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Scientific Article

Biochemical composition of seminal plasma of agoutis in different environmental conditions of Caatinga

Composição bioquímica do plasma seminal de cutias em diferentes condições ambientais da Caatinga

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ABSTRACT - This study aims to advance knowledge of the reproductive aspects of the red-rumped agouti (*Dasyprocta leporina*). The objective was to describe the organic and inorganic constituents present in the seminal plasma of agouti specimens analyzed under different environmental conditions in a semiarid region. Seminal plasma was collected from six adult males via electroejaculation during both the dry and rainy seasons. Organic components (total proteins, albumin, cholesterol, triglycerides, fructose, and glucose) and inorganic components (phosphorus, magnesium, calcium, iron, chloride, sodium, and potassium) were analyzed. Significantly higher concentrations of glucose (88.24 vs. 26.27 mg/dL), phosphorus (66.40 vs. 3.67 mg/dL), and potassium (92.67 vs. 19.68 mmol/L) were detected during the dry season compared to the rainy season ($p < 0.05$). Conversely, chloride concentrations (43.04 vs. 201.40 mEq/L) were significantly higher during the rainy season ($p < 0.05$). This study provides the first description of the biochemical composition of seminal plasma in this species, revealing important seasonal variations in these constituents within the Caatinga biome.

Keywords: Seasonality. *Dasyprocta leporina*. Reproductive physiology.

RESUMO - Este estudo visa contribuir para o avanço do conhecimento sobre os aspectos reprodutivos das cutias (*Dasyprocta leporina*). O objetivo foi descrever os constituintes orgânicos e inorgânicos presentes no plasma seminal de cutias analisados sob diferentes condições ambientais em uma região semiárida. O plasma seminal foi coletado de seis machos adultos por meio de eletroejaculação durante os períodos seco e chuvoso. Foram analisados componentes orgânicos (proteínas totais, albumina, colesterol, triglicerídeos, frutose e glicose) e inorgânicos (fósforo, magnésio, cálcio, ferro, cloretos, sódio e potássio). Detectaram-se maiores concentrações de glicose (88,24 e 26,27 mg/dL), fósforo (66,40 e 3,67 mg/dL) e potássio (92,67 e 19,68 mmol/L) na estação seca em comparação à estação chuvosa. As concentrações de cloreto (43,04 e 201,40 mEq/L) foram maiores na estação chuvosa. Este é o primeiro estudo a descrever a composição bioquímica do plasma seminal nesta espécie, revelando importantes correlações entre esses constituintes e os períodos seco e chuvoso no bioma Caatinga.

Palavras-chave: Sazonalidade. *Dasyprocta leporina*. Fisiologia reprodutiva.

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INTRODUCTION

The red-rumped agouti (*Dasyprocta leporina*) is a Neotropical hystricomorphic rodent that inhabits South America, Central America, Mexico, and the Caribbean Antilles, and plays a fundamentally important ecological role (JONES; LALL; GARCIA, 2019). This species contributes to seed dispersal and soil aeration through its digging behavior. It also serves as a key link in the food chain, acting as prey for foxes and medium to large felines, thereby helping to maintain ecological balance (EMMONS; REID, 2016). Although globally classified as stable (EMMONS; REID, 2016), in some regions, populations may be declining due to anthropogenic pressures such as predatory hunting and habitat destruction (BROWN-UDDENBERG et al., 2004).

Although red-rumped agoutis are capable of reproducing year-round, reproductive peaks in wild rodent populations are often closely associated with variations in food availability and climatic conditions. In tropical and semiarid regions, such environmental factors can regulate the timing and intensity of reproductive activity, with more favorable conditions promoting higher reproductive success (DUBOST; HENRY, 2017; DINIZ et al., 2019). Therefore, understanding how reproductive seasonality interacts with environmental variables is critical for species conservation and management.

Over the years, several studies have aimed to develop effective assisted reproduction protocols for *D. leporina*, focusing particularly on semen collection (LIMA et al., 2023) and male germplasm preservation (SILVA et al., 2022). A major challenge in advancing semen technology for this species is the low success rate in obtaining spermatozoa. Most ejaculates (50–60%) collected via electroejaculation are azoospermic and consist only of secretions from the accessory glands (CASTELO et al., 2015; LIMA et al., 2023). While this poses a challenge for improving semen collection methods, it also presents an opportunity

to study the seminal plasma particularly the products of the accessory glands in isolation, thereby enriching our understanding of agouti reproductive physiology. In this species, the typical accessory structures found in most mammals vesicular, prostatic, and bulbourethral glands are complemented by an additional coagulating gland (MOLLINEAU et al., 2006).

Seminal fluid plays a critical role in maintaining sperm function, facilitating the movement of male gametes within the female reproductive tract. Its biological activity, influenced by its complex biochemical composition, can be affected by a variety of factors, including seasonal climatic changes (MOREIRA et al., 2019). In the Caatinga biome a natural habitat for agoutis characterized by a semiarid climate temperature significantly impacts biological rhythms and can limit reproductive efficiency (MOREIRA et al., 2019). In such environments, variations in rainfall and temperature can influence rodent reproduction due to limited availability of water and energy during the dry season. A recent study has shown that seasonal climate variations in the Caatinga affect agouti sperm parameters (DANTAS et al., 2024); however, their effect on the biochemical composition of seminal plasma remains unknown.

Understanding the biochemical composition of seminal plasma is essential for improving semen preservation protocols (MOREIRA et al., 2022). Analyzing the seminal plasma of captive animals offers a unique opportunity to isolate the effects of environmental variables on reproductive parameters, while minimizing confounding factors such as food and water scarcity. By maintaining consistent nutritional and hydration conditions year-round, observed physiological changes can be more confidently attributed to climatic variations.

In this context, the objective of this study was to describe the organic and inorganic biochemical constituents of seminal plasma derived from the accessory glands of red-rumped agoutis, evaluated under different environmental conditions in a semiarid region.

MATERIALS AND METHODS

Bioethics and experimental animals

The experimental procedures were conducted following approval from the Ethics Committee for Animal Use of the Federal Rural University of the Semiarid Region (CEUA/UFERSA; no. 11/2019) and the Chico Mendes Institute for Biodiversity (ICMBIO; no. 66618-5). Six sexually mature adult males, approximately 12 months old and with an average weight of 2.31 ± 0.46 kg, were sourced from the Center for the Multiplication of Wild Animals – CEMAS/UFERSA, a scientific breeding facility registered with the Brazilian Institute for the Environment – IBAMA (no. 1478912).

The animals were housed individually in paddocks measuring 2.25 m² and 1.5 m in height, roofed with ceramic tiles and maintained under consistent rearing conditions throughout the experiment. The paddocks were enclosed with wire mesh on the sides to allow for adequate air circulation. All animals were kept in captivity and fed a diet consisting of fruits, corn, and commercial rabbit feed once daily, with water provided *ad libitum*. The same management practices were maintained throughout both seasonal periods of the experiment.

Environmental conditions

CEMAS/UFERSA is located in the city of Mossoró (latitude 5°11'S, longitude 37°20'W, 18 m above sea level), in the northeastern Brazilian hinterland, within the Caatinga biome. This region has a semiarid tropical climate, characterized by eight to nine dry months and three to four rainy months, with a natural photoperiod of approximately 12 hours throughout the year (ESPÍNOLA SOBRINHO et al., 2011).

To identify the peak periods of the dry (September, October, and November 2019) and rainy (February, March, and April 2020) seasons, rainfall (mm) and solar radiation (W/m²) data were obtained from the National Meteorological Institute station located on the UFERSA campus. In addition, air temperature (°C), relative humidity (%), and wind speed (m/s) were recorded using a thermohygrometer-anemometer (INSTRUTERM-HT300; São Paulo, Brazil) installed near the animal pens. These parameters were measured every 30 minutes over a 24-hour period, and average, minimum, and maximum values were calculated for each season.

Obtaining fluids from accessory glands

During both seasons, the same individuals underwent a solid fasting period of twelve hours and a water fasting period of eight hours before seminal plasma collection. On the collection day, the animals were physically restrained using a capture net. They were then anesthetized via intramuscular administration of ketamine (35 mg/kg; Cetamin, Syntec, Tamboré, SP, Brazil) and xylazine (5 mg/kg; Xilazin, Syntec, Tamboré, SP, Brazil), following the protocol for agoutis (MOLLINEAU; ADOGWA; GARCIA, 2010).

Seminal plasma, representing the product of the accessory glands, was obtained through electroejaculation using a previously established protocol for the species (CASTELO et al., 2015). The animals were positioned in lateral recumbency, and the procedure was performed using a device emitting sine waves, coupled with a probe fitted with ring electrodes. The electroejaculation protocol consisted of three stimulation cycles, with 5-minute intervals between each cycle. The first cycle involved 10 stimuli at 2, 3, and 4 V successively; the second cycle involved 10 stimuli at 5, 6, and 7 V sequentially; and the third cycle involved 10 stimuli at 8, 9, and 10 V. The ejaculates were collected in plastic tubes, and their volume was measured using micropipettes. Ejaculates were evaluated under a light microscope to confirm the absence of spermatozoa. Since the goal of the study was to obtain isolated fluid from the accessory glands, only azoospermic ejaculates were used.

It is important to note that in this species, electroejaculation frequently results in azoospermic ejaculates, which contain primarily accessory gland secretions. This phenomenon has been well-documented in previous studies (CASTELO et al., 2015; LIMA et al., 2023), with approximately 50–60% of samples collected via electroejaculation lacking spermatozoa. To ensure that the azoospermia observed in our study was due to limitations of the collection method and not intrinsic infertility, all animals were euthanized at the end of the study, and epididymal sperm

analysis confirmed the presence of viable spermatozoa. The reproductive capacity of the individuals was confirmed, revealing an average of 823.47 ± 137.9 × 10^6 sperm in the epididymal cauda region. Details of these sperm analyses can be found in the work published by Dantas et al. (2024).

Biochemical analysis

After the initial analysis, the seminal fluid was separated from cells and debris by centrifugation at 700 × g for 10 minutes at room temperature (~27°C). The supernatant was transferred to 1.5 mL microtubes and stored at -20°C until evaluation. Analyses were performed using commercial biochemical kits (Labtest Diagnóstica SA, Lagoa Santa, MG, Brazil) and Espermoteste (InVitro Diagnostic S/A, Itabira-MG, Brazil), as previously reported by Moreira et al. (2019).

To determine and quantify organic constituents (total protein, albumin, cholesterol, triglycerides, fructose, and glucose) and inorganic constituents (phosphorus, magnesium, calcium, iron, chloride, sodium, and potassium), the absorbance of the samples was measured using a spectrophotometer (Biospectrum model SP-22, Curitiba, PR, Brazil) at the wavelengths specified for each kit. The absorbance values for each constituent were measured, and the final concentrations of the samples were calculated according to the instructions provided with each commercial kit.

Statistical analysis

Each individual underwent three collections of seminal fluid per season, totaling 18 samples per season and 36 samples for the entire experiment. Data are presented as means and standard errors and were analyzed using SAS statistical software (version 8.0; SAS Institute, Inc., Cary, NC, USA). Data normality and homoscedasticity were assessed using the Shapiro–Wilk test and Levene's test, respectively. To evaluate seasonal and environmental changes in seminal plasma biochemical parameters, a one-way ANOVA F-test was performed using PROC GLM in SAS. A P-value of < 0.05 was considered statistically significant for all analyses.

RESULTS AND DISCUSSION

Characterizing the environmental conditions

The maximum air temperature (Figure 1A), solar radiation (Figure 1B), and wind speed (Figure 1D) were significantly higher during the dry season compared to the rainy season ($P < 0.05$). However, the mean and minimum air temperatures were similar between both periods ($P > 0.05$). Relative air humidity (Figure 1C) and total rainfall (Figure 1E) were considerably higher in the rainy season ($P < 0.05$), with precipitation averaging 443.6 mm during this period, compared to nearly 0 mm in the dry season. These environmental characteristics align with the typical range for the region, as reported in previous studies (MOREIRA et al., 2019). It is important to note that the results of this study are based on a small captive population, which may limit the generalization of findings to wild agouti populations. Further studies involving wild specimens are necessary to confirm these patterns.

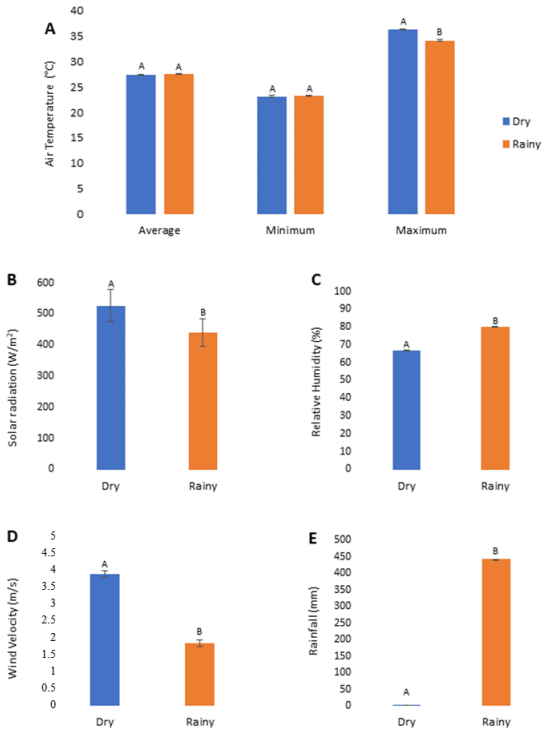

Figure 1. Mean values of the environmental variables during the dry (September, October, and November 2019) and rainy (February, March, April, and May 2020) periods in the equatorial semiarid region. A) Air temperature (°C); B) solar radiation (W/m^2); C) relative humidity (%); D) wind velocity (m/s); E) rainfall (mm). $^{A, B}$ Different letters in the same column indicate significant differences for the same observed variable ($P < 0.05$).

Electroejaculation efficiency

The electroejaculation protocol effectively obtained fluids from the accessory glands of agoutis, providing an adequate volume of seminal plasma regardless of the degree of erection. Three samples were collected from each animal per season, resulting in 18 ejaculates per season. None of the 18 ejaculates used contained spermatozoa, and no urine contamination was observed. The average volume obtained during the dry season (575.0 ± 162.5 µL) was significantly greater ($P < 0.05$) than that obtained during the rainy season (484.0 ± 88.6 µL).

Despite the absence of sperm, the azoospermic ejaculates allowed for a detailed analysis of the biochemical composition of the fluids. Unlike other mammals, the contribution of accessory glands to seminal plasma formation in this species is composed not only of the products from the prostate, seminal vesicles, and bulbourethral glands but also includes the coagulating gland. This uncommon accessory gland is essential for the reproductive process in this rodent species (CAVAZOS, 1975). Its presence may influence the composition of the seminal plasma, as seen in other rodents such as rats, mice, and guinea pigs, where semen coagulation and the formation of the copulatory plug result from enzyme-substrate interactions between secretions from coagulating glands and seminal vesicles (MANN; LUTWAK-MANN, 1981).

It should be noted that seminal plasma in ejaculates containing sperm includes remnants of fluids from the epididymis and testis, in addition to the fluids from the accessory glands (RODRIGUEZ-MARTINEZ et al., 2021). These contributions may subtly alter its biochemical composition, though this remains to be investigated in agoutis.

Organic compounds in seminal fluids

The same organic constituents (Table 1) were present in all samples analyzed during both the dry and rainy seasons. Similar concentrations ($P > 0.05$) of total proteins (2.98 g/dL and 1.90 g/dL), particularly albumin (5.43 g/dL and 6.64 g/dL), were detected between the two seasons in the Caatinga. Generally, seminal plasma proteins play crucial roles in key fertilization processes in mammals, such as regulating sperm capacitation, establishing the sperm reservoir in the fallopian tube, modulating the uterine immune response, facilitating sperm transport in the female reproductive tract, and enabling gamete interaction (TÖPFER-PETERSEN et al., 2005; RODRIGUEZ-MARTÍNEZ et al., 2021).

Table 1. Mean, median, and range of the organic biochemical constituents of the seminal plasma of agouti (*D. leporina*) collected during the dry and rainy periods in a semiarid region (n = 18 ejaculates per period).

Biochemical components	Dry period			Rainy period		
	Mean	Median	Range	Mean	Median	Range
Total proteins (g/dL)	2.98 ± 0.8[a]	2.60	0.38 - 5.8	1.90 ± 0.6[a]	1.49	0.44 - 4.4
Albumin (g/dL)	5.43 ± 1.2[a]	5.09	1.96 - 9.2	6.64 ± 2.3[a]	6.27	1.19 - 15.6
Cholesterol (mg/dL)	119.91 ± 21.6[a]	107.86	76.31 - 217.0	125.16 ± 34.3[a]	112.02	38.12 - 270.5
Triglycerides (mg/dL)	100.11 ± 34.4[a]	70.17	10.10 - 231.0	282.04 ± 83.5[a]	256.25	33.01 - 556.9
Fructose (mg/dL)	29.66 ± 7.1[a]	26.94	8.95 - 58.1	26.92 ± 8.0[a]	26.00	4.16 - 57.3
Glucose (mg/dL)	88.24 ± 10.3[a]	97.36	39.07 - 106.3	26.27 ± 9.8[b]	20.44	2.02 - 60.0

[ab]Different superscripts in the same line indicate significant differences ($P<0.05$).

Regarding the lipid composition of fluids from agouti accessory glands, no differences were found in cholesterol and triglyceride levels between the dry and rainy seasons. These lipids are essential for sperm quality, as their oxidation provides the energy necessary for cellular metabolism. Cholesterol, in particular, is positively related to semen quality in humans (GAROLLA et al., 2018) and stallions (BRINSKO et al., 2007). Unlike agoutis, seasonal variation in seminal plasma lipid composition has been observed in bulls, with higher cholesterol and triglyceride levels during winter compared to spring in temperate regions like Croatia (BEER-LJUBIĆ et al., 2009). Such interspecies differences may be related to adaptation mechanisms to environmental conditions.

Sugars in seminal plasma serve as metabolic energy sources for sperm and are directly linked to cell motility (MOREIRA et al., 2019). In agoutis, there were no differences between the dry and rainy seasons regarding the fructose content in the fluids derived from accessory glands. Similar to our findings in azoospermic ejaculates, Mollineau, Adogwa, and Garcia (2008) reported low fructose levels in ejaculates containing sperm in the same species, demonstrating that increased fructose concentrations correlated with higher sperm morphological defects. Therefore, maintaining low fructose levels in seminal plasma during seasonal changes in semiarid environments seems beneficial for preserving sperm morphology integrity in this species.

About sugars, glucose concentrations in agouti accessory gland fluids exceeded fructose levels only during the dry season, with significantly higher values ($P < 0.05$) in the dry season (88.24 mg/dL) compared to the rainy season (26.27 mg/dL). In rats and hamsters, glucose concentration also predominates over fructose, with coagulation glands secreting glucose at relatively high levels (FOUQUET, 1971). The presence of coagulation glands in agoutis (MOLLINEAU et al., 2006) may explain the glucose levels observed in this study. Furthermore, glucose concentration in agouti seminal plasma was notably higher during the dry period. These results align with Dantas et al. (2024), who reported decreased epididymal sperm motility in the dry period compared to the rainy season for agoutis reared under similar conditions.

Therefore, increased glucose concentrations in seminal plasma during the dry period may compensate for low epididymal sperm motility by providing additional energy for cell metabolism.

Inorganic compounds in seminal fluids

For inorganic constituents (Table 2), sodium was detected in only two ejaculates during the dry season and in four ejaculates during the rainy season. Potassium was present in all samples analyzed during the dry season but in only three ejaculates during the rainy season. Chloride concentrations were significantly higher ($P < 0.05$) in the rainy season (201.40 ± 36.99 mEq/L) compared to the dry season (43.04 ± 8.28 mEq/L). Conversely, phosphorus and potassium concentrations were significantly higher ($P < 0.05$) during the dry season (66.40 ± 17.64 mg/dL and 92.67 ± 8.45 mmol/L) than during the rainy season (3.67 ± 0.59 mg/dL and 19.68 ± 5.61 mmol/L).

Table 2. Mean, median, and range of the inorganic biochemical constituents of the seminal plasma of agouti (*D. leporina*) collected during the dry and rainy periods in a semiarid region (n = 18 ejaculates per period).

Biochemical components	Dry period			Rainy Period		
	Mean	Median	Range	Mean	Median	Range
Phosphorus (mg/dL)	66.40 ± 17.64[a]	67.66	6.11 - 114.77	3.67 ± 0.59[b]	3.75	1.77 - 5.52
Magnesium (mg/dL)	4.70 ± 0.41[a]	4.44	3.42 - 6.06	4.24 ± 0.38[a]	3.98	3.29 - 5.98
Calcium (mg/dL)	8.85 ± 1.73[a]	9.76	1.09 - 12.60	12.47 ± 1.85[a]	13.39	6.73 - 18.28
Iron (µg/dL)	601.76 ± 203.05[a]	495.50	49.00 - 1492.86	620.63 ± 266.33[a]	449.57	126.00 - 1902.35
Chlorides (mEq/L)	43.04 ± 8.28[a]	43.95	12.44 - 70.57	201.40 ± 36.99[b]	163.57	99.07 - 339.77
Sodium (mEq/L)	158.60 ± 9.39[a]	165.47	131.12 - 172.34	267.42 ± 112.94[a]	267.42	154.48 - 380.36
Potassium (mEq/L)	92.67 ± 8.45[a]	84.48	83.96 - 109.58	19.68 ± 5.61[b]	18.58	4.91 - 36.60

[ab]Different superscripts in the same line indicate significant differences ($P<0.05$).

As reported by Silva, Dode, and Unanian (1993), potassium levels are directly related to sperm concentration, supporting the findings of Dantas et al. (2024), who observed a higher number of epididymal sperm in red-rumped agoutis during the dry season compared to the rainy season. In their study of agouti sperm parameters in the same semiarid region, Dantas et al. (2024) also reported better sperm quality in the rainy season, which coincided with higher chloride concentrations in seminal plasma. This could be related to a higher demand for these ions to enhance reproductive efficiency during the rainy season.

Additionally, Purohit, Laloraya, and Kumar (1999) described the modulation of ion channels, such as chloride, sodium, and potassium, in semen as being associated with sperm capacitation and the acrosomal response in mammalian spermatozoa. The role of ions in the seminal plasma of agoutis and their contribution to reproductive physiology remains unclear. However, in the phylogenetically related Phodopus hamsters, a recent study revealed that sperm capacitation does not seem to depend on calcium bicarbonate as it does in other mammalian species, suggesting the need for further investigation of alternative pathways (SANCHEZ-RODRIGUEZ et al., 2023). On the other hand, it is known that extracellular Na^+ can suppress hamster sperm hyperactivation by reducing intracellular $Ca2^+$ levels (TAKEI et al., 2023). Whether this phenomenon could apply to agoutis remains unknown, and specific studies on this subject are needed. There is a significant lack of knowledge regarding the biochemical composition of semen in wild rodents, both from the Neotropics and the Old World, and consequently, little is known about the physiological mechanisms in which these ions participate. Therefore, the novelty of the present study and its potential to contribute to understanding the reproductive biology of wild rodent species is particularly valuable.

Wild rodent reproduction in tropical regions is influenced by various factors, beyond just environmental conditions, such as food and water availability, sexual competition, and predation (DUBOST; HENRY; COMIZZOLI, 2005). In the humid tropical Brazilian Amazon (GUIMARÃES; MOREIRA; VALE, 1997) and French Guiana (DUBOST; HENRY, 2017), the black-rumped agouti (Dasyprocta prymnolopha) reproduces year-round and is classified as polyestrous, both in captivity and the wild, likely due to constant food availability. Based on the present study conducted in a semiarid region, we hypothesize that the composition of fluids from accessory glands may have a compensatory effect, enabling the red-rumped agouti to reproduce year-round, as previously demonstrated (PINHEIRO; ANDRADE; CUNHA, 1989; DINIZ et al., 2019). This hypothesis is particularly relevant considering the low epididymal sperm quality observed during the dry season by Dantas et al. (2024), during which an increase in important accessory gland fluid components, such as glucose, phosphorus, and chloride, was noted. These components may support sperm viability in the dry season. However, further investigation is needed to understand the interactions between seminal plasma and spermatozoa, especially in ejaculates containing spermatozoa. Although our results suggest a possible compensatory effect of seminal plasma during the dry season, this hypothesis requires direct experimental validation in future studies to explore the functional impacts of seasonal changes in seminal plasma composition on sperm viability and fertilization success in *Dasyprocta leporina*.

CONCLUSIONS

This study offers the first comprehensive description of the biochemical composition of seminal plasma, specifically derived from accessory gland secretions, in *Dasyprocta leporina*. Significant seasonal differences were observed, with higher concentrations of glucose, phosphorus, and potassium during the dry season, and higher chloride concentrations during the rainy season. These biochemical adjustments may reflect adaptive strategies that help maintain reproductive viability throughout the year under the challenging conditions of the semiarid Caatinga biome. Our findings provide valuable insights that can enhance assisted reproduction and conservation programs for this species.

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