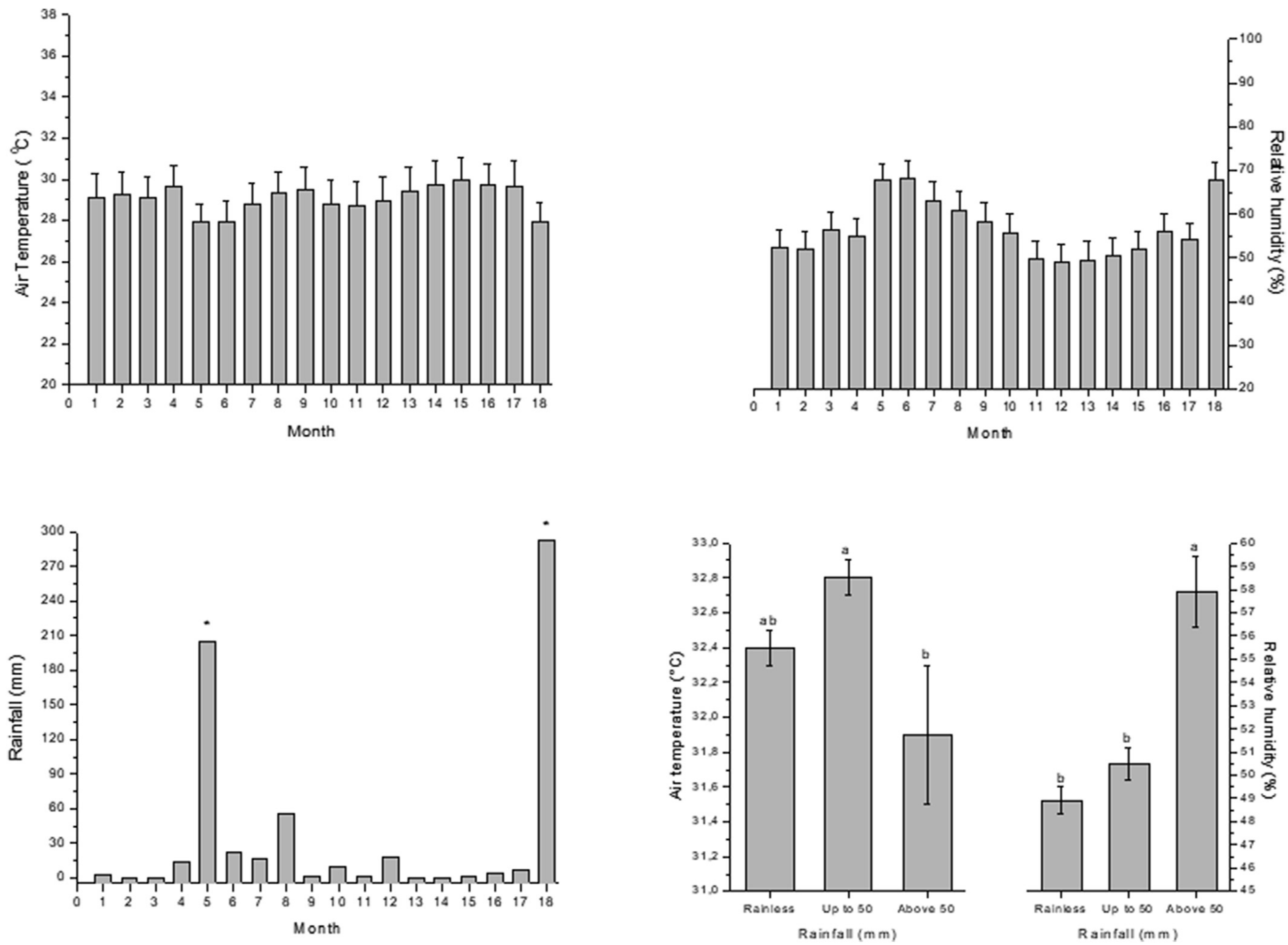

Fig. 1. Mean values (±SEM) for the air temperature (° C) relative humidity (%), and cumulative rainfall regime per month, and for air temperature (° C) and relative humidity (%) obtained in the day of semen collection in collared peccaries (n = 10 males) under different rainfall regimes (class 1 – rainless; class 2 – up to 50 mm; class 3 – above 50 mm), from September 2015 (month 1) to February 2017 (month 18) in the Caatinga biome. Asterisks (*) evidence rainy peaks (*) in January 2016 (month 5) and February 2017 (month 18); [a,b] Means with different letters differ statistically ($P < 0.05$).

Immediately after restraint, a digital thermometer (Mesure Technology, Park Dongting Town, China) determined the rectal temperature of the individuals [19]. Moreover, surface temperature of each individual was obtained through a surface infrared thermometer (Fluke, Everett, Washington, USA.) at each hour from 6 a.m. to 6 p.m. This infrared thermometer was positioned at 2 m from the individual and the laser sensor was pointed to its dorsum [19].

2.3. Testicular morphometry

When animals were restrained to collect semen, their scrotal circumference was measured in the largest portion of the testicular sac, using a metric tape measure [8]. Then, testicular ecotexture and echogenicity were evaluated by ultrasonography, using a 7.5 MHz sector transducer coupled to a portable ultrasound equipment (Aquila vet, Pie Medical®, Nutricell, Campinas, Brazil) (Peixoto et al., 2012). The testicular biometry was evaluated by taking the measurements of length (L), width (W) and height (H) of each testicle. These measures were used to calculate the testicular volume (V) by the Lambert formula ($V = L \times H \times W \times 0.71$ [20]). This analysis was performed on each animal, monthly, on semen collection days, shortly after animal restraint, for 12 months (September 2015 to August 2016).

2.4. Blood collection and assessment of serum testosterone

For the occasion of semen collection, to determine serum concentrations of total testosterone, two blood samples from each animal were collected by puncture of the saphenous vein, at intervals of 30 min between each one. The first sample was obtained immediately after the animal was restrained, and the second sample was taken at the end of the semen collection process. Blood was passed to 3 mL vials (Vacutainer®, BD Diagnostics, Franklin Lakes, NJ, USA), and immediately placed in an icebox. After collection, the tubes were taken to the laboratory and centrifuged (5000 g, 60 min, 4 °C) for serum separation, which was aliquoted into microtubes (1.5 mL) and stored (−20 °C) until the hormonal analysis [21].

For analysis of serum testosterone, it was used the liquid phase radioimmunoassay (RIA) and a commercial kit (ImmuChem™ Double Antibody Testosterone RIA-MP Biomedicals) in a Wizard device detector (PerkinElmer of Brazil Ltda). The intra-assay coefficient was 8.54% and 7.31% inter-assay. All concentrations determined were between the minimum and maximum points of the curve [22].

2.5. Semen collection and evaluation

For 18 months, animals were subjected to a semen collection per month, always in the early morning (6–8 a.m.). On these occasions, anesthetized animals were placed in lateral decubitus position, and submitted to an electroejaculation protocol previously described for the species [23]. A portable device (Autojac®, Neovet, Campinas, SP, Brazil) connected to a 12 V source was used, to which a rectal probe 15 cm long and 1.3 cm in diameter was attached. The probe was inserted approximately 12 cm into the rectum of the animal. The semen of the animals was collected in plastic tubes and immediately evaluated.

Semen volume was measured with micropipettes. Sperm concentration (in millions of sperm/mL) was determined in a Neubauer chamber. From this concentration and volume, the total number of sperm in the ejaculate was calculated [8]. For analysis of sperm morphology (%), smears stained in Rose Bengal were evaluated by light microscopy (1000x), counting 200 cells per slide [24].

For the analysis of sperm plasma membrane integrity (%), a fluorescent solution of carboxyfluorescein diacetate (CFDA) and propidium iodide (PI) was used. Samples were incubated for 10 min at 27 °C in the CFDA + PI solution and then evaluated by epifluorescence microscopy (Episcopic Fluorescent attachment "EFA" Halogen Lamp Set; Leica, Kista, Sweden). For each sample, 200 sperm were counted and those marked totally green (CFDA) were classified intact, while those marked totally or partially red (PI) were considered with the non-intact membrane [24].

The kinetic patterns of sperm motility were determined by computerized analysis (IVOS 7.4G, Hamilton-Thorne ResearchTM, Beverly, MA, USA), according to previously determined configurations for the species [25]. The following parameters were evaluated: total motility (%), VAP velocity average pathway (μm/s), velocity straight line (VSL; μm/s), velocity curvilinear (VCL; μm/s), amplitude lateral head (ALH, μm), beat cross frequency (BCF, Hz), straightness (STR; %), and linearity (LIN; %) as well as the sperm subpopulations: rapid, medium, slow and static.

2.6. Experimental design and statistical analysis

The effect of rainfall regimes (distributed in classes) and meteorological conditions on reproductive metrics of collared peccaries located in the Caatinga biome was analyzed. All variables were expressed as average and standard error. The data were first examined for normality by the Shapiro–Wilk test and for homoscedasticity by the Levene's test and were transformed by log (x + 1) or arc-sine ($\sqrt{}$ (x/100)), when necessary. An analysis of variance (ANOVA) was performed using the least squares method, considering rainfall index classes (1, 2 or 3) as the only source of variation on the reproductive parameters studied. Tukey-Kramer test ($P < 0.05$) was used to compare the averages. In these statistical procedures the general linear model procedure (PROC GLM) of the Statistical Analysis System [26] was used.

In order to verify the relation between semen metrics and meteorological variables, the Pearson correlation test ($P < 0.01$) was performed using the SAS PROC CORR procedure. To test the difference in collection efficiency (%) between collection classes, Pearson's Chi-square test ($\chi 2$) was applied, with $P < 0.05$ using SAS PROC FREQ procedure. All graphs were constructed using the OriginPro 8 software. For all analyzes, $P < 0.05$ was used.

3. Results

3.1. Influence of body temperature on sperm metrics

Rainfall regimes in different seasons did not influence average values of rectal and surface temperatures (Fig. 2). The surface temperature did not influence sperm metrics. However, rectal temperature showed a negative correlation ($P < 0.05$) with total motility ($R = -0.20$), VAP ($R = -0.20$), and rapid sperm subpopulations ($R = -0.23$), as well as positive correlation with static spermatozoa ($R = 0.20$, $P = 0.02$).

3.2. Influence of season on serum testosterone profile and testicular morphometry

The values for serum testosterone concentration of the animals (Fig. 2) remained constant throughout the different rainfall regimes that characterized the different seasons. In addition, scrotal circumference and testicular morphometry (right, left and total testicular volume) did not change along the seasons (Fig. 2). Based on values obtained on the days of semen collections, a significant correlation was found between serum testosterone concentrations and scrotal circumference ($R = 0.21$, $P < 0.05$).

3.3. Influence of season on semen metrics

Overall rainfall amount did not influence the success of semen collection. Regarding rainfall regimes, 70 collection attempts were performed in class 1 (typical dry season), and 47 semen samples were obtained (67.1%). In class 2 (intermediary season), 80 attempts were performed, which resulted in 59 semen samples (73.8%). Finally, in class 3 (typical rainy season), 19 samples were obtained from 30 attempts of semen collection (63.3%).

Following different seasons, rainfall classes did not lead to significant differences in semen volume, sperm concentration, total sperm count, sperm morphology, functionality and membrane integrity (Table 1). In addition, the computerized analysis of motility did not identify significant influence of seasons on the sperm kinetic metrics (Table 1).

Relations between semen metrics and meteorological conditions (rainfall, relative humidity and air temperature) are presented in Table 2. On the day before semen collection, there was a positive correlation ($P < 0.05$) between average relative humidity and BCF ($R = 0.29$), STR ($R = 0.28$) and medium sperm subpopulation ($R = 0.25$). On the other hand, a negative correlation ($P < 0.05$) was identified between air temperature and BCF ($R = -0.31$), STR ($R = -0.20$) and medium sperm subpopulation ($R = -0.25$). When evaluating meteorological variables over the week prior to semen collection, relative humidity was positively correlated ($P < 0.05$) with volume ($R = 0.20$), BCF ($R = 0.28$), STR ($R = 0.39$) and LIN ($R = 0.21$). Besides, negative correlations ($P < 0.05$) were identified between air temperature and volume ($R = -0.27$), normal morphology ($R = -0.31$), BCF ($R = -0.31$), STR ($R = -0.31$), and medium sperm subpopulation ($R = -0.19$). When evaluating meteorological variables over 51–55 days before semen collection, relative humidity was positively correlated ($P < 0.05$) with total motility ($R = 0.23$) and negatively correlated ($P < 0.05$) with static sperm ($R = -0.24$). Lastly, negative correlations ($P < 0.05$) were found between air temperature and BCF ($R = -0.23$).

Regarding radiant head load (RHL) in the sun, which was evaluated every 6 h in the day of semen collection, a negative correlation between it and STR ($R = -0.21$, $P < 0.05$) was identified. RHL did not influence any other semen metric.

4. Discussion

Even though no seasonal effect of Caatinga's semiarid weather was observed on peccary reproduction, short to medium term influence of meteorological conditions on semen metrics were evident. Moreover, serum levels of testosterone from the collared peccaries remained constant against the different seasons of the Caatinga biome. It is probable that the meteorological metrics of this biome do not vary sufficiently during the year to the point of

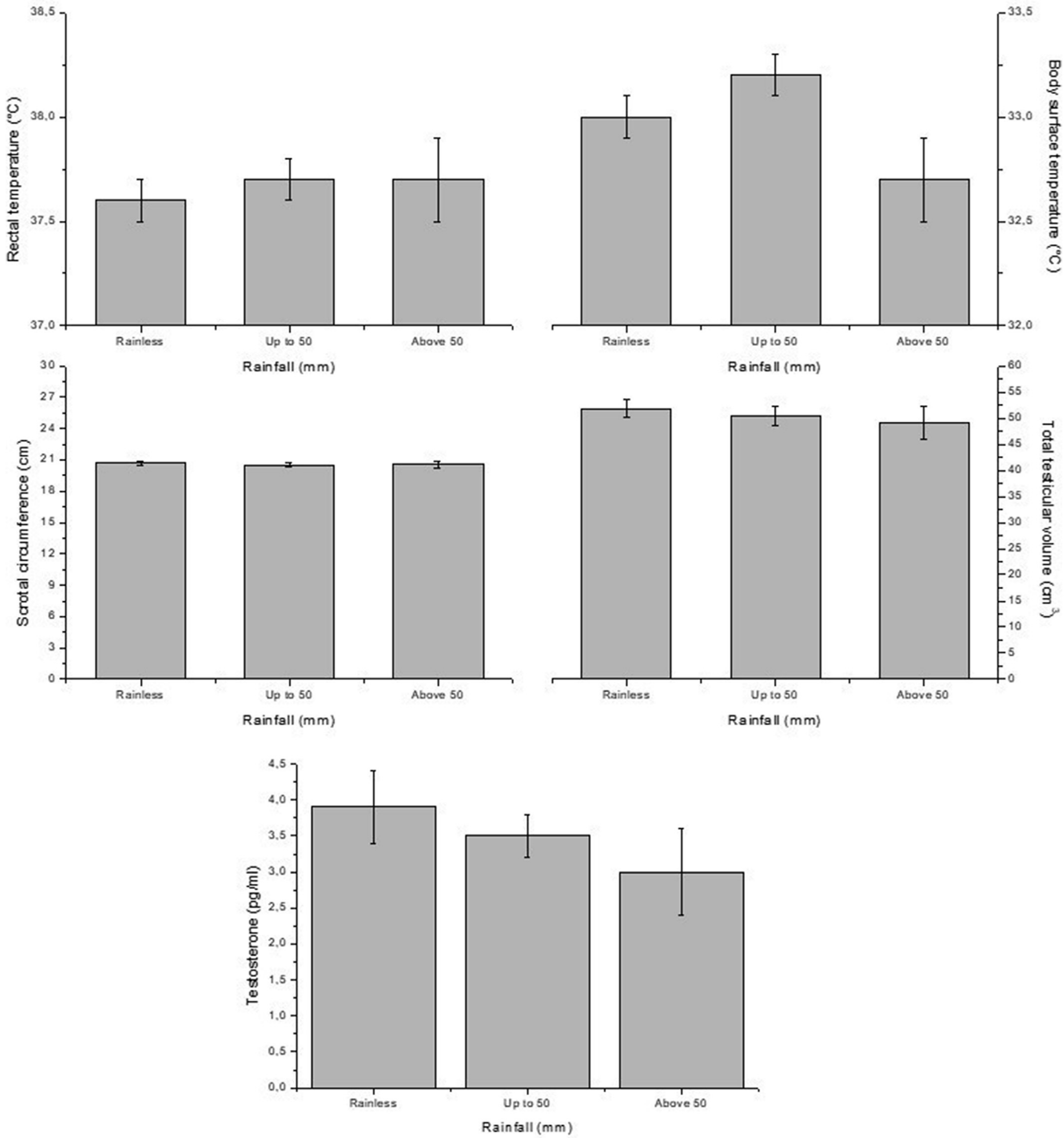

Fig. 2. Mean values (±SEM) for the rectal and surface temperatures (°C), scrotal circumference (cm), total testicular volume (cm3) and serum testosterone (pg/mL) of collared peccaries (n = 10 males) obtained according to different rainfall classes (class 1 – rainless; class 2 – up to 50 mm; class 3 – above 50 mm), P > 0.05.

affecting the reproductive hormones of these males, since these findings differ from those reported for the same species when settled in Texas, USA, under arid climate conditions [5]. In that region, the animals showed a seasonal trend in serum testosterone concentrations (P < 0.05), with an increase in values during the winter and a decrease in these values during the summer. It is worth noting, however, that there are discrepancies among researchers regarding the variations in testosterone concentrations in males of mammalian species, since some animals with seminal quality affected by environmental thermal stress may not present any hormonal alteration [27]. Similar evidences have been reported for domestic pigs because if their testicular tissues show a degree of degeneration that is not so severe, the hormone production of the boars may not be affected [28]. In addition, the constancy of the

Table 1
Mean values (±SEM) of semen metrics and sperm motility kinetic parameters evaluated by computerized analysis in collared peccaries (*Pecari tajacu;* n = 10 males) raised in the Caatinga biome. Data obtained from September 2015 to February 2017 under different rainfall classes (rainless, up to 50 mm, and above 50 mm).

Semen metrics	Rainfall Classes*		
	Rainless	Up to 50 mm	Above 50 mm
Number of ejaculates evaluated	39	46	16
Volume (mL)	3.1 ± 0.4	3.72 ± 0.6	3.5 ± 0.8
Concentration (x10^6 sperm/mL)	339.8 ± 53.3	280.9 ± 28.2	226.3 ± 55
Sperm/ejaculate (x10^6)	817.6 ± 149.8	912.1 ± 140.0	742.96 ± 212.1
Normal morphology (%)	86.6 ± 1.4	86.3 ± 1.1	91.2 ± 1.3
Membrane integrity (%)	78.5 ± 2	79.9 ± 2	76.7 ± 2.6
Sperm kinetic parameters			
Total motility (%)	80.9 ± 2.8	81.4 ± 2.3	74.1 ± 5.9
Velocity average pathway (μm/s)	47.5 ± 1.7	50.7 ± 1.9	49.8 ± 2.7
Velocity straight line (μm/s)	26.8 ± 1.1	30.4 ± 1.5	29.2 ± 1.8
Velocity curvilinear (μm/s)	105.7 ± 3.1	110.1 ± 3.1	105.1 ± 4.6
Amplitude lateral head (μm)	6.5 ± 0.1	6.6 ± 0.1	6.7 ± 0.2
Beat cross frequency (Hz)	35.5 ± 0.7	36.1 ± 0.7	33.9 ± 1.1
Straightness (%)	55.3 ± 1.3	57.2 ± 1	55.7 ± 1.4
Linearity (%)	27.1 ± 1	30.0 ± 0.7	27.6 ± 0.7
Subpopulations			
Rapid (%)	59.0 ± 3.4	61.4 ± 2.9	54.9 ± 5.8
Medium (%)	16 ± 1.7	14.6 ± 1.1	13.7 ± 1.8
Slow (%)	5.9 ± 0.5	5.6 ± 0.4	5.3 ± 0.6
Static (%)	19 ± 2.5	18.5 ± 2.1	25.9 ± 5.6

*No significant difference was evidenced among rainfall classes, P > 0.05.

Table 2
Correlation between semen metrics in collared peccaries (*Pecari tajacu*; n = 10 males) and meteorological conditions (Rainfall, Relative Humidity – RH, Air Temperature – AT) one day, one week, or 55 days before semen collection.

Semen metrics	Meteorological Conditions								
	One day before			A week before			Over 55 days before		
	Rainfall	RH	AT	Rainfall	RH	AT	Rainfall	RH	AT
Volume (mL)	0.03	0.06	−0.08	0.13	0.20*	−0.27*	0.17	−0.02	−0.13
Concentration (x10^6 sperm/mL)	−0.02	−0.10	−0.05	0.05	0.08	−0.16	−0.14	−0.07	−0.05
Sperm/ejaculate (x10^6)	0.00	−0.01	−0.17	0.09	0.14	−0.31*	−0.00	−0.03	−0.02
Normal morphology (%)	0.08	0.04	0.00	−0.07	0.05	0.08	0.13	0.08	0.03
Membrane integrity (%)	−0.08	0.02	0.03	−0.22	−0.10	0.11	−0.02	−0.16	0.10
CASA metrics									
Total motility (%)	−0.10	−0.13	0.02	−0.08	−0.05	−0.09	0.05	0.23*	0.07
Velocity average pathway (μm/s)	−0.18	−0.25	0.31	−0.12	−0.11	0.19	0.11	0.13	0.07
Velocity straight line (μm/s)	−0.10	−0.13	0.24	−0.05	0.00	0.09	0.11	0.18	0.09
Velocity curvilinear (μm/s)	−0.20	−0.20	0.23	−0.10	−0.10	0.11	0.05	0.11	−0.04
Amplitude lateral head (μm)	−0.20	−0.15	0.32	−0.10	−0.15	0.27	0.12	−0.01	−0.01
Beat cross frequency (Hz)	0.11	0.29*	−0.31*	0.15	0.19*	−0.31*	−0.16	−0.08	−0.23*
Straightness (%)	0.18	0.28*	−0.20*	0.18	0.27*	−0.30*	0.02	0.17	−0.00
Linearity (%)	0.14	0.17	−0.08	0.12	0.21*	−0.17	0.05	0.13	0.05
Subpopulations									
Rapid (%)	−0.15	−0.22	0.12	−0.12	−0.10	0.01	0.07	0.19	0.08
Medium (%)	0.13	0.25*	−0.25*	0.12	0.13	−0.19*	−0.07	0.01	−0.04
Slow (%)	−0.01	0.09	0.02	0.02	0.02	0.10	−0.01	−0.11	−0.10
Static (%)	0.12	0.12	−0.02	0.08	0.05	0.08	−0.05	−0.24*	−0.06

*Superscript asterisk indicates significant correlation (P < 0.05).

testosterone levels in the Caatinga biomes confirms peccaries' adaptability, since even under stressful conditions (high temperatures and very low relative humidity, typical of the dry season of the semiarid region), the hormonal production of these animals remained stable.

Like the serum testosterone levels, the scrotal circumference and testicular morphometry of the collared peccaries located in the Caatinga biome were not affected by the seasons of the semiarid climate. These findings also differ from those reported for the peccaries settled in Texas, USA, under arid climate conditions [5]. In these individuals, the estimated testicular volume and scrotal circumference presented significantly higher values during the rainier periods when compared to the records performed in the driest period, correlating directly with serum testosterone values. The authors suggested that this male remains reproductively fertile throughout the year but may suffer an optional quiescence in the dry season, which may be influenced by the ambient temperature and social factors [5]. In the Caatinga, a positive correlation of testosterone with scrotal circumference was observed in peccaries, as described by Hellgren et al. [5], however, there were no fluctuations of these metrics along the different rainfall indexes, and no type of reproductive quiescence was identified, denoting its possibility of reproduction throughout the year in this biome.

In fact, we demonstrated that it is possible to obtain semen from the peccaries by means of electroejaculation, independently of the rainfall regime. During all seasons, their semen quality was constant and with values like those previously described for individuals raised in the same biome [8,25]. Like verified for caprine natives of Caatinga [29], it seems that changes in semen quality remain within the limits of the physiological measures to overcome the thermal stress, allowing the maintenance of their reproductive performance throughout the year [30]. In fact, peccaries are rustic species apparently already acclimated to the semiarid region. This is mainly due to their adaptive flexibility in their physiological metrics, which allows them to adjust to the seasonal changes in their environment, through adaptation in metabolic heat production, adjustments in evaporative and heat flow capacities, and changes in tolerance to extreme temperatures of the dry season [31]. However, under climatic conditions of a different biome, in the case of the Eastern Amazon with its semi-humid Equatorial climate, Kahwage et al. [6] identified fluctuations in the semen parameters of the species throughout the year, evidencing a significant improvement of these parameters during periods of higher rainfall. It is necessary to point out that in the eastern Amazonia there is a total annual rainfall of 2900 mm^3, ranging from 2000 to 3800 mm^3 [32], well above the total rainfall of 650 mm^3 observed in the Caatinga during present study. It is emphasized that although the influence of the semi-humid climate of the Eastern Amazon on the reproduction of the male collared peccaries was identified, this influence was not of sufficient magnitude to indicate reproductive seasonality in these animals [6], which remain apt to perform activities through all the years as that observed in the Caatinga.

The low and irregular rainfall regimes that characterizes this biome is known for its elevated temperatures [33]. In this context, air temperature seems to be the more important meteorological parameter at impairing peccaries' semen quality at short or medium-term by exerting negative effect on BCF, STR and sperm subpopulations, as well as on semen volume. At this point, necessary to highlight the importance of CASA at detecting subtle differences on peccaries' sperm motility metrics as those provoked by meteorological parameters, like previously reported for bovine [34].

Similar to peccaries, domestic swine presents reduced semen quality when exposed to high ambient temperatures [35], especially with relation to the sperm motility [36]. Stress due to high temperatures may be the main cause of the problem, as stress can induce excess corticosteriod production [37]. Probably, a decrease in estradiol-17b levels is also involved [38], since this hormone is notably associated to the epidydimal function and spermatogenesis [39]. Moreover, the fluctuations in temperature between the day and the night may be a significant factor in creating stress for boars during the hot season [28], and maybe also for peccaries. Recently, Gong et al. [40] tried to elucidate the molecular basis of heat stress-induced decrease of sperm motility in swine. Authors affirm that temperature affects sperm motility through downregulation of mitochondrial activity and ATP synthesis yield, which involves dephosphorylation of glycogen synthase kinase-3α (GSK3α) and interference of mitochondrial remodeling. In this context, we hypothesize that a similar event could happen on peccaries when subjected to high temperature which impairs sperm metrics determined by CASA, especially those related to the stability of a progressive motion as BCF and STR, highlighting the commitment of the mitochondrial function.

On the other hand, relative humidity of a semiarid weather exerted a positive influence on various peccaries' kinetic metrics as total motility, STR, BCF, LIN and sperm subpopulations both on a short and medium-term, as well as positively affect semen volume on a short term. Similarly, a positive effect of relative humidity on sperm motility was evidenced for bulls raised in semiarid region [41]. It is possible that these changes in sperm metrics could be related to the activity of accessory glands that produce the seminal plasma, mainly because a positive influence of relative humidity on semen volume was detected for peccaries. In fact, the composition of seminal plasma can be influenced by seasonal climatic changes as recently reported for bison [42]. The presence of a heat-shock protein (HSP-70), which play important roles in thermotolerance in cells, was identified in the boar spermatozoa during the hot season, being associated with semen quality [43]. In peccaries, initial studies described the proteomic composition [44] and the antioxidant enzyme activity [45] of their seminal plasma, but the presence of no heat shock protein is highlighted. Moreover, the existence of relationships among plasma seminal activities and meteorological conditions remains unknown for this species.

On the day of semen collection, a negative influence of the radiant heat load (RHL) in the sun on peccaries' sperm STR was verified. RHL is an important meteorological variable usually used as an indicator of thermal comfort [17]. The adverse effect of increased RHL on reproductive capacity of mammals have been discussed for a long time [46]. In fact, it is known that the home-okinetic changes to regulate body temperature can compromise reproductive function [47]. Probably because of RHL interference, negative correlations between rectal temperature and important kinetic metrics of peccaries' sperm motility were verified, thus showing that high body temperatures could affect sperm quality. This is mainly evidenced because as higher was the peccaries' rectal temperature, higher was the amount of static sperm, maybe as a consequence of the impaired mitochondrial function [40]. Similarly, thermal discomfort is reported for decreasing reproductive efficiency in different mammals as rams [13] and boars [48], maybe by causing testicular degeneration and thus impairing the sperm production [35].

In collared peccaries, spermatogenesis lasts approximately 55.1 ± 0.7 days [7], a period very similar to spermatogenesis in pigs, which are extremely sensitive to high temperatures [49]. In boars, the effect of season on the initiation of spermatogenesis was recently investigated, and a positive influence of the climatic variables on sperm concentration was highlighted [50]. Such influence on peccaries' sperm concentration, however, was not evidenced. In general, there is limited studies that relate different meteorological factors to spermatogenesis in mammals, and these studies mainly focus on the effect of temperature alone [49,51,52]. It is suggested that heat stress results in caspase-9 proteolysis and redistribution of the pro-apoptotic protein Bax from the cytosol to perinuclear regions [53] thus inducing sperm production damage. In amphibians, the association of air temperature and rainfall regimes may influence the success of the spermatogenesis process [54], as observed for the collared peccary in which relative humidity during rainy periods improved the sperm motility metrics and air temperature negatively influenced it. In this sense, the unpublished information presented here will allow monitoring of the sperm production of the species during the future climate changes estimated for the Caatinga biome [11].

In conclusion, no seasonal changes on reproductive metrics of collared peccaries raised in the Caatinga biome could be detected overt the 18-month period; however, meteorological conditions of this semi-arid region have a short to medium term impact on the semen production of the species. Therefore, it is expected that long term environmental changes in a biome like the Caatinga will influence the reproductive physiology of species leaving in that habitat. The information generated here can be applied to the management of collared peccaries within the said biome, either in captivity or in free life, to develop strategies for its reproduction and conservation.

Acknowledgments

The authors thank the Brazilian Council of Scientific Development (CNPq) (Process n° 407.302/2013-1) and the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES, Brazil) (Financial Code 001) for financial support. We also thank the Center for Multiplication of Wild Animals (CEMAS/UFERSA) for providing the animals used for this study. Alexandre R. Silva and Felipe Z. Brandão received a grant from the CNPq. Ana Liza P. Souza, Andreia M. Silva and João B.F. Souza-Jr. received a grant from CAPES.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.theriogenology.2018.12.032.

References

[1] Gongora J, Reyna-Hurtado R, Beck H, Taber A, Altrichter M, Keuroghlian A. Pecari tajacu. The IUCN Red List of Threatened Species 2011: e.T41777A10562361. https://doi.org/10.2305/IUCN.UK.2011-2.RLTS.T41777A10562361 Cited 15 June 2018. [accessed 13 July 2018].

[2] Desbiez ALJ, Keuroghlian A, Beisiegel BM, Medici EP, Andressa G, Antônio RM, et al. Evaluation of the risk of extinction of the collared peccary (*Pecari tajacu* – Linnaeus, 1758) in Brazil. Biodiversidade Bras 2012;2:74–83.

[3] Rankin DJ, Koko H. Do males matter? The role of males in population dynamics. Oikos 2007;116:335–48. https://doi.org/10.1111/j.0030-1299.2007.15451.x.

[4] Bellatoni E. Habitat use by mule deer and collared peccaries in an urban environment. Tucson AZ: University of Arizona; 1991.

[5] Hellgren EC, Lochmiller RL, Amoss Jr MS, Seager SWJ, Magyar SJ, Coscarelli KP, et al. Seasonal variation in serum testosterone, testicular measurements and semen characteristics in the collared peccary (*Pecari tajacu*). J Reprod Fertil 1989;85:677–86.

[6] Kahwage PR, Garcia AR, Guimarães DAA, Ohashi OM, Luz-Ramos RS, Dias HLT, et al. Biometria testicular, eletroejaculação e características seminais de caititus, *Tayassu tajacu* Linnaeus, 1758 (Mammalia, Artiodactyla, Tayassuidae) mantidos em cativeiro na Amazônia Oriental. Acta Amazonica 2010;40:771–8.

[7] Costa DS, Henry M, Paula TAR. Spermatogenesis in the collared peccary (*Tayassu tajacu*). Arq Bras Med Vet Zootec 2004;56:46–51. https://doi.org/10.1590/S0102-09352004000100008.

[8] Peixoto GCX, Silva MA, Castelo TS, Silva AM, Bezerra JA, Souza AL, et al. Individual variation related to testicular biometry and sêmen characteristics in collared peccaries (*Tayassu Tajacu* Linnaeus, 1758). Anim Reprod Sci 2012;134:191–6. https://doi.org/10.1016/j.anireprosci.2012.08.026.

[9] Thompson LG. Climate change: the evidence and our options. Behav Anal 2010;33:153–70.

[10] Ogutu JO, Owen-Smith N, Piepho H-P, Dublin HT. How rainfall variation influences reproductive patterns of African Savanna ungulates in an equatorial region where photoperiod variation is absent. PLoS One 2015;10(8), e0133744. https://doi.org/10.1371/journal.pone.0133744.

[11] Painel Brasileiro de Mudanças Climáticas – PBMC. Contribuição do Grupo de Trabalho 1 ao Primeiro Relatório de Avaliação Nacional do Painel Brasileiro de Mudanças Climáticas [Contribution of Working Group 1 to the First National Assessment Report of the Brazilian Panel on Climate Change] [Cited 8 Jul 2018]. Rio de Janeiro, Brasil: Sumário Executivo GT1. PBMC; 2013. Available from:, http://www.pbmc.coppe.ufrj.br/pt/noticias/82-destaque.

[12] Carvalho CAC, Silva EO, Bezerra MA. Impact of climate changes in plants, fruits and grains. Revista Caatinga 2014;27(1):205–12.

[13] Maia MS, Medeiros IM, Lima CAC. Reproductive characteristics of rams in the Northeast Brazil: semen parameters. Rev Bras Reprod Anim 2011;35:175–9.

[14] Van Tilburg MF, Salles MGF, Silva MM, Moreira RA, Moreno FB, Monteiro-Moreira AC, et al. Semen variables and sperm membrane protein profile of Saanen bucks (*Capra hircus*) in dry and rainy seasons of the northeastern Brazil (3°S). Int J Biometeorol 2015;59:561–73. https://doi.org/10.1007/s00484-014-0869-6.

[15] Aubréville A. Étude écologique des principales formations végétales du Brésil, et contribuition à la connaissance des forêts de l' Amazonie. Paris: Comtre Techique Forestier Tropical; 1961.

[16] Silva RG. Biofísica Ambiental: Os animais e seu Ambiente. Jaboticabal: FUNEP/FAPESP; 2008.

[17] Silva RG, Guilhermino MM, Morais DAEF. Thermal radiation absorbed by dairy cows in pasture. Int J Biometeorol 2010;54:5–11. https://doi.org/10.1007/s00484-009-0244-1.

[18] Souza ALP, Castelo TS, Queiroz JPAF, Barros IO, Paula VV. Evaluation of anesthetic protocol for the collection of semen from captive collared peccaries (*Tayassu tajacu)* by eletroejaculation. Anim Reprod Sci 2009;116:370–5. https://doi.org/10.1016/j.anireprosci.2009.02.017.

[19] Lima LR, Souza Jr JBF, Ricarte ARF, Santos EAA, Costa LLM, Oliveira MF, Barbosa Filho JAD. Application of infrared thermography to determine the temperature patterns of the scrotum in collared peccary (*Tayassu Tajacu* Linnaeus, 1758). J Anim Behav Biometeorol 2014;2:85–9.

[20] Lambert B. The frequency of mumps and of mumps orchitis and the consequences for sexuality and fertility. Acta Genet Stat Med 1951;2:1–166.

[21] Maia KM, Peixoto GCX, Campos LB, Bezerra JAB, Aracelly RFR, Moreira N, et al. Estrus cycle monitoring of captive collared peccaries (*Pecari tajacu*) in semi-arid conditions. Pesqui Vet Bras 2014;34:1115–20. https://doi.org/10.1590/S0100-736X2014001100014.

[22] Aquino-Cortez A, Pinheiro BQ, Silva HVR, Lima DBC, Silva T, Souza MB, et al. Serum testosterone, sperm quality, cytological, physicochemical and biochemical characteristics of the prostatic fraction of dogs with prostatomegaly. Reprod Domest Anim 2017;52:998–1003. https://doi.org/10.1111/rda.13009.

[23] Castelo TS, Bezerra FSB, Souza ALP, Moreira MAP, Paula VV, Oliveira MF, et al. Influence of the thawing rate on the cryopreservation of semen from collared peccaries (*Tayassu tajacu*) using Tris-based extenders. Theriogenology 2010;74:1060–5. https://doi.org/10.1016/j.theriogenology.2010.05.002. https://doi.org/10.1590/S0100-736X2013000700014.

[24] Sousa PC, Santos EAA, Souza ALP, Lima GL, Barros FFPC, Oliveira MF, et al. Sperm morphological and morphometric evaluation in captive collared peccaries (*Pecari tajacu*). Pesqui Vet Bras 2013;33:924–30.

[25] Souza ALP, Lima GL, Peixoto GCX, Castelo TS, Oliveira MGC, Paula VV, et al. Sperm characteristics following freezing in extenders supplemented with whole egg yolk and different concentrations of low-density lipoproteins in the collared peccary (*Pecari tajacu*). Reprod Biol 2015;15:223–8.

[26] SAS. Statistical Analysis System User' Guide Statistics. Cary: SAS Institute Inc.; 1999.

[27] Coelho LA, Sasa A, Bicudo SD, Balieiro JCC. Plasma concentrations of testosterone, triiodothyronine (T3), and thyroxine (T4) in bucks submitted to heat stress. Arq Bras Med Vet Zootec 2008;60(6):1338–45. https://doi.org/10.1590/S0102-09352008000600007.

[28] Kunavongkrit A, Suriyasomboon A, Lundeheim N, Heard TW, Einarsson S. Management and sperm production of boars under differing environmental conditions. Theriogenology 2005;63:657–67.

[29] Aguiar GV, Van Tilburg MF, Catunda AGV, Celes CKS, Lima ICS, Campos ACN, et al. Sperm parameters and biochemical components of goat seminal plasma in the rainy and dry seasons in the Brazilian Northeast: the season's influence on the cooling of semen. Arq Bras Med Vet Zootec 2013;65:6–12. https://doi.org/10.1590/S0102-09352013000100002.

[30] Marai IFM, El-Darawany AA, Abou-Fandoud EI, Abdel-Hafez MAM. Reproductive traits and the physiological background of the seasonal variations in Egyptian Suffolk ewes under the conditions of Egypt. Ann Arid Zone 2004;42:1–9.

[31] Zervanos SM. Seasonal effects of temperature on the respiratory metabolism of the collared peccary (*Tayassu tajacu*). Comp Biochem Physiol Physiol 1975;50:365–71. https://doi.org/10.1016/0300-9629(75)90027-4.

[32] Pachêco NA. Boletim agrometeorológico de 2009 para Belém, PA [Agrometeorological Bulletin of 2009 for Belém, PA]. Belém, PA: Embrapa Amazônia Oriental; 2011. https://ainfo.cnptia.embrapa.br/digital/bitstream/item/44214/1/Doc-371.pdf. [Accessed 13 July 2018].

[33] Araújo EL, Castro CC, Albuquerque UP. Dynamics of Brazilian Caatinga – A review concerning the plants, environment and people. Funct Ecosyst Communities 2007;1:15–28.

[34] Malama E, Zeron Y, Janett F, Siuda M, Roth Z, Bollwein H. Use of computer-assisted sperm analysis and flow cytometry to detect seasonal variations of bovine semen quality. Theriogenology 2017;87:79–90. https://doi.org/10.1016/j.theriogenology.2016.08.002.

[35] Kunavongkrit A, Prateep P. Influence of ambient temperature on reproductive efficiency in pigs: (1) boar semen quality. Pig J 1995;35:43–7.

[36] McNitt J, First NL. Effects of 72-hour heat stress on semen quality in boars. Int J Biometeorol 1970;14:373–80. https://doi.org/10.1007/BF01462914.

[37] Malmgren L. Experimentally induced testicular alteration in boars [thesis]. The Swedish University of Agricultural Science; 1988.

[38] Louis GF, Lewis AJ, Weldon WC, Miller PS, Kittok RJ, Stroup WW. The effect of protein intake on boar libido, semen characteristics and plasma hormone concentration. J Anim Sci 1994;72:2038–50.

[39] Menad R, Smaïa S, Bonnetd X, Gernigon-Spychalowicza T, Moudiloue E, Khammarb F, Exbrayate JM. Seasonal variations of aromatase and estrogen receptors expression in the testis of free-ranging sand rats. Acta Histochem 2017;119:382–91. https://doi.org/10.1016/j.acthis.2017.03.008.

[40] Gong Y, Guo H, Zhang Z, Zhou H, Zhao R, He B. Heat stress reduces sperm motility via activation of glycogen synthase kinase-3α and inhibition of mitochondrial protein import. Front Physiol 2017;22:718. https://doi.org/10.3389/fphys.2017.00718.

[41] Biniová Z, Ducháček J, Stádník L, Doležalová M, Hájek R. Effects of climatic conditions on bovine semen characteristics. Acta Univ Agric Silvic Mendelianae Brunensis 2017;65:17–23. https://doi.org/10.11118/actaun201765010017.

[42] Krishnakumar S, Whiteside DP, Elkin B, Thundathil JC. A preliminary assessment of the effects of reproductive seasonality on seminal plasma characteristics in North American bison (*Bison bison*). J Zoo Wildl Med 2017;48(1):91–101. https://doi.org/10.1638/2016-0041.1.

[43] Huang SY, Kuo YH, Lee YP, Tsou HL, Lin EC, Ju CC, Lee WC. Association of heat shock protein 70 with semen quality in boars. Anim Reprod Sci 2000;63:

231–40.
[44] Santos EAA, Sousa PC, Martins JAM, Moreira RA, Monteiro-Moreira AC, Oliveira MF, Moura AAA, Silva AR. Protein profile of the seminal plasma of collared peccaries (*Pecari tajacu*). Reproduction 2014;147:753–64. https://doi.org/10.1530/REP-13-0220.
[45] Santos EAA, Sousa PC, Silva AM, Paiva ALC, Lago AEA, Paula VV, Pereira AF, Moura AAAN, Silva AR. Superoxide dismutase and catalase activity in collared peccary (*Pecari tajacu*) seminal plasma and their relation to sperm quality. Semina 2018;39:787–96. https://doi.org/10.5433/1679-0359.2018v39n2p787.
[46] Meyerhoeffer DC, Coleman SW. The influence of radiant heat load and temperature stress on bull growth and fertility. Ani Sci Res Rep 1978;1:233–6.
[47] Silva TPD, Torreão JNC, Marques CAT, Araújo MJ, Bezerra LR, Dhanasekaran DK, Sejian V. Effect of multiple stress factors (thermal, nutritional and pregnancy type) an adaptive capability of native ewes under semi-arid environment. J Therm Biol 2016;59:39–46. https://doi.org/10.1016/j.jtherbio.2016.05.001.
[48] Macchi E, Starvaggi-Cucuzza A, Badino P, Odore RF, Re F, Bevilacqua L, et al. Seasonality of reproduction in wild boar (*Sus scrofa*) assessed by fecal and plasmatic steroids. Theriogenology 2010;73:1230–7. https://doi.org/10.1016/j.theriogenology.2009.12.002.
[49] Cameron RDA, Blackshaw AW. The effect of elevated ambient temperature on spermatogenesis in the boar. J Reprod Fertil 1980;59:173–9.
[50] Chinchilla-Vargas J, Kerns K, Rotschild MF. Lunar and climatic effects on boar ejaculate traits. Anim Reprod Sci 2018;193:117–25. https://doi.org/10.1016/j.anireprosci.2018.04.006.
[51] Yaeram J, Setchell BP, Maddocks S. Effect of heat stress on the fertility of male mice in vivo and in vitro. Reprod Fertil Dev 2006;18:647–53.
[52] Shadmehr S, Tabatabaei SFR, Hosseinifar S, Tabandeh MR, Amiri A. Attenuation of heat stress-induced spermatogenesis complications by betaine in mice. Theriogenology 2018;106:117–26. https://doi.org/10.1016/j.theriogenology.2017.
[53] Vera Y, Rodriguez S, Castanares M, Lue Y, Atienza V, Wang C, et al. Functional role of caspases in heat-inducedtesticular germ cell apoptosis. Biol Reprod 2005;72:516–22.
[54] Chaves MF, Moura GJB, Tenório FCMA, Batista JS, Clovis JCLN, Valeria WT, et al. Influence of rainfall and temperature on the spermatogenesis of *Leptodactylus macrosternum* (Anura: Leptodactylidae). Zoologia 2017;34:1984–4689. https://doi.org/10.3897/zoologia.34.e20782.