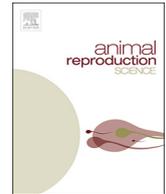




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# Composition of collared peccary seminal plasma and sperm motility kinetics in semen obtained during dry and rainy periods in a semiarid biome

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## ABSTRACT

The aim of this study was to evaluate environmental effects in a semiarid region on collared peccary seminal plasma content and sperm motility. Ejaculates from 12 mature males were obtained during the peak of rainy and dry periods of the Caatinga biome. Samples were evaluated for semen volume, pH, as well as sperm concentration, morphology, osmotic response, membrane integrity, chromatin condensation, and kinetic motility. Seminal plasma was evaluated for ions and organic compounds. The values for chloride, iron, magnesium, phosphorus, citric acid, cholesterol, triglycerides, total proteins, albumin, and fructosamine were similar during the dry and rainy periods; however, concentrations of fructose (849.2 mg/dL compared with 119.4 mg/dL) and calcium (32.3 mg/dL compared with 15.6 mg/dL) were greater during the rainy compared with dry period ( $P < 0.05$ ). There were correlations ( $P < 0.05$ ) among values for semen variables and biochemical contents, particularly between fructose and sperm velocity average pathway ( $r = 0.65$ ), velocity straight line ( $r = 0.78$ ), velocity curvilinear ( $r = 0.57$ ), amplitude lateral head ( $r = 0.62$ ), linearity ( $r = 0.41$ ), and subpopulation with a medium velocity ( $r = -0.75$ ). Furthermore, values for relative humidity were positively correlated with concentrations of fructose ( $r = 0.49$ ), while air temperature ( $r = -0.43$ ) and wind velocity values ( $r = 0.66$ ) were negatively affected by concentration of fructose ( $P < 0.05$ ). There were novel results regarding collared peccary seminal plasma biochemistry indicating there are important correlations with values for semen variables that are affected by the environment in a semiarid climate.

## 1. Introduction

The collared peccary (*Pecari tajacu* Linnaeus, 1758), a wild ungulate native from the Americas, conducts an important ecological effect as a seed disperser and as prey for large carnivores (Desbiez et al., 2012). The global population has been classified as a species of least concern with regard to extinction; however, considering the continuous rates of habitat destruction and excessive poaching by

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humans, along with a decrease of biomes (Gongora et al., 2011), such as the Atlantic Forest for habitat, there is concern about sustaining the viability of the species (Desbiez et al., 2012).

To safeguard collared peccary germplasm, techniques for the cryopreservation of its semen have been developed (Castelo et al., 2010; Souza et al., 2016). Peccaries are usually considered a non-seasonal breeding species, therefore, females can produce offspring throughout the year (Mayor et al., 2010). It, however, was recently reported that even if the environmental parameters of a semiarid climate in terms of air temperature and relative humidity did not affect the quality of collared peccary fresh sperm, these parameters affect the freezability of the sperm in terms of a reduction of sperm motility and membrane integrity in samples collected and cryopreserved during the dry period of the Caatinga (Maia et al., 2018). In this biome, which is characterized by a semiarid climate with a short rainy period (approximately 3 months) and a long dry period (approximately 9 months), there needs to be regulation of the physiological functions of collared peccaries to adapt to the thermal stress (Zervanos, 1975).

Seminal plasma is a fluid resulting from the combination of secretions originating from the testes, epididymis, and accessory glands such as the vesicular, prostatic, and bulbourethral glands as described for the collared peccary (García et al., 2015). In this species, information regarding seminal plasma composition is limited to two studies. In the first study, there was identification of 23 different proteins in seminal plasma, with porcine spermadhesin PSP-1 (20.9%), clusterine (19.8%), and bodhesin-2 (10.2%) being the most abundant (Santos et al., 2014). There has also been identification of antioxidant activity of the enzyme superoxide dismutase during the dry season of the Caatinga but there was not any correlation between the values for activities of this enzyme and those for sperm variables (Santos et al., 2018).

In addition to proteins and antioxidant enzymes, mammalian seminal plasma is typically rich in many other constituents. In the domestic pig, the species most closely related to the peccaries, various inorganic compounds, such as calcium and magnesium, as well as organic contents such as proteins and lipids, were identified (Zaja et al., 2016), with some of these compounds being affected by meteorological conditions (Strzezek et al., 2000; Argenti et al., 2018). In the present study, the aim was to characterize the biochemical profile of seminal plasma from collared peccaries located in semiarid conditions in the Caatinga biome. Furthermore, there was determination of the effect of the environmental period (dry or rainy) on the seminal plasma biochemical contents and an ascertaining of the correlations among such contents and the environmental variables.

## 2. Materials and methods

The ethics committee of the Universidade Federal Rural do Semi-Árido (UFERSA) approved the experimental protocols, as well as the animal care procedures used for conducting this study (n°. 23091.008820/2016-03). The study was authorized by the Chico Mendes Institute for Biodiversity (n°. 37329). All chemicals used were purchased from Sigma Chemical Co. (St. Louis, MO, USA), unless otherwise indicated.

### 2.1. Experimental conditions and animals

Sexually mature animals ( $n = 12$ ) were used, with a mean age of  $22.8 \pm 1.9$  months, weighing on average  $20.1 \pm 0.5$  kg at the commencement of the experiment. The animals belonged to the Centre for Wild Animals Multiplication (n°. 1478912), located in the city of Mossoró, RN, Brazil (latitude:  $5^\circ 10'S$ , longitude:  $37^\circ 10'W$ ; altitude: 16 m, typical semiarid climate). The collared peccaries were assigned to three groups of four animals, housed in an enclosure (20 m  $\times$  3 m) containing a covered area (3 m  $\times$  3 m) with there being a natural 12 h photoperiod during the experimental period. During both climatic periods, animals were fed isocaloric (3300 kcal/kg) and isoproteic (14% protein) diet consisting of corn (79.8%), soy bean meal (15.4%), wheat bran (1.45%), calcium (2.6%), and a vitamin (0.2%) and mineral premix (0.05%), supplemented with tropical fruits, such as melon. Water was provided *ad libitum*.

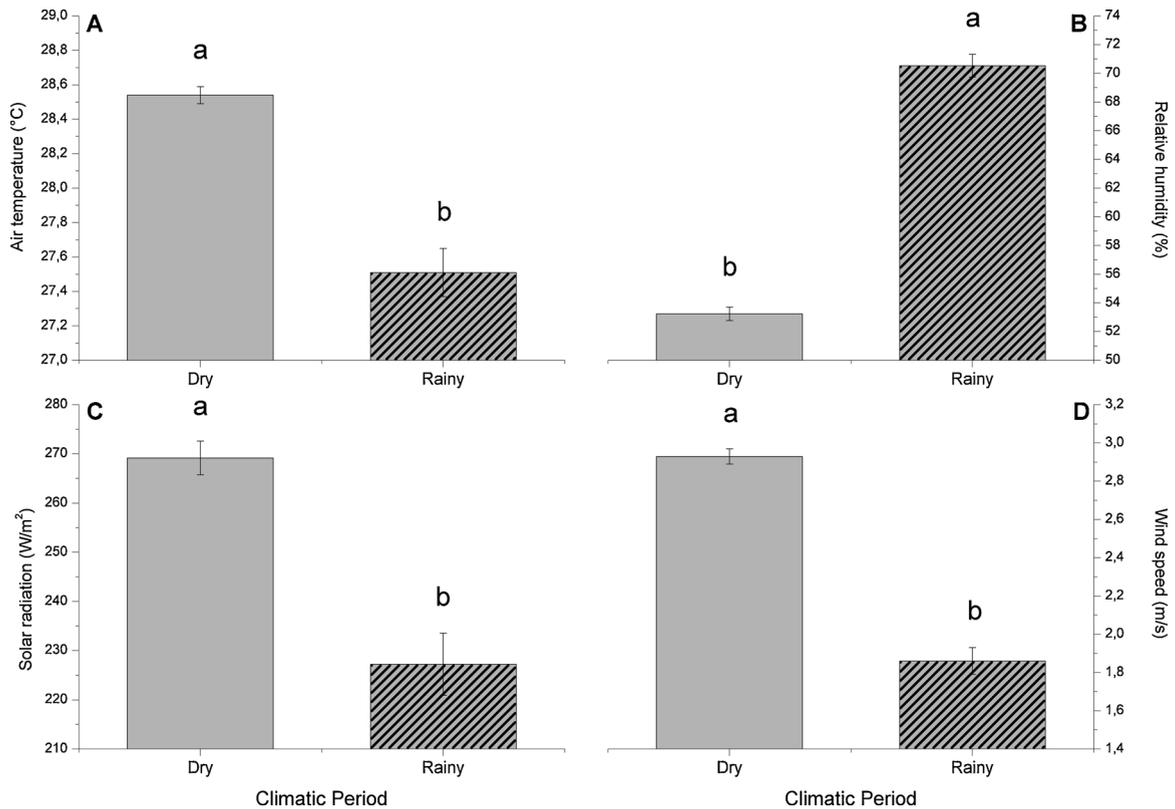
### 2.2. Environmental conditions

To characterize the peak of dry (September, October, and November 2016) and rainy (February, March, and April 2017) periods of the semiarid climate in the Caatinga, data were obtained regarding total rainfall (in mm) during each period from the automatic station of the National Institute of Meteorology (INMET), located in Mossoró, RN, Brazil. During the experiment, there were differences in the total rainfall between peaks of the dry (0.01 mm) and rainy (266.08 mm) periods ( $P < 0.05$ ).

Furthermore, there were differences in the average air temperature ( $T_a$ ,  $^\circ C$ ), relative humidity (RH, %), solar radiation ( $W/m^2$ ), and wind speed (m/s), as determined by the automatic weather station (Campbell Scientific Brasil, São Paulo, Brazil) located 50 m from the experimental site between the dry and rainy periods (Fig. 1).

### 2.3. Semen collection

For each climatic period, one ejaculate was obtained from each animal. For semen collection, the animals were fasted for 12 h and were initially restrained with a hand net, and were then anesthetized (Souza et al., 2009) using propofol (Propovan®, Cristália, Fortaleza, Brazil) in a bolus with administration occurring using an intravenous dose of 5 mg/kg ( $100.6 \pm 2.3$  mg/individual) into the lateral saphenous vein. Throughout the experimental procedure, the animals were submitted to fluid therapy (sterile saline solution 0.9%) and their rectal temperature, heart and respiratory rates were monitored (Souza et al., 2009). The animals were then placed in a lateral decubitus position and electroejaculated using procedures previously described for the species (Castelo et al.,



**Fig. 1.** Values for environmental variables (mean  $\pm$  SEM) related to air temperature ( $^{\circ}$ C), relative air humidity (%), solar radiation ( $W/m^2$ ), and wind speed (m/s) verified during peaks of the dry (September, October and November 2016) and rainy (February, March and April 2017) periods in a semiarid region of the Caatinga biome.

<sup>a,b</sup>Lowercase superscript letters indicate difference among periods ( $P < 0.05$ )

2010) with a portable device (Autojac<sup>®</sup>, Neovet, Campinas, SP, Brazil) connected to a 12 V source. The stimulatory cycle was comprised of ten stimuli at each voltage, starting from 5 V, followed by a voltage increase in steps of 1 V, up to 12 V. Each electrical stimulus lasted 3 s, with intermittent breaks of 2 s. The stimuli cycles were maintained for 10 min from the beginning of the procedure. The dimension of the electroejaculator probe was 15  $\times$  1.3 cm, and 12 cm of the probe was inserted into the animal rectum. The ejaculates were collected in plastic tubes and there was an immediate evaluation of the semen.

#### 2.4. Semen evaluation

Semen volume was measured using micropipettes. The pH was determined using pH-indicator strips (Neutralit<sup>®</sup>, Merck, Bucharest, Romania). Sperm concentration (in millions of sperm/mL) was determined in a Neubauer counting chamber (Souza et al., 2009). For the sperm morphology analysis, smears stained in Bengal Rose were evaluated using light microscopy (1000  $\times$ ), with assessments of 200 cells per slide occurring, in five randomly selected fields. There was verification of percentages of normal and abnormal sperm in each smear (Souza et al., 2016).

Sperm membrane functionality was determined by evaluating the osmotic response of the sperm to the hypo-osmotic test using distilled water (0 mOsm/L). A total of 200 cells were examined and those with swollen and coiled tails were considered to have a functional membrane (%; Souza et al., 2016).

The chromatin integrity was evaluated using a smear stained with toluidine blue dye. Briefly, sperm aliquots (5 mL) were used for placement of smears on microscopic slides. The smears were fixed in 3:1 ethanol:acetic acid for 1 min and then in 70% ethanol for 3 min; smears were then immersed in HCl 4 M solution for 25 min. Samples were subsequently washed in distilled water and dried at room temperature. Smears were submerged in the toluidine blue dye [0.025% dye in McIlvaine buffer (sodium citrate: phosphate pH  $\frac{1}{4}$  4.0)], with a coverslip placed over the smear and immediately evaluated using bright field microscopy with a 1000  $\times$  magnification. A total of 500 sperm were evaluated per smear in duplicate. Cells slightly stained blue were classified as normal (negative) and those stained with a violet to dark blue color were considered to have altered chromatin (positive; Beletti and Mello, 2004).

Plasma membrane integrity was assessed using a fluorescent solution containing fluorophores 6-carboxyfluorescein diacetate (0.46 mg C-FDA/1 mL dimethylsulfoxide) and propidium iodide (0.5 mg PI/1 mL 0.9% saline solution). One aliquot of 10  $\mu$ L semen was extended in 40  $\mu$ L fluorescent solution. After 10 min, slides with the stained samples were evaluated using epifluorescence microscopy (Episcopic Fluorescent attachment "EFA" Halogen Lamp Set, Leica, Kista, Sweden). For each sample stained with CFDA/

PI, 200 sperm were counted and classified as having or not having an intact plasmalemma (based on color). Cell membranes stained green (CFDA) were considered intact, whereas those stained (PI) or partially stained red were considered non-intact (Souza et al., 2016).

Values for sperm motility variables were analyzed using computer assisted sperm assessment (IVOS 7.4 G; Hamilton-Thorne Research, Beverly, MA, USA), utilizing settings previously validated for collared peccaries (Souza et al., 2016), at a temperature of 37 °C; 60 frames/s; minimum contrast, 45; straightness threshold, 30%; low-velocity average pathway (VAP) cutoff, 10 m/s; and medium VAP cutoff, 30 m/s. Five independent and non-consecutive microscopic fields were randomly selected and evaluated using scanning procedures. The values for the following endpoints were analyzed: number of counted cells, total motility (%), VAP (mm/s), velocity straight line (mm/s), curvilinear velocity (mm/s), amplitude of lateral head (mm), beat cross frequency (Hz), straightness (STR; %), and linearity (LIN; %). When there was a low VAP (LVV) and medium VAP (MVV) cutoff, the overall sperm population was subdivided into four categories: rapid, with VAP > MVV; medium, with LVV < VAP < MVV; slow, with VAP < LVV; and static when there was no cell motility detected. For a reliable evaluation of the sperm motility patterns, the Edit Tracks Option of the IVOS 7.4 G system was used to exclude the debris derived from the extenders. There was a further dilution in salt solution (1:1) only if necessary (Souza et al., 2016).

### 2.5. Seminal plasma separation and analysis

After the initial analysis, the seminal plasma was separated from cells and other debris using centrifugation at 700 × g for 10 min at room temperature (~27 °C). The supernatant was transferred to microtubes (1.5 mL) and stored at -20 °C until analysis. The samples were analyzed by evaluation of the absorbance determined using photolorimetry utilizing spectrophotometry (SP-22®, Biospectro, Curitiba, PR, Brazil; Aquino-Cortez et al., 2017). The concentrations of ions such as calcium, chloride, iron, magnesium, and phosphorus; and organic compounds such as cholesterol, triglycerides, total protein, albumin, and fructosamine, were estimated using commercial biological kits (Labtest Diagnóstica SA, Lagoa Santa, MG, Brazil), while the concentrations of fructose and citric acid were estimated using the Espermoteste® kit (InVitro Diagnostic S/A, Itabira-MG, Brazil).

### 2.6. Statistical analysis

Data were expressed as the mean and standard error and evaluated using the Statistical Analysis Software version 8.0 (SAS Institute Inc., Cary, NC, USA). Values were assessed for normality using the Shapiro-Wilk test and for homocedasticity using the Levene test. When data did not meet the parametric assumptions, the data were transformed logarithmically or with use of arc sine transformation. All variables were subjected to repeated-measures ANOVA to analyze the data using PROC MIXED of SAS, where the climatic period and the animals were considered as fixed and random effects, respectively.

When parametric assumptions were not met, even after data transformation, a nonparametric test was used. Thus, the Wilcoxon test was applied to evaluate the effect of the climatic period on pH, and concentrations of cholesterol, total protein, magnesium, chloride, albumin, phosphorus, iron, and fructose.

To determine correlations among values for biochemical variables and semen metrics or environmental characteristics, the Spearman's correlation test was applied using the PROC CORR of SAS. For all analyses,  $P < 0.05$  was considered to indicate a significant difference.

## 3. Results

### 3.1. Semen variable evaluation

Semen samples were obtained from all the experimental peccaries during both climatic periods. The ejaculates were of a milky-white color. The mean values for semen variables related to volume, pH, sperm concentration, normal morphology, osmotic response, membrane integrity, and chromatin condensation did not differ between periods (Table 1). Regarding the means for the kinetic variables (Table 2), velocity average pathway (VAP), velocity straight line (VSL), velocity curvilinear (VCL), and amplitude lateral

**Table 1**

Mean values ( ± SEM) for collared peccary ( $n = 12$ ) semen collected by electroejaculation during peaks of dry and rainy periods in a semiarid biome.

Semen variables	Dry period	Rainy period
Volume (mL)	1.9 ± 0.4	1.9 ± 0.5
pH (0 – 14)	7.9 ± 0.3	8.5 ± 0.3
Concentration ( × 10 <sup>6</sup> sperm/mL)	245.8 ± 50.2	295.8 ± 60.6
Normal morphology (%)	80.4 ± 4.0	72.1 ± 7.1
Osmotic response (%)	75.3 ± 5.5	80.3 ± 2.9
Membrane integrity (%)	84.9 ± 2.3	81.8 ± 1.3
Chromatin condensation (%)	99.6 ± 0.1	99.1 ± 0.3

<sup>a,b</sup>Superscript lowercase letters indicate differences in values in different columns ( $P < 0.05$ ).

**Table 2**

Mean values ( $\pm$  SEM) for collared peccary ( $n = 12$ ) sperm kinetic motility variables in ejaculates obtained by electroejaculation during peaks of dry and rainy periods in a semiarid biome.

Sperm kinetic motility variables	Dry period	Rainy period
Total motility (%)	64.3 $\pm$ 6.4	58.5 $\pm$ 9.1
Progressive motility (%)	24.4 $\pm$ 4.6	29.1 $\pm$ 7.0
Velocity average pathway (mm/s)	38.0 $\pm$ 1.3 <sup>b</sup>	49.5 $\pm$ 2.2 <sup>a</sup>
Velocity straight line (mm/s)	21.3 $\pm$ 0.8 <sup>b</sup>	30.1 $\pm$ 1.3 <sup>a</sup>
Velocity curvilinear (mm/s)	80.0 $\pm$ 3.1 <sup>b</sup>	102.4 $\pm$ 4.1 <sup>a</sup>
Amplitude lateral head (mm)	5.7 $\pm$ 0.1 <sup>b</sup>	6.7 $\pm$ 0.2 <sup>a</sup>
Beat cross frequency (Hz)	30.3 $\pm$ 1.0	29.8 $\pm$ 1.5
Straightness (%)	54.6 $\pm$ 2.0	57.4 $\pm$ 1.7
Linearity (%)	27.2 $\pm$ 1.5	29.1 $\pm$ 1.5
Sperm subpopulations		
Rapid (%)	42.2 $\pm$ 5.5	43.2 $\pm$ 8.8
Medium (%)	22.1 $\pm$ 3.1 <sup>a</sup>	10.5 $\pm$ 1.3 <sup>b</sup>
Slow (%)	7.4 $\pm$ 1.2	9.3 $\pm$ 4.4
Static (%)	28.4 $\pm$ 6.0	37.0 $\pm$ 9.0

<sup>a,b</sup>Superscript lowercase letters indicate differences in values in different columns ( $P < 0.05$ ).

head (ALH) values were greater during the rainy than dry season ( $P < 0.05$ ); however, there was a larger sperm subpopulation with medium velocity during the dry, as compared with the wet season ( $P < 0.05$ ).

### 3.2. Biochemical contents

For the biochemical contents of the seminal plasma (Table 3), all ejaculates contained all the compounds evaluated, with the exception of fructosamine, which was detected in only six individuals during the dry period and four individuals during the rainy period. Furthermore, the seminal plasma of two individuals did not contain iron in any of the ejaculates collected from these individuals, regardless of the climatic period. In addition, there was verification that there was a greater concentration of fructose and calcium during the rainy compared with the dry period ( $P < 0.05$ ).

### 3.3. Correlations among values of semen variables and biochemical contents

There were correlations ( $P < 0.05$ ) among values of semen and some biochemical variables of the collared peccary seminal plasma (Table 4). Among the compounds, there were greater positive correlations of fructose concentrations with various sperm metrics such as VAP ( $r = 0.65$ ), VSL ( $r = 0.78$ ), VCL ( $r = 0.57$ ), ALH ( $r = 0.62$ ), and linearity ( $r = 0.41$ ), and there was also a negative correlation of fructose concentration with the sperm subpopulation having the medium velocity ( $r = -0.75$ ).

### 3.4. Effect of environmental variables on semen biochemical contents

There were negative correlations among fructose concentrations and values for ambient temperature ( $r = -0.43$ ;  $P = 0.04$ ) and

**Table 3**

Mean values, medians and range for the biochemical contents of collared peccary ( $n = 12$ ) seminal plasma in ejaculates obtained during peaks of dry and rainy periods in a semiarid biome.

Biochemical components	Dry period			Rainy period		
	Mean	Median	Range	Mean	Median	Range
Ions						
Calcium (mg/dL)	15.6 <sup>b</sup>	11.0	0.3 – 49.4	32.3 <sup>a</sup>	29.3	4.5 – 57.7
Chloride (mEq/L)	315.6	139.2	16.5 – 1218.2	271.3	234.8	59.8 – 535.4
Iron ( $\mu$ g/dL)	210.9	205.4	0 – 625.6	423.2	267.2	0 – 1470
Magnesium (mg/dL)	5.7	6.1	0.6 – 7.0	5.9	5.95	4.6 – 7.0
Phosphorus (mg/dL)	12.3	6.8	1.6 – 47.4	72.1	4.6	1.7 – 342.2
Organic compounds						
Citric acid (mg/dL)	140.8	132.3	2.1 – 358.3	169.7	158.9	95.5 – 300
Fructose (mg/dL)	119.4 <sup>b</sup>	46.9	3.3 – 450	849.2 <sup>a</sup>	827.8	150 – 1800
Cholesterol (mg/dL)	152.3	132.8	35.9 – 328	332.0	323.0	7.9 – 867.3
Triglycerides (mg/dL)	296.1	127.2	5.0 – 918.8	306.8	299.2	9.2 – 676.7
Total proteins (g/dL)	8.0	6.8	0.3 – 23.38	7.0	5.1	0.8 – 18.8
Albumin (g/dL)	9.4	6.9	0.4 – 34.2	3.0	2.4	0.6 – 7.4
Fructosamine (mmol/L)	403.6	377.5	0 – 780.1	198.4	125.6	0 – 798.3

<sup>a,b</sup>Superscript lowercase letters indicate differences in values in different columns ( $P < 0.05$ ).

**Table 4**Correlations (*r*) between the biochemical components of the seminal plasma and the semen metrics in collared peccaries.

Semen variables	Organic compounds			
	Fructose	Triglycerides	Total Proteins	Frutosamine
Membrane integrity	n.s	n.s	0.48 <i>P</i> = 0.022	n.s
Normal morphology	n.s	n.s	n.s	0.61 <i>P</i> = 0.021
Sperm concentration	n.s	0.51 <i>P</i> = 0.043	n.s	n.s
Velocity average pathway	0.65 <i>P</i> < 0.001	n.s	n.s	n.s
Velocity Straight Line	0.78 <i>P</i> < 0.001	n.s	n.s	n.s
Velocity curvilinear	0.57 <i>P</i> < 0.01	n.s	n.s	n.s
Amplitude lateral head	0.62 <i>P</i> < 0.01	n.s	n.s	n.s
Linearity	0.41 <i>P</i> = 0.049	n.s	n.s	n.s
Medium sperm subpopulation	-0.75 <i>P</i> < 0.001	n.s	n.s	n.s

n.s.- non significant.

wind speed ( $r = -0.66$ ;  $P > 0.01$ ), but fructose concentration was positively correlated with values of relative humidity ( $r = 0.49$ ;  $P = 0.02$ ). In addition, values for solar radiation was positively correlated to concentrations of citric acid ( $r = 0.46$ ;  $P = 0.04$ ) but were negatively correlated with chloride concentrations ( $r = -0.42$ ;  $P = 0.04$ ).

#### 4. Discussion

With consideration of the great importance of semen chemical compounds to sperm viability, in the present study there were concentrations of inorganic and organic biochemical compounds in collared peccary seminal plasma similar to those previously reported for other mammals (Mann, 1954). Among the inorganic compounds, important ions such as calcium, magnesium, chloride, iron, and phosphorus were identified in collared peccary seminal plasma.

At physiological concentrations, calcium regulates intracellular ATP, and thus contributes to sperm motility (Baker et al., 2004) while magnesium functions in more than 300 enzymatic reactions related to sperm energy metabolism (Wong et al., 2001), also involved in regulation of sperm motility as a result of induction of adenylcyclase activity (Lapointe et al., 1996). Regarding the proportion of calcium (15.6 mg/dL) and magnesium (5.7 mg/dL) concentrations in peccary seminal plasma, it is important to highlight that it is inverse of what occurs in pigs, which have relatively lesser calcium (3.2 mg/dL) compared to magnesium (37.8 mg/dL) concentrations (Pipan et al., 2017). In this regard, it can be inferred that the seminal plasma of the collared peccary is similar to that of other domestic species such as the bull, ram, and stallion, in which there are greater calcium relative to magnesium concentrations in seminal plasma (Mann, 1954). This could be one of the reasons why semen technologies, previously developed for the domestic swine, such as the use of the Beltsville extender (Castelo et al., 2010) or small amounts of low density lipoproteins (Souza et al., 2015), typically are not effective when used for storing of collared peccary sperm, while those protocols adapted from ruminants are effective for peccary sperm storage (Castelo et al., 2010).

Regarding the other ions identified in the seminal plasma of collared peccaries, chloride, for which concentrations were negatively correlated with values for solar radiation has been reported to regulate sperm membrane potential (Catunda et al., 2009), while iron functions in the transport of electrons and oxygen, and is also essential for DNA synthesis (Hermes-Lima, 2004). Furthermore, phosphorus is known to have various cellular actions related to cell signaling and sperm energy metabolism as a result of enzyme activity regulation (Abdel-Rahman et al., 2000).

Concerning the organic compounds, citric acid functions in the maintenance of the osmotic equilibrium and affects sperm motility (Mann, 1954). Furthermore, fructose is a carbohydrate that is used to produce ATP, which is also an important energy source for sperm motility (Williams and Ford, 2001). This may be why the fructose concentration in the present study was positively correlated with some kinetic patterns of the peccary sperm movement, such as VAP, VSL, VCL, ALH and linearity. Although mammalian sperm can utilize most monosaccharides such as glucose and fructose, the specific manner of metabolism of these compounds varies among species (Rodriguez-Gil and Bonet, 2016). In collared peccaries, results in the study in which there was the first attempt of semen cryopreservation indicated the sperm had the capacity to metabolize both fructose and glucose contained in the extender (Castelo et al., 2010); however, the importance of presence of glucose in collared peccary seminal plasma remains to be ascertained.

Triglycerides of seminal plasma affect sperm motility and viability by being an energetic source (Juyena and Stelletta, 2012). In domestic swine, triglyceride concentration in the seminal plasma is positively correlated with the sperm concentration of sperm in ejaculates (Zaja et al., 2016) as occurred in peccaries in the present study. Peccary seminal plasma also contains cholesterol, which is

the main esterol present in the sperm membrane, contributing to its fluidity (Yeagle, 1985), and functioning as a protective agent against the thermal shock as a result of changes in environmental temperature (Sofikitis and Miyagawa, 1991).

Various proteins, including albumin (Santos et al., 2014), have been previously detected in collared peccary seminal plasma similar to observations in the present study. Some proteins are involved in the protective mechanisms of the sperm membrane (Santos et al., 2014), with the content of these proteins being correlated with protein concentrations in collared peccary seminal plasma and sperm membrane integrity. In addition, fructosamine is a glycosylated protein that is not commonly investigated in the seminal plasma of animals, but in humans, when there are relatively greater concentrations of this protein, the presence of fructosamine is indicative of infertility (Tomaszewski et al., 1992). In collared peccaries, fructosamine concentrations were positively correlated with values for normal sperm morphology; however, the effect of this biochemical compound on peccary reproductive performance remains to be elucidated, mainly because it was not identified in the ejaculates of all the individuals in the present study.

Climatic changes have a major effect on the reproductive physiology of animals (Qu et al., 2016). In collared peccary seminal plasma, there were relatively greater fructose and calcium concentrations during the rainy period, while for other compounds, there was no difference in values between the rainy and dry seasonal periods. There was also a greater fructose concentration in the seminal plasma in goats during the rainy, as compared with the dry, season in the same biome (Catunda et al., 2009). Because fructose metabolism results in the transfer of energy to ATP which is an energy source that is used for sperm movement (Williams and Ford, 2001), the larger amount available during the rainy season could contribute to the improved freezability of the collared peccary sperm obtained during this period compared to that obtained during the dry season, as reported by Maia et al. (2018). Seasonal alterations in the seminal plasma calcium concentration, however, were observed neither in goats raised in the Caatinga biome (Aguar et al., 2013) nor in pigs raised in Japan's climatic conditions (Murase et al., 2007). According to Strzerek et al. (2000), variations in boar seminal plasma contents could be attributed to alterations in the secretions produced by the seminal vesicle gland, the function of which is dependent on testosterone concentrations that can vary according to different meteorological conditions. In collared peccaries, seasonal variations in testosterone concentrations were reported to occur (Hellgren et al., 1989), which could potentially lead to alterations in seminal vesicle secretions and thus variations in seminal plasma contents.

In addition to variations in the biochemical compounds in the seminal plasma, there were increases in some kinetic patterns of sperm movement, which are correlated with fructose increases which were verified in the collared peccaries during the rainy period in the present study. Importantly, however, seasonal variations in these sperm kinetic patterns in fresh semen were not consistent when the patterns detected in the present study were compared with those reported by Maia et al. (2018). When conducting the previous study, the total recorded rainfall was only 73.2 mm during the rainy period (March-May 2016), while in the present study there was a total rainfall of 266.1 mm (February-April 2017) in the same biome. These inconsistencies indicate that the reduction by 40%–50% in rainfall and the predicted temperature increase of 3.5 °C that is predicted to occur at the end of the present century, as estimated for the Caatinga (Painel Brasileiro de Mudanças Climáticas PBMC, 2013), will likely impair collared peccary reproductive performance. Indeed, the positive correlations between environmental variables, such as the values for relative humidity and the fructose concentrations, highlights this seasonal effect on the biochemical composition of the seminal plasma, particularly during the warmer months, when temperatures exceed the maximal temperatures of the thermoneutral zone (Catunda et al., 2009).

There is an importance of the results in the present study for the monitoring of reproduction of peccaries, specifically as related to climatic changes estimated for the Caatinga, and mainly for individuals in their natural habitat that have the seasonal variation in food intake. Such information could also be used as a basis for the management and conservation of closely related species to the peccary that are vulnerable to extinction, such as the white-lipped peccary (*Tayassu pecari*) and Chacoan peccary (*Catagonus wagneri*). Based on the results from the present study, there was development of strategies for peccary conservation by scheduling the semen collections during the rainy season to obtain semen with a greater quality for biobank storage. In addition, the results from the present study may contribute knowledge for development of semen extenders with a composition conducive for peccary sperm viability, especially in the samples obtained during the dry periods.

## 5. Conclusions

In summary, there are effects of environmental parameters in a semiarid climate during the dry, as compared with the rainy period, on biochemical contents of collared peccary seminal plasma. Furthermore, results of the present study indicated concentrations of biochemical compounds are correlated with various seminal characteristics of collared peccaries.

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## Declaration of Competing Interest

The authors declare that there are no conflicts of interest.

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