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MAIKO ROBERTO TAVARES DANTAS

CARACTERÍSTICAS ESPERMÁTICAS DE CUTIAS (*Dasyprocta leporina* LINNAEUS, 1758) DURANTE O TRÂNSITO EPIDIDIMÁRIO E NAS ESTAÇÕES SECA E CHUVOSA DO BIOMA CAATINGA

MOSSORÓ
2022

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Tese apresentada ao Doutorado em Ciência Animal do Programa de Pós-Graduação em Ciência Animal da Universidade Federal Rural do Semi-Árido como requisito para obtenção do título de Doutor em Ciência Animal.

Linha de Pesquisa: Morfofisiologia e Biotecnologia Animal

Orientador: Prof. Dr. Alexandre Rodrigues Silva

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À minha esposa, filho e meus pais,
pelo amor incondicional e apoio.

Dedico

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“Por que caímos, Bruce? Para aprendermos a levantar”

Thomas Wayne

RESUMO

Uma vez que as características reprodutivas dos roedores silvestres são afetadas por fatores ambientais, entender a influência sazonal sobre as características espermáticas de cutias durante a maturação espermática é essencial para a conservação desta e de outras espécies filogeneticamente próximas. Diante disso, objetivou-se avaliar os efeitos causados pelas condições climáticas dos períodos seco e chuvoso de uma região semiárida sobre os parâmetros espermáticos morfofuncionais de cutia (*Dasyprocta leporina*) ao longo do trânsito epididimário. Para tanto, o estudo foi dividido em três experimentos, os quais utilizaram-se animais adultos oriundos do Centro de Multiplicação de Animais Silvestres da UFERSA. No experimento I, objetivou-se descrever as mudanças ultraestruturais, cinéticas, morfológicas e morfométricas do espermatozoide de cutia ao longo do trânsito epididimário, compreendendo sua maturação. Para tanto, 7 complexos de testículo-epidídimo de cutias sexualmente maduras foram removidos e os espermatozoides de diferentes regiões do epidídimo (caput, corpus e cauda) foram coletados através da técnica de flutuação. Não houve diferença significativa entre as regiões do epidídimo e a morfologia normal dos espermatozoides. As médias do comprimento da cabeça, largura da cabeça e comprimento da cauda dos espermatozoides foram maiores na cabeça do epidídimo (5,15 μm, 3,44 μm e 32,04 μm, respectivamente), diminuindo ao longo do trânsito epididimário. Os espermatozoides da cauda do epidídimo apresentaram os maiores valores de integridade de membrana com atividade mitocondrial (79,71%). Houveram aumentos significativos na motilidade total e vários parâmetros cinéticos, seguindo a direção cabeça-corpo-cauda. Nós fornecemos dados sem precedentes relacionados ao trânsito epididimário de espermatozoides em cutias. O experimento II objetivou avaliar a integridade funcional da membrana celular do espermatozoide de cutia ao longo do trânsito com diferentes soluções hiposmóticas e verificar suas associações com os parâmetros espermáticos. Foram usados os espermatozoides epididimários dos animais do experimento I (n = 6), seguindo-se as análises dos parâmetros espermáticos, correlacionando-as com o teste HOST (0 mOsm/L, 50 mOsm/L e 200 mOsm/L). Houveram correlações significativas entre os parâmetros espermáticos das regiões do caput e corpus e as soluções hiposmóticas, mas não com a cauda. A integridade funcional da membrana espermática de cutias pode ser avaliada com solução à base de citrato de sódio e frutose com osmolaridade de 50 mOsm/L. Por fim, o experimento III objetivou avaliar os impactos sazonais de uma região semiárida sobre as características espermáticas da cutia. Foram avaliados os parâmetros morfofuncionais dos espermatozoides da cauda do epidídimo de 7 cutias criadas no período seco do experimento I e 7 no período chuvoso, comparando-os como as variáveis ambientais aferidas em cada estação. A temperatura máxima do ar (ºC), umidade relativa (%), velocidade do vento (m/s) e a precipitação total (mm) para as estações seca e chuvosa foram, respectivamente: 36,2 e 34,1 ºC, 66,8 e 80,1%, 4,0 e 1,9 m/s, 0,2 e 517,7 mm. Os parâmetros relativos à morfofuncionalidade espermática apresentaram um padrão de melhores resultados durante o período chuvoso: redução nos defeitos do acrossomo (0,1% durante a estação chuvosa e 1,43% durante a seca), maiores comprimento de cabeça, largura da cabeça e comprimento da peça intermediária (5,42 μm, 3,61 μm e 5,78 μm, respectivamente) e melhores padrões de motilidade (motilidade total, progressiva, VAP, VSL, VCL e subpopulação de velocidade rápida). Em resumo, as variações ambientais relacionadas ao período chuvoso de uma região semiárida influenciaram positivamente a qualidade espermática de cutias. No entanto, a maior quantidade de gametas masculinos foi obtida durante a estação seca, provavelmente devido a mecanismos compensatórios.

Palavras-chave: Biotécnicas. Mudanças climáticas. Influência do ambiente. Maturação espermática. Vida selvagem.

ABSTRACT

Since the reproductive characteristics of wild rodents are affected by environmental factors, understanding the seasonal influence on the sperm characteristics of agoutis during sperm maturation is essential for the conservation of this and other phylogenetically related species. Therefore, the objective was to evaluate the effects caused by the climatic conditions of the dry and rainy periods in a semiarid region on the morphofunctional sperm parameters of agouti (*Dasyprocta leporina*) during epididymal transit. Therefore, the study was divided into three experiments, using adults animals from the Center for Multiplication of Wild Animals of UFERSA. In experiment I, the objective was to describe the ultrastructural, kinetic, morphological, and morphometric changes of agouti sperm during epididymal transit, including its maturation. For this, seven testis-epididymis complexes of sexually mature agoutis were removed, and sperm from different regions of the epididymis (caput, corpus, and cauda) were collected using the flotation technique. There were no significant differences between epididymal regions and normal sperm morphology. The mean head length, head width, and tail length of sperm were higher in the epididymal caput (5.15 μm, 3.44 μm, and 32.04 μm, respectively), decreasing along the epididymal transit. Spermatozoa from the cauda of the epididymis showed the highest values of membrane integrity with mitochondrial activity (79.71%). There were significant increases in total motility and several kinetic parameters following the caput-corpus-cauda direction. We provide unprecedented data related to epididymal transit of sperm in agoutis. Experiment II aimed to evaluate the functional integrity of the sperm cell membrane of agouti during the transit with different hypoosmotic solutions and verify their associations with sperm parameters. Epididymal sperm from the animals in experiment I (n = 6) were used, followed by analyzing sperm parameters, correlating them with the HOST test (0 mOsm/L, 50 mOsm/L, and 200 mOsm/L). There were significant correlations between the sperm parameters of the caput and corpus regions and the hypoosmotic solutions, but not with the cauda. The functional integrity of the sperm membrane of agoutis can be evaluated with a solution based on sodium citrate and fructose with an osmolarity of 50 mOsm/L. Finally, experiment III aimed to evaluate the seasonal impacts of a semiarid region on the sperm characteristics of the agouti. The morphofunctional parameters of spermatozoa from the cauda of the epididymis of 7 agoutis reared in the dry period of experiment I and 7 in the rainy period were evaluated, comparing them with the environmental variables measured in each season. The maximum air temperature (ºC), relative humidity (%), wind speed (m/s) and total precipitation (mm) for the dry and rainy seasons were, respectively: 36.2 and 34.1 ºC, 66 .8 and 80.1%, 4.0 and 1.9 m/s, 0.2 and 517.7 mm. The parameters related to sperm morphofunctionality showed a pattern of better results during the rainy season: reduction in acrosome defects (0.1% during the rainy season and 1.43% during the dry season), greater head length, head width, and midpiece length (5.42 μm, 3.61 μm, and 5.78 μm, respectively), and better motility patterns (total motility, progressive, VAP, VSL, VCL, and rapid speed subpopulation). In summary, weather changes related to the rainy season of a semiarid region positively influenced the sperm quality in red-rumped agouti. Furthermore, the largest amount of male gametes was obtained during the dry season, probably due to compensatory mechanisms.

Keywords: Biotechniques. Changes climate. Environmental Influence. Sperm maturation. Wild animal.

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LISTA DE ABREVIATURAS E SIGLAS

10^6 sperm	Milhões de espermatozoides
10^6 sperm/mL	Milhões de espermatozoides por mililitro
ALH	Amplitude do deslocamento lateral da cabeça
ANOVA	Análise de Variância
ART	Técnica reprodutiva assistida
BCF	Frequência cruzada de batimento
CASA	Análise espermática assistida por computador
CEMAS	Centro de Multiplicação de Animais Silvestres
CEUA	Comitê de Ética para Uso de Animais
g	grama
H342	Hoechst 342
HOS test	Teste de intumescência hiposmótico
Hz	Hertz
°C	Grau Celsius
h	Horas
IBAMA	Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis
ICMBIO	Instituto Chico Mendes de Biologia
IM	Intramuscular
IV	Intravenoso
IUCN	União Internacional pela Conservação da Natureza
LIN	Linearidade
M	Molar

m^2	Metro quadrado
mg/kg	Miligramas por quilo
mg/ml	Miligramas por mililitro
mL	Mililitro
mL/kg	Mililitro por quilo
mm	Milímetro
min	Minutos
m/s	Metros por segundo
mOsm/L	Miliosmol por litro
nM	Nanomolar
µm	Micrômetro
µm/s	Micrômetros por segundo
µL	Microlitro
PI	Iodeto de propídio
RN	Rio Grande do Norte
PBS	Solução salina tamponada fosfatada
SEM	Microscopia eletrônica de varredura
STR	Retilinearidade
VAP	Velocidade média de trajetória
VCL	Velocidade curvilínea
VSL	Velocidade em linha reta
UFERSA	Universidade Federal Rural do Semi-Árido

LISTA DE SÍMBOLOS

>	Maior
±	Mais ou menos
®	Marca Registrada
<	Menor
%	Porcentagem
ρ	Correlação pelo teste de Spearman
P	Nível de significância

SUMÁRIO

1. INTRODUÇÃO

A conservação de recursos genéticos é uma medida que visa diminuir a perda contínua da vida silvestre (COSTA; MARTINS, 2008). Tanto em seus próprios habitats, como em cativeiros legalizados, a criação de espécies silvestres contribui com a conservação da espécie, minimizando os impactos ambientais provocados por ação antrópica, como a caça predatória e destruição de suas áreas naturais de ocorrência (SILVIUS; FRAGOSO, 2003). Assim, a manutenção de espécies silvestres em cativeiro traz a vantagem da integração de programas de manejo eficientes, aliado à possibilidade de obtenção de informações relativas à fisiologia e o comportamento da espécie, assim possibilitando a exploração sustentável do seu potencial zootécnico destes (RIBEIRO et al., 2008).

Na reprodução de espécies silvestres em cativeiro, é importante estabelecer o uso de técnicas de reprodução assistida com intuito de aumentar o número de indivíduos, para que estes sejam transferidos para programas de reintrodução de espécimes ao meio ambiente (FOOSE; WIESE, 2006). No entanto, ainda há muito o que se conhecer sobre a fisiologia reprodutiva de diversas espécies de animais silvestres, visto que a maioria das pesquisas são voltadas para os animais domésticos ou de produção (BRONSON; HEIDEMAN, 1994).

Em roedores silvestres como as cutias (*Dasyprocta sp.* Linnaeus, 1758; Rodentia: Dasyprotidae), o conhecimento detalhado a respeito de sua morfofisiologia reprodutiva é ainda muito limitado, o que também limita o desenvolvimento de técnicas de reprodução assistida que poderiam fomentar o seu manejo em cativeiro. Embora a caracterização da anatomia reprodutiva dos machos (MOLLINEAU et al., 2006) já tenha sido realizada e, inclusive, tenha sido identificado que as cutias apresentam testículos e epidídimos intra-abdominais, pouco ou nada se sabe a respeito dos mecanismos fisiológicos que regem o seu trânsito espermático. A aquisição de tais informações torna-se essencial, principalmente para fomentar os estudos voltados para o processamento espermático, uma vez que a membrana espermática sofre diversas modificações durante sua maturação, decorrentes de mudanças funcionais, estruturais e bioquímicas nos espermatozoides durante o trânsito epididimário, às quais são complexas e bem orquestradas (OLSON et al., 2003).

Além disso, sabe-se que a reprodução em roedores silvestres é fortemente influenciada por fatores ambientais como o fotoperíodo (MUTEKA et al., 2006; TRILLMICH et al., 2009; TAVOLARO et al., 2015) a pluviosidade (DUBOST; HENRY, 2017; SARLI et al., 2016) e a temperatura (SARLI et al., 2016; SALMAN et al., 2017; FABIO-BRAGA; KLEIN, 2018). Neste sentido, é necessário analisar os diversos níveis fisiológicos e ambientais por eles

afetados durante a maturação espermática em uma dada espécie, como em cutia, uma vez que a existência de uma possível sazonalidade reprodutiva na espécie permanece por ser comprovada.

Uma vez obtidos os conhecimentos fundamentais sobre a maturação espermática e da influência do das condições ambientais sobre o espermatozoide da cutia (*D. leporina*), poder-se-á estabelecer biotécnicas reprodutivas, sendo que suas aplicações poderão contribuir para a manutenção de ecossistemas viáveis, criação racional em cativeiro, bem como a recuperação e a conservação desta espécie. Para tanto, segue-se uma breve revisão de literatura a respeito do tema.

2. REVISÃO DE LITERATURA

2.1. Aspectos ecológicos da cutia

O maior número de espécies de mamíferos placentários pertence a ordem dos roedores, contendo mais de 2000 espécies catalogadas, correspondendo no total 40% das espécies na classe dos mamíferos, habitando em grande número todos os continentes e ilhas, menos na Antártida (MUSSER; CARLETON, 2005). A cutia é um roedor silvestre que pertence à família Dasyproctidae, onde estão catalogadas onze espécies no gênero *Dasyprocta*. Dentro destas, sete espécies habitam naturalmente em ecossistemas brasileiros: *Dasyprocta azarae*; *Dasyprocta coibae*; *Dasyprocta fuliginosa*; *Dasyprocta guamara*; *Dasyprocta prymnolopha*; *Dasyprocta punctata*; *Dasyprocta leporina* (EMMONS; REID, 2016), sendo *D. leporina* e *D. prymnolopha* as espécies com populações mais frequentes no Nordeste brasileiro. Ainda que ocorra caça predatória, as cutias são classificadas como animais em estado pouco preocupante (EMMONS; REID, 2016), segundo os critérios da IUCN (The World Conservation Union).

As cutias são peças vitais na manutenção de muitos ecossistemas em regiões tropicais (SILVIUS; FRAGOSO, 2003; GARCIA, 2004). Estas, são animais herbívoros que contribuem na disseminação de diversas espécies vegetais, uma vez que se alimentam de folhas, raízes, flores, e principalmente de frutos caídos no solo, sendo excelentes dispersores de sementes (MUSSER; CARLETON, 2005; LANGE; SCHMIDT, 2007).

Estes animais reproduzem-se muito rápido e são parte da alimentação de predadores, como carnívoros de médio e grande, mantendo o equilíbrio na cadeia alimentar (HOSKEN; SILVEIRA, 2001; MUÑOZ; BONAL, 2011). Ainda, existem relatos de que as cutias exercem outros papéis de fundamental importância na natureza, como o fato de depositarem fezes e restos alimentares em tocas por elas construídas, servindo de fonte de nutrientes e sais minerais para outros pequenos animais e vegetais (SILVIUS; FRAGOSO, 2003; LOPES et al., 2004), bem como seus hábitos constantes de cavar, contribuindo para a aeração do solo (GORCHOV et al., 2004; MUÑOZ; BONAL, 2011), Estes animais ainda são utilizados como fonte proteica na alimentação humana, exercendo potencial para exploração econômica (HOSKEN; SILVEIRA, 2001). Além disso, os pesquisadores podem utiliza-las como modelo experimental viável, uma vez que já fora relatado a sua importância nas pesquisas biomédicas (BAAS et al., 1976).

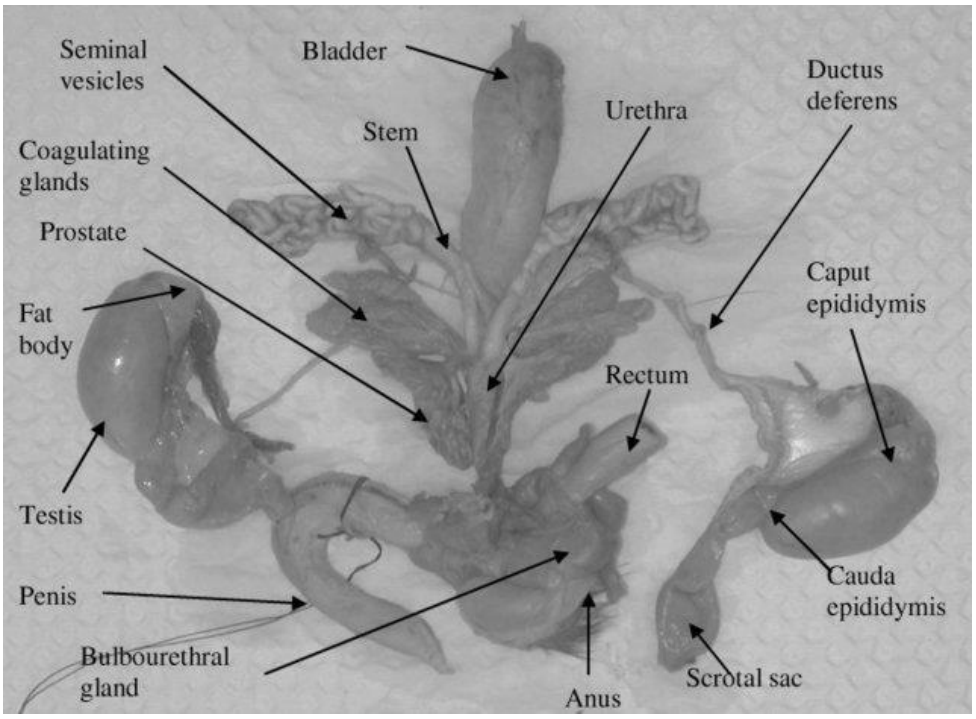
2.2. Aspectos reprodutivos da cutia macho

No tocante às características anatômicas, as cutias adultas possuem altura média de 23 cm e comprimento médio de 50 cm, do focinho a base da cauda, sendo esta última entre 1,5 a 3,5 cm e geralmente desprovida de pelos. Pesam entre 2,0 e 3,0 kg e seus corpos são alongados, com os membros torácicos mais curtos que os pélvicos (DEUTSCH; PUGLIA, 1990). Os primeiros dispõem de quatro dedos funcionais, utilizados para levar o alimento à boca, enquanto que os segundos são dotados de fortes unhas, semelhantes a cascos, e exercem função adaptativa de deslocamento. Seus pelos são estruturas ásperas, duras e compridas, onde as cores predominantes são o marrom-avermelhado, claro ou escuro, e amarelado ou dourado, variando dentre as diversas espécies em seus respectivos habitats. Sua cabeça é estreita e alongada, focinho achatado, olhos grandes e orelhas arredondadas e pequenas (DEUTSCH; PUGLIA, 1990).

A cutia macho possui órgãos genitais internos compostos por glândulas acessórias e um par de testículos e de epidídimos emparelhados, encontrados em contato direto com músculos abdominais, sendo que o "caput" epididimário delimita-se por tecido adiposo (MOLLINEAU et al., 2006) (Figura 1). Os testículos possuem as médias de peso testicular, comprimento e diâmetro de 5,03 ± 0,52 g, 1,67 ± 0,04 cm e 3,67 ± 0,12 cm, respectivamente. Os ductos deferentes possuem, respectivamente, comprimento e diâmetro médios de 10,98 ± 0,40 e 0,14 ± 0,01 cm. Seus órgãos sexuais acessórios são compostos pela próstata, glândulas vesiculares, glândula coaguladora e glândulas bulbouretrais (MOLLINEAU et al., 2006).

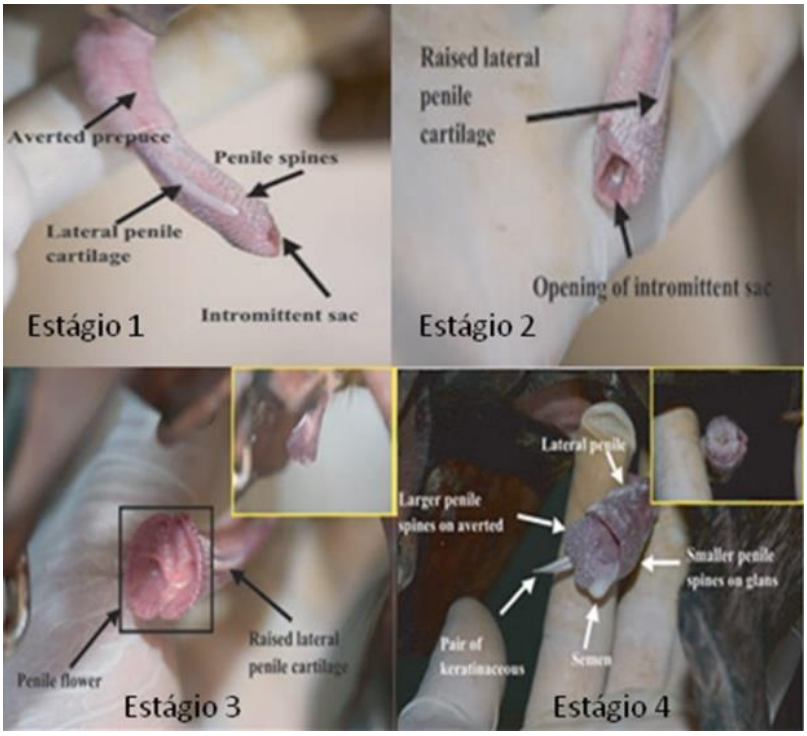
O pênis da cutia é do tipo fibrocavernoso, o qual é dobrado caudalmente, contendo uma flexura em formato de U deitado e um comprimento médio de 9,90 ± 0,43 cm (MOLLINEAU et al., 2012). A glande, no momento da ereção, apresenta uma dilatação arredondada de formato peculiar, a qual é denominada de toro uretral ou flor peniana. Nela, existe um par de cartilagens dispostas lateralmente, contendo espículas queratinosas ventrais e emparelhadas, às quais são facilmente identificadas durante a ereção (MOLLINEAU et al., 2012). No tocante à ereção peniana, ela é descrita em quatro estágios distintos: no primeiro, ocorre a protusão do pênis do orifício prepucial; no segundo, a abertura das cartilagens laterais do pênis; no terceiro, a florescência da cabeça da glande, denominada flor peniana; no quarto, a eversão do saco intromitente e a protrusão dos espículos queratinosos. Apenas no quarto estágio a ejaculação ocorre (MOLLINEAU et al., 2012) (Figura 2).

Figura 1: Estrutura do sistema reprodutor de uma cutia macho sexualmente madura (*Dasyprocta leporina*).



Fonte: MOLLINEAU et al. (2012).

Figura 2: Ereção do pênis de cutia (*Dasyprocta leporina*) em seus quatros estágios.



Fonte: MOLLINEAU et al. (2012).

No que diz respeito à puberdade da cutia macho, alguns trabalhos relataram a existência de períodos distintos onde se observam o desenvolvimento dos órgãos sexuais (ASSIS-NETO, 2003a), a quantificação dos túbulos seminíferos e espermatogênese (ASSIS-NETO, 2003b) e das células de sertoli e leydig (SIMÕES et al., 2017), assim como a caracterização ultraestrutural do epidídimo e vasos deferentes (ARROYO et al., 2014) ao longo de diferentes idades. Assim, foi possível estabelecer nestes animais os diferentes estágios de desenvolvimento sexual e verificar as mudanças morfológicas e funcionais no trato reprodutivo até a maturidade sexual. O estágio compreendido desde o nascimento até os cinco meses de idade, corresponde ao período pré-púbere, onde se verifica a presença de gonócitos e ausência de lúmen tubular nos cordões testiculares. Entre os seis e oito meses, observa-se uma fase de transição à puberdade, denominada de pré-puberdade, onde surgem os primeiros espermatócitos primários e espermátides arredondadas, com 40 a 90% dos cordões testiculares em processo de luminação tubular. A puberdade, ou maturidade sexual, é alcançada a partir dos nove meses de idade. Ainda, percebe-se que dos doze meses de idade em diante, é possível notar a um crescimento rápido dos testículos (estágio de pós-puberdade), a qual coincide com o período onde o epitélio seminífero encontra-se completamente formado.

2.3. A maturação espermática

O órgão responsável pela maturação espermática é o epidídimo, o qual está situado longitudinalmente na borda posterior do testículo, composto de um ducto único, altamente convoluto e revestido por um epitélio pseudoestratificado complexo. Ele é composto por células principais, basais, apicais e claras, com epitélio pseudoestratificado, colunar e estereociliado (ARROYO et al., 2014). O órgão é segmentado, podendo ser dividido ultraestruturalmente e histologicamente em: caput - região dilatada que ultrapassa o pólo superior do testículo; corpus - o segmento intermediário; cauda - a porção inferior e mais estreitada, denominada de cauda (HAFEZ; HAFEZ, 2004). Cada segmento apresenta contribuições específicas em sua atuação no microambiente luminal, fundamentais para que o espermatozoide amadureça no momento em que este alcance a região da cauda, sendo então armazenado (CORNWALL, 2009).

As células principais do caput são responsáveis pela secreção de proteínas, às quais, são adsorvidas na superfície da membrana do espermatozoide, modificando sua composição proteica. Quanto às células principais no corpus, devido a grande quantidade de lipídios presentes da região supranuclear, estas atuam modificando a composição lipídica da membrana

plasmática do espermatozoide. Finalmente, na cauda do epidídimo, seu epitélio é muito mais curto do que os segmentos anteriores e as células claras são predominantes, às quais atuam na secreção de glicoconjugados. As células claras fagocitam as gotas citoplasmáticas que são liberadas do esperma ao longo do trânsito epididimário, além de fagocitarem outros detritos luminais. É na região da cauda que as proteínas luminais em excesso são reabsorvidas, enquanto a imobilina é secretada para manter o esperma quiescente (ROBAIRE et al., 2006). Durante o trânsito epididimário, sabe-se que os espermatozoides perdem ou modificam várias de suas proteínas de superfície, bem como ganham proteínas de superfície transitórias ou permanentes durante a maturação (GERVASI; VISCONTI, 2017).

2.4. Métodos de estudo da maturação espermática

A maturação espermática tem sido investigada cientificamente em diversas espécies de mamíferos devido à sua importância ao fornecer a capacidade fertilizante e as propriedades relacionadas a motilidade dos espermatozoides (SULLIVAN; MIEUSSET, 2016). Em roedores, esse fenômeno acorre ao longo do trânsito epididimário, decorrente de uma ação conjunta de processos fisiológicos específicos e bem orquestrados, os quais promovem uma série de modificações morfofuncionais nos gametas masculinos, nas diferentes regiões do epidídimo (caput, corpus e cauda) (GERVASI; VISCONTI, 2017).

As modificações espermáticas ao longo da maturação afetam o reposicionamento de componentes proteicos, lipídicos e glicoproteicos em diferentes regiões específicas das membranas intracelulares e da membrana plasmática, remodelando-a ao longo do trânsito epididimário, conferindo motilidade e capacidade de fertilização durante o processo (SOLER et al., 2017). Além disso, sabe-se que existe uma associação morfofisiológica intimamente relacionada às dimensões e formato dos espermatozoides com a sua motilidade (GARCÍA-VÁZQUEZ et al., 2016). Uma variação fora do padrão da espécie pode prejudicar a progressão dos espermatozoides ao longo do trato reprodutor feminino em direção ao oócito e inviabilizando a fertilização. Assim, diversas técnicas podem ser utilizadas para avaliar os parâmetros inerentes à morfoestrutura da membrana e motilidade dos espermatozoides a fim de compreender sua fisiologia reprodutiva.

É fundamental estabelecer biotécnicas mais adequadas para conservar e preservar espécies de animais silvestres (SILVA et al., 2010). Para tanto, é necessário compreender a fisiologia reprodutiva da espécie estudada. Assim, quanto aos machos de cutias (*D. leporina*),

é fundamental que seja realizada a avaliação e caracterização morfofisiológica dos espermatozoides ao longo da maturação. Esta informação é a base para a tomada de decisões futuras sobre biotécnicas reprodutivas. Dessa forma, essas medidas contribuem para a formação de criobiobancos que possibilitam a preservação do germoplasma e o estabelecimento de técnicas de reprodução assistida (SILVA et al., 2012, 2013).

2.4.1. Avaliação por meio de análise espermática assistida por computador (CASA)

Um dos métodos mais eficientes e fidedignos para se avaliar os parâmetros cinéticos do espermatozoide é através da análise computadorizada performada pelo CASA (AMANN; WABERSKI, 2014). O sistema avalia os espermatozoides através da captura de imagem das células através de um microscópio e, através dos algoritmos de softwares, converte as imagens em medidas das variáveis cinéticas. O software não só avalia os espermatozoides individuais, mas também promovem o agrupamento das células espermáticas com base em um ou mais atributos, para que os resultados reflitam em subpopulações de espermatozoides com propriedades semelhantes.

Os sistemas CASA podem ser uma ótima ferramenta para comparar objetivamente a motilidade e morfologia espermática (AMANN; WABERSKI, 2014). Além disso uma vez que a motilidade espermática está intimamente relacionada à sua capacidade fertilizante, estudos têm sido desenvolvidos no sentido de avaliar estes parâmetros ao longo do trânsito epididimário, caracterizando a aquisição da motilidade nos diferentes estágios durante a maturação (ANGRIMANI et al., 2017; SOLER et al., 2017). Desta foram, os sistemas CASA fornecem informações importantes para mensurar a qualidade do sêmen, tanto para a comercialização, como para a avaliação das respostas espermáticas frente às mudanças físicas e químicas no microambiente de pesquisa.

Os principais parâmetros espermáticos avaliados através do CASA são: a motilidade total (%), motilidade progressiva (%), velocidade média de trajetória (VAP, μm/s), velocidade em linha reta (VSL, μm/s), velocidade curvilínea (VCL, μm/s), amplitude lateral de cabeça (ALH, μm), frequência de batimento cruzamento (BCF, Hz), retilinearidade (STR, %) e linearidade (LIN, %). Ainda, quanto a intensidade de sua velocidade, a população total de espermatozoides pode ser subdividida em quatro categorias: rápida, média, lenta e estática (%).

2.4.2. Avaliação da funcionalidade da membrana espermática pelo teste hiposmótico

O teste hiposmótico (HOST) avalia a capacidade da membrana espermática de se adaptar ao influxo de fluidos para dentro da célula, a fim de proporcionar um equilíbrio osmótico entre os fluidos extra e intracelular (SANTOS et al., 2011). O teste mostra, por meio do transporte de água para dentro da célula, quais espermatozoides possuem plena funcionalidade e integridade de membrana (NEILD et al., 1999), sendo identificado naqueles que apresentam como resposta osmótica, o enrolamento da cauda devido ao aumento do volume celular. Portanto, o teste é baseado em um conceito fisiológico simples: células vivas incham quando expostas a um ambiente hiposmótico, e células com membranas danificadas não (ZUBAIR et al., 2015). Apesar da relativa simplicidade do teste de HOS, existem alguns pontos que precisam ser esclarecidos para se obter maior confiabilidade, como a osmolaridade ideal da solução hiposmótica (MELO et al., 2003), pois cada espécie pode apresentar uma resposta osmótica espermática diferente (MATSON et al., 2009).

Nesse contexto, o teste hiposmótico tem sido aplicado para avaliar a funcionalidade da membrana espermática de várias espécies domésticas, como cães (KUMI-DIAKA, 1993), suínos (LECHNIAK et al., 2002), ovinos (OBERST et al., 2003), equinos (ALVES et al., 2004) e bovinos (MARTINS et al., 2011). No entanto, quanto aos animais silvestres, o seu uso do HOST é limitado, uma vez que porque a resposta osmótica do espermatozóide é desconhecida para a maioria das espécies selvagens. A validação do HOST já fora realizada para algumas espécies selvagens, como o tatu-bola (SANTOS et al., 2011), o urso pardo (PÉREZ-GARNELO et al., 2013) e o queixada (VIEIRA et al., 2022).

2.4.3. Avaliação da integridade estrutural de membrana e atividade mitocondrial do espermatozoide

Ao mesmo tempo que ocorre a remodelação da membrana plasmática do espermatozoide durante a maturação espermática, as células germinativas sofrem modificações proporcionais à aquisição de motilidade e capacidade de fertilização. Estas alterações são devidas principalmente à absorção e reposicionamento de glicoproteínas e componentes lipídicos em diferentes domínios da membrana ao longo o trânsito epididimário (COOPER, 1995). Tudo isso é cuidadosamente processado em diferentes regiões do epidídimo, afetando indiretamente as membranas intracelulares, nucleoproteínas e organelas, como as mitocôndrias (JONES, 1998). Para prever a qualidade da motilidade espermática, é necessário avaliar sua

função mitocondrial, uma vez que o potencial de movimento da célula é altamente dependente da energia metabólica derivada das funções mitocondriais (MARCHETTI et al., 2004). A análise da integridade estrutural da membrana e da atividade mitocondrial geralmente é realizada por meio de sondas fluorescentes e microscópios específicos para esse tipo de abordagem.

Ao estudarem a maturação espermática no epidídimo de camundongos, Yuan et al. (2013) relataram que durante o processo, foi identificado uma relação proporcional entre a função mitocondrial e a motilidade espermática. Os autores identificaram que isso foi devido ao aumento e a maturação da bainha mitocondrial ao longo do trânsito espermático. Isto está associado ao amadurecimento do complexo da cadeia respiratória mitocondrial, bem como ao desenvolvimento de cristas (PARK; PANG, 2021).

2.4.4. Avaliação morfológica do espermatozoide

Para avaliar o espermatozoide de uma espécie, um dos métodos basilares é a análise morfológica, que consiste em identificar e descrever a estrutura fisiológica do espermatozoide e suas possíveis anormalidades, o que afetam direta ou indiretamente a fertilização (CHEMES; SEDO, 2012). No entanto, a microscopia de luz convencional limita-se à ampliação da objetiva e por que é feita com base na observação visual, o que acaba não permitindo observações com alto padrão de detalhes. Assim, a avaliação subjetiva da morfologia espermática pode levar a resultados de grande variação (HIDALGO et al., 2005). Dessa forma, a avaliação ultraestrutural por microscopia eletrônica minimiza esses problemas de subjetividade ao proporcionar a observação detalhada da superfície, a disposição e a integridade das organelas e estruturas celulares, bem como possíveis anormalidades que podem passar despercebidas na microscopia de luz (CHEMES; RAWE, 2003).

Os principais defeitos morfológicos encontrados em espermatozoides de mamíferos são àqueles relacionados ao enrolamento da cauda e a presença de gota citoplasmática (GU et al., 2019). Estudos com espermatozoide de roedores demonstram que a migração progressiva da gota citoplasmática está positivamente relacionada com a aquisição da motilidade ao longo do trânsito epididimário (GOYAL et al., 2001; GU et al., 2019).

2.4.5. Avaliação morfométrica do espermatozoide

Além da análise morfológica convencional, pode-se utilizar conjuntamente a avaliação morfométrica espermática. Essa abordagem é essencial, uma vez que permite identificar e estabelecer as métricas espermáticas padrões para cada espécie nos diferentes estágios da maturação, a fim de determinar os defeitos que ocorrem principalmente na cabeça, peça intermediária e cauda do esperma, bem como associar empiricamente as funções espermáticas com sua morfometria (IMMLER et al., 2010). Outra aplicação da morfometria é fornecer valores de referência para a espécie, que podem ser aplicados a softwares de análise espermática assistida por computador (CASA), estabelecendo a padronização de suas configurações (setups), sendo que poucas espécies possuem referências padronizadas para esse tipo de análise (SOLER et al., 2017).

Os espermatozoides de diferentes espécies podem apresentar padrões morfométricos bem distintos, uma vez que existe uma considerável variação de formatos e tamanhos, como observado em espécies de roedores. Por exemplo, existem espermatozoides com cabeça de formato falciforme, como em ratos (*Rattus norvegicus*) e hamsters (*Mesocricetus auratus*) (GU et al. 2019), de formato oval, como em cutias (*D. leporina*) (CASTELO et al., 2015a) e de formato espatular, como em cobaia (*Cavia porcellus*) (GU et al. 2019). Em cutias (*D. leporina*), a morfometria da cabeça, da peça intermediária e da cauda, obtida por eletroejaculação e com origem na cauda do epidídimo já foram descritas (Castelo et al., 2015b). No entanto, as dimensões dos espermatozoides de outros segmentos do epidídimo permanecem desconhecidas.

2.5. Efeito das variáveis ambientais sobre a reprodução de roedores

As informações acerca da influência dos fatores climáticos sobre as características reprodutivas dos roedores silvestres, bem como seus efeitos, geraram o artigo de revisão que se configura no Capítulo I desta tese.

3. JUSTIFICATIVA

Embora o semiárido nordestino Brasileiro tenha um grande potencial econômico, científico e tecnológico, pouco tem sido feito a respeito de políticas e estudos que visem a conservação das espécies silvestres, a multiplicação em cativeiro e sua sustentabilidade. As cutias são roedores silvestres que apresentam valor ecológico e econômico para a região e vitais na manutenção de muitos ecossistemas, visto que contribuem na dispersão de sementes de diversas espécies vegetais, são presas de carnívoros de médio e grande porte, além de poderem ser explorados zootecnicamente como fonte proteica na alimentação humana. No entanto, assim como a maioria das espécies silvestres, existem diversas lacunas a serem compreendidas acerca de fisiologia reprodutiva da cutia.

Nesse contexto, justifica-se conhecer a fisiologia espermática da cutia ao longo de sua maturação no epidídimo, bem como conhecer como as características espermáticas são afetadas pelas variáveis climáticas durante os períodos seco e chuvoso do semiárido nordestino. A compreensão das modificações morfofuncionais ocasionadas ao longo do trânsito epididimário, bem como dos fatores ambientais que as influenciam, permitem identificar e descrever os padrões espermáticos durante a maturação, possibilitando o estabelecimento e melhoramento de protocolos de biotécnicas para a conservação e preservação da espécie. Além disto, em utilizando a cutia como modelo experimental, tais estudos seriam importantes não apenas para biotécnicas e formação de bancos de germoplasma desta espécie, mas também para a conservação de espécies correlatas filogeneticamente, como a *Dasyprocta ruatanique*, *Dasyprocta coibae* e *Dasyprocta mexicana*.

4. HIPÓTESES

O espermatozoide de cutia sofre diversas modificações ao longo do trânsito epididimário, promovendo o desenvolvimento da funcionalidade e motilidade à medida que este sofre a maturação.

Embora os epidídimos de cutias sejam intra-abdominais, as modificações espermáticas ao longo do trânsito epididimário são similares aos padrões verificados em espécies roedoras escrotais.

A avaliação da integridade funcional do espermatozoide da cutia pelo HOST com diferentes osmolaridades apresentará melhores resultados a 0 mOsm/L.

A resposta osmótica da membrana espermática se modifica ao longo do trânsito epididimário.

Os parâmetros morfológicos, morfométricos, ultraestruturais e funcionais do espermatozoide de cutia apresentarão diferenças quantitativa e qualitativas, a depender da estação em que forem coletados, se a seca ou chuvosa.

Os espermatozoides de cutia coletados durante o período chuvoso de uma região semiárida apresentarão melhores resultados, quando comparados aos do período seco.

5. OBJETIVOS

Objetivo Geral

Descrever as características espermáticas de cutias (*Dasyprocta leporina*), ao longo do trânsito epididimário, e identificar a existência de interações destas com as variáveis ambientais de uma região semiárida.

Objetivos Específicos

- Descrever detalhadamente as modificações morfológicas no espermatozoides de cutias durante o trânsito epididimário, identificando o perfil de alterações espermáticas mais frequente em cada região do epidídimo;
- Evidenciar a existência de modificações morfométricas e ultraestruturais em espermatozoides de cutias ao longo do trânsito epididimário;
- Avaliar as modificações estruturais de membrana espermática, bem como o potencial mitocondrial de espermatozoides de cutias durante o processo de maturação no epidídimo;
- Compreender a evolução do processo de aquisição de motilidade espermática ao longo do trânsito epididimário em cutias;
- Comparar diferentes soluções hiposmóticas no sentido de estabelecer o teste hiposmótico para avaliação da funcionalidade de membrana plasmática em cutias;
- Apresentar a evolução da aquisição da funcionalidade de membrana espermática ao longo do trânsito epididimário em cutias;
- Verificar a existência de interações entre função de membrana e outros parâmetros espermáticos em cutias;
- Caracterizar os parâmetros espermáticos de cutias criadas em uma região semiárida de acordo com os diferentes períodos climáticos;
- Identificar relações entre as variáveis ambientais da região semiárida e os parâmetros espermáticos de cutias.

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CAPÍTULO I

Understanding how environmental factors can influence reproductive aspects of wild myomorphic and hystricomorphic rodents

Entendendo como fatores ambientais podem influenciar aspectos reprodutivos de roedores miomórficos e histricomórficos selvagens

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REVIEW ARTICLE

Understanding how environmental factors influence reproductive aspects of wild myomorphic and hystricomorphic rodents

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Abstract

Myomorphic and hystricomorphic rodents are vital for maintaining various ecosystems around the planet. This review enables a better understanding of how these rodents respond to environmental factors and adapt to climate adversities. Innumerable factors, such as photoperiod, rainfall, and temperature, can impair or contribute to the quality of rodent reproductive parameters. Prolonged animal exposure to high ambient temperatures alters thermoregulation mechanisms and causes testicular and ovarian tissue degeneration and hormonal deregulation. Photoperiod influences the biological circannual rhythm and reproductive cycles of rodents because it strongly regulates melatonin secretion by the pineal gland, which modulates gonadotropic hormone secretion. Rainfall quantity directly regulates the abundance of fruits in an ecosystem, which modulates the reproductive seasonality of species which are most dependent on a seasonal fruit-based diet. Species with a more diversified fruit diet have smaller reproductive seasonality. As such, habitats are chosen by animals for various reasons, including the availability of food, sexual partners, intra-and inter-specific competition, and predation. This knowledge allows us to monitor and establish management plans to aid in conservation strategies for wild rodent species.

Keywords: climate, seasonality, reproductive physiology, Rodentia, wildlife.

Introduction

According to the International Union for Conservation of Nature (IUCN), animal extinction occurs at a much higher rate than speciation, which is estimated to occur in at least 25% of endangered mammal species (IUCN, 2019). This event is probably a consequence of climate change caused by global warming, which limits the survival of species sensitive to large variations in climate elements. Anthropic actions also exert a strong influence on species extinctions due to habitat destruction by burning, deforestation (Comizzoli et al., 2009; Comizzoli, 2015), and predatory hunting (Bodmer et al., 2018; El Bizri et al., 2018). These factors may induce the disappearance of a single species in an equilibrated ecosystem, which can compromise the functioning of an entire food chain, harming several directly or indirectly involved populations.

The largest number of placental mammalian species belong to the rodent order, with over 2,000 cataloged species, accounting for a total of 40% of the species in the mammal class, inhabiting all continents and islands, except Antarctica (Carleton and Musser, 2005). The

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importance of rodents is enormous, as they are vital to the maintenance of many ecosystems around the world because these animals reproduce quickly and are part of the diet of various predatory species (Muñoz et al., 2009). In addition, rodents such as those belonging to Myomorpha and Hystricomorpha suborders act as excellent seed dispersers in various biomes (Muñoz and Bonal, 2011).

Various environmental factors influence reproduction in rodents, including photoperiod (Muteka et al., 2006c; Trillmich et al., 2009; Tavolaro et al., 2015), rainfall (Dubost et al., 2005; Sarli et al., 2016; El Bizri et al., 2018), and temperature (Sarli et al., 2016; Salman et al., 2017; Fabio-Braga and Klein, 2018) Understanding how these environmental factors affect rodents is essential for identifying the physiological and behavioral responses of these animals, thus making it possible to establish strategies that can mitigate the deleterious effects on reproductive activity (Rezende and Bozinovic, 2019), whether caused by climate change, or direct anthropic actions that harm ecosystems.

Studies on the reproductive characteristics of wildlife animals are more complex than those of farm and domestic animals, and for many species, the literature is still scarce. To fill the gaps in these characteristics, many wild species are bred in captivity, either for conservation, preservation, the establishment of biotechnology protocols, or to study their biology (Frankham, 2008; Comizzoli et al., 2009; Comizzoli, 2015; Queiroz et al., 2020). In this context, studies of captive wild rodents may become an important strategy for their conservation (Praxedes et al., 2018), particularly if the rodent species show good acclimatization to captivity and relatively easy management (Slade et al., 2014; Castelo et al., 2015; Queiroz et al., 2019). This practice allows us to understand more efficiently how environmental variables influence the characteristics and behavior of rodents, thus improving our understanding of their reproductive biology.

This review addresses the different responses of reproductive aspects of wild rodents of the suborders Myomorpha and Hystricomorpha to the climatic elements of different biomes, highlighting how the main abiotic factors can affect their reproduction in different weather conditions, whether as free-living animals or bred in captivity. This information could assist in the monitoring of these animals by providing support for the improvement of management strategies and assisted reproductive techniques aimed at their preservation in the face of climate adversity.

Myomorphic and hystricomorphic rodents – general aspects

Myomorpha is the largest suborder of rodents, containing around 1130 species, almost a quarter of all mammalian species (Carleton and Musser, 2005). This group includes the superfamilies Muroidea (bamboo rats, hamsters, true rats, true mice, gerbils, spiny dormice, lemmings, and voles) and Dipodoidea (jerboas and jumping mice). They are classified according to the structure of the mandible and molar teeth, where part of the medial masseter muscles is inserted into the mandible, in addition to crossing the infraorbital foramen to insert into the rostral muzzle (O'Malley, 2005). Myomorphs are cosmopolitan and generally nocturnal granivores.

In a broader sense, the suborder Hystricomorpha refers to rodents with a hystricomorphous zygomasseteric system, being medium to large rodents. This large group includes the superfamily Ctenodactylidae (comb rats), and the infraorders Caviomorpha (agoutis, pacas, capybaras, guinea pigs, spiny rats, chinchillas, and viscachas) and Phiomorpha (dassie rats and mole-rats) (Carleton and Musser, 2005). They are widely distributed in South America and Africa and act as excellent seed dispersers (Muñoz and Bonal, 2011) because they feed on fruits and leaves (Dubost and Henry, 2017).

For the present review, a keyword search for relevant literature presenting data related to the influence of abiotic factors, such as photoperiod, rainfall, and temperature, on rodent reproduction was conducted, focusing on the suborders Myomorpha and Hystricomorpha. For the search, we used PubMed, Science Direct, Google Scholar, Web of Science, and SCOPUS. Date limitations were removed, but Boolean terms "AND" and "OR" were included wherever possible. Additional relevant articles were sought from the reference lists of all included studies using the snowball sampling method. The authors independently screened the manuscripts to reduce bias and improve the reliability of the findings.

Photoperiod

The period of daylight hours in a given geographic region, represented by day length, is called the photoperiod (Silva, 2000). Its length depends on latitude and season, with greater variation the further away from the equator. The ability of animals to react to the photoperiod duration is called photoperiodism, which can affect its behavior and reproduction (Santos, 2002; Muteka et al., 2006c; Tavolaro et al., 2015).

The photoperiod duration (Figure 1) is one of the most important drivers of the biological circannual rhythm of an animal (Table 1), because it strongly regulates melatonin secretion by the pineal gland (Tavolaro et al., 2015; Gallol et al., 2020). Melatonin is a neurotransmitter responsible for informing the body of the daylight hours and corresponding time of year (Rocha et al., 2013). It modulates the secretion of follicle stimulating hormone (FSH) and luteinizing hormone (LH) through membrane receptors in hypothalamic-pituitary-gonadal axis cells, regulating the seasonal rhythms and reproductive cycles of several mammals, including rodents (Muteka et al., 2006c; Tavolaro et al., 2015). In viscachas (*Lagostomus maximus*), which are rodents of two genera (*Lagidium* and *Lagostomus*) in the family Chinchillidae, native to South America, it is well demonstrated that melatonin variation strongly affects endocrine physiology, thus causing photoperiod-dependent seasonality in both males (Acosta and Mohamed, 2011) and females (Busolini et al., 2017).

Table 1. Influence of different climatic factors on reproductive aspects of rodent species.

Species	Gender	Abiotic factor	Geographic location	Main outcomes	Authors
Aethomys ineptus	Male and female	Photoperiod	South Africa	This is a reproductive seasonal species restricted to the summer and fall months, in the south hemisphere.	Muteka et al. (2006a)
Aethomys namaquensis	Male and female	Photoperiod	Southern Africa	Pregnant and lactating females were observed only during spring and summer, besides a significant increase in testicular volume, seminiferous tubule diameter and testosterone plasma concentration in males.	Muteka et al. (2006b)
Aethomys ineptus	Male	Photoperiod	South Africa	Both species exhibited significantly higher testicular mass when exposed to high photoperiod than during short light hours.	Muteka et al. (2006c)
Aethomys namaquensis					
Acomys dimidiatus	Male and female	Rainfall and temperature	Saudi Arabia	This animal is a seasonal breeder that can breed opportunistically. Male and female correlate reproductive recrudescence to rainfall. Pregnancies occur in most seasons apart from the winter.	Sarli et al. (2016)

Table 1. Continued...

Species	Gender	Abiotic factor	Geographic location	Main outcomes	Authors
C57BL/6J mice	Male	Temperature	Under laboratory conditions	Heat caused by high temperature cause damage to the testicles, such as testicular atrophy, presence of vacuolization and perforations in the seminiferous tubule epithelium, germ cell death.	Zhang et al. (2020)
Cavia aperea	Female	Photoperiod	Under laboratory conditions	Female pups kept in groups of two matured at about 47 days when born into lengthening and 79 days when born into shortening day length. They kept under identical short-day conditions after weaning on day 20 of life.	Trillmich et al. (2009)
Cuniculus paca	Female	Rainfall	Amazon rainforest	The precipitation of upland forest fruiting was positively correlated with precipitation, which was causally related to higher rates of pregnancy, lactation, and weaning of offspring.	El Bizri et al. (2018)
Fischer 344 rat (F344)	Male	Photoperiod	Under laboratory conditions	Rats held under photoperiods of ≥ 12 h of light/day showed increased growth, food intake and higher paired testes weight relative to rats held under photoperiods of ≤ 10 h of light/day.	Tavolaro et al. (2015)
Gerbilliscus leucogaster	Male and female	Rainfall and temperature	Namibia	The ovarian activity increased at the end of the dry period and throughout the wet months. During the wet months, pregnant and lactating females were found, besides a increase of testicular mass relative to body mass, testicular volume, and seminiferous tubule diameter in males.	Muteka et al. (2018)

Table 1. Continued...

Species	Gender	Abiotic factor	Geographic location	Main outcomes	Authors
Lagostomus maximus maximus	Male	Reproductive seasonality	Argentina	Greater number of morphological defects was observed in the period of decreased gonadal activity (33.8%, winter) than in the activity period (7.8%, summer-autumn). The morphological characteristics of sperm undergo significant changes during their reproductive cycle.	Cruceño et al. (2013)
Lasiopodomys brandtii	Male	Photoperiod	China	It was displayed a synchronous peak in gonadal activity with annual day length around summer solstice. The hypothalamic photoperiod genes studied regulate seasonal breeding in a natural rodent population.	Wang et al. (2019)
Myoprocta exilis, Dasyprocta leporina, Cuniculus paca	Male and female	Rainfall and temperature	French Guiana forest	The species tended to breed in the period corresponding to the largest supply of fruits from their diets. It was linked to the seasonal importance of fruits in diets, the most aseasonal species having the most diversified diet during the poor fruit season.	Dubost et al. (2005)
Wistar rat	Female	Temperature	Under laboratory conditions	The high temperature is responsible for suppression of ovarian function by decreasing the expression of steroidogenic enzymes, estrogen and gonadotropin receptors in the ovary.	Zheng et al. (2019)

In equatorial regions, which have little difference in daylight length throughout the year, animals are minimally influenced by this phenomenon. In contrast, in regions closer to the tropics, where photoperiod fluctuation has a greater influence on animals, reproductive seasonality is more evident, and is also related to thermal stress and nutritional deficiencies depending on the time of year (Muteka et al., 2006a, b, c).

Photoperiod can influence the onset of puberty in female rodents, as demonstrated by Trillmich et al. (2009) in guinea pigs (*Cavia aperea*) (Rodentia, Caviidae) under laboratory conditions. The study showed that individuals that were exposed to many hours of light per day (14:10 h, light:dark), reached puberty within 47 days, while under the lowest light exposure per day, individuals reached puberty in 79 days (10:14 h, light:dark).

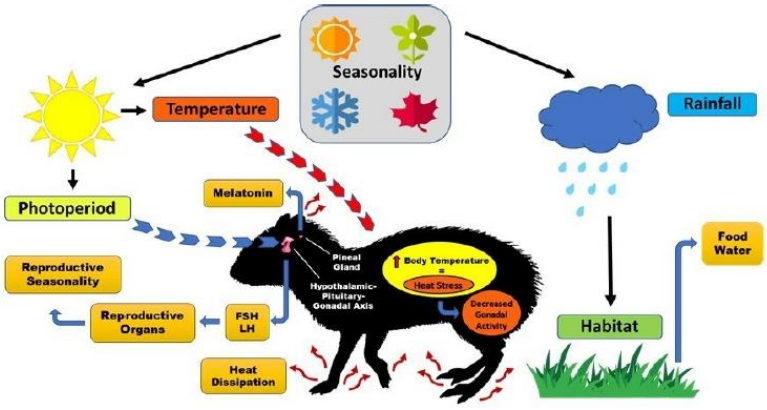

Figure 1. Schematic design of how the main environmental elements influence the reproduction of rodents. In general, the more distant a region is from the equator, the greater the variations in temperature and rainfall between the seasons, thus representing the seasonality. Moreover, photoperiod is related to the presence/absence of light that causes variations in melatonin production by the pineal gland, which modulates the FSH and LH levels, resulting in the regulation of gonadal activity and reproductive seasonality. Rainfall refers to the precipitation that is intricately linked to the supply of water and food, which in some cases may cease the reproductive cycle if the habitat does not have an abundance and variety of resources throughout the year. Finally, the excessive and prolonged heat stress tends to increase body temperature, which decreases gonadal activity; however, some rodents can dissipate this heat through body regions that are usually rich in blood vessels and low in hair, such as the ears, paws, and tail.

In males, the influence of photoperiod on various reproductive aspects is also evident. Studying the Tete veld rat (*Aethomys ineptus)* and the Nomaq rock rat *(Aethomys namaquensis*), Muteka et al. (2006c) demonstrated that during long days (16 h photoperiod), both species exhibited significantly greater testicular mass in relation to body mass, in addition to greater testicular volume and seminiferous tubule diameter, than during short days (8 h photoperiod). However, it is necessary to emphasize, that different rodent species can present different responses to photoperiod, since only in *A. namaquensis*, circulating plasma testosterone concentrations showed higher values on long days (Muteka et al., 2006c). In addition, Wang et al. (2019) onducted an interesting study, which demonstrated that hypothalamic photoperiod genes (*Dio2/3*, *Rfrp-3*, *Kiss-1*, and *GnRH*) regulate seasonal breeding in a natural wild rodent population of male Brandt's voles (*Lasiopodomys brandtii*) from inner Mongolia, China. Over the four years of study, the researchers verified that these rodents exhibited a synchronous peak in photoperiod-related gonadal activity around the summer solstice, as evidenced by the high expression of hypothalamic genes and better reproductive parameters in these periods. It was observed that testicular mass, epididymis mass, and fecal testosterone levels were positively correlated with day length, with the best values obtained between June and July (summer).

Rainfall

The rain precipitation quantity (Figure 1) for a region in a given time is called rainfall (Silva, 2000). Unpredictable rain and large variations in air temperature influence the reproduction of small mammals (Table 1). Harsh conditions associated with arid environments, where energy and water are severely restricted, may limit or even cease reproduction in rodents during the favorable period.

Sarli et al. (2016) observed that the free-living rat *Acomys imidiatus* found in Saudi Arabia reproduces seasonally, stopping reproduction during the dry season, which corresponds to autumn and winter in that region. This is closely linked to the rainfall quantity and indirectly to other factors such as salinity in the vegetation of this desert, which affects the availability of

food for these rodents (Figure 1). The researchers also found that the number of ovarian follicles in the females was significantly lower during the dry autumn and winter (0 mm rainfall) than during the spring and summer rainy season (61.8 mm rainfall). In males, testicular volume and seminiferous tubule diameter were also significantly higher during spring and summer. Plasma testosterone concentrations in males and progesterone in females were also significantly higher during the rainy (summer) period than during the dry period (Sarli et al., 2016).

Similar results were found by Dubost et al. (2005), who demonstrated an improvement in gestation rates in three free-living wild rodent species, the acouchi (*Myoprocta exilis)*, paca (*Cuniculus paca)*, and crown-rumped agouti (*Dasyprocta leporina)*, during increased rainfall in the French Guiana rainforest. The researchers attributed the reproductive seasonality in these species to the production of some forest fruits, which are important in the diet of these animals (Figure 1). These fruits depended directly on the rainfall amount to regulate their abundance, and thus, the less seasonal species had a more diverse diet during the scarcer fruit season.

For tropical regions, in a 15-year participatory study in two areas in the northwest of the Amazon, El Bizri et al. (2018) analyzed reproductive organs of pacas (*C. paca*) obtained from voluntary donations by hunters. They used data on precipitation, river water level, and fruiting phenology. They concluded that the upland forest fruiting was positively correlated with precipitation (Figure 1), which was directly linked to higher rates of pregnancy, lactation, and weaning of offspring.

Temperature

The study of animal thermal performance curves is generally used to anticipate the effects of ambient temperature on characteristics of interest in these organisms and is one of the means to predict the potential effects of global warming on ecological systems (Rezende and Bozinovic, 2019), since ambient temperature (Table 1) is essential for maintaining animal health (Silva, 2000; Santos, 2002).

Sudden changes in temperature cause heat stress, usually causing immunological depreciation, which makes animals more susceptible to infections (Silva, 2000). Homeothermic animals, such as rodents, are known to respond to environmental thermal variations by modulating thermogenesis, thereby activating sensible and evaporative heat transfer mechanisms, either for loss or gain of thermal energy (El-Sabrout, 2018; Mascarenhas et al., 2018). In some rodent species, such as the mole rats (*Fukomys mechowii* and *Heliophobius argenteocinereus*) (Šumbera et al., 2007) and agoutis (*Dasyprocta aguti*) (Queiroz et al., 2019), there is a physiological pathway for heat dissipation through a body area that is normally rich in blood vessels and low in hair, that facilitates heat dissipation (Figure 1); this is called the thermal window (Romanovsky et al., 2002). In this regard, Queiroz et al. (2020), studying Spix's yellow-toothed cavy (*Galea spixii*) in a semi-arid environment in northeastern Brazil, recently identified that the pinnae and vibrissae regions act as thermal windows, thus being the first line of defense against overheating.

Most small rodents have a thermal comfort range of 21-24 °C, while laboratory animals such as guinea pigs adapt better to the temperature range of 18-20 °C (Santos, 2002). Prolonged exposure to high temperatures alters thermoregulatory mechanisms, rendering them inefficient in dissipating excess body heat (Rashamol et al., 2018; Maurya et al., 2019), which is the main cause of infertility in male animals (Setchell, 2006). In fact, some free-living African wild rodents cease reproductive activities during periods of high temperatures, as observed for *A. ineptus* (Muteka et al., 2006a), *A. namaquensis* (Muteka et al., 2006b), and *Gerbilliscus leucogaster* (Muteka et al., 2018). In addition, as demonstrated under laboratory conditions, exposure to low temperatures causes respiratory problems (Santos, 2002) and reduces basal metabolism in free-living rodent species (Muteka et al., 2018), thus indirectly decreasing their reproductive potential.

Depending on the intensity of the thermal injury, testicular parenchyma degeneration and serious damage to spermatogenesis may occur (Kleisner et al., 2010; Durairajanayagam et al.,

2014; Fabio-Braga and Klein, 2018). To illustrate this, Zhang et al. (2020) subjected male C57BL/6J mice to two overheating treatments at 39 and 42 ºC, submerging the lower parts of the body (hind legs, tail, and scrotum) in a thermostatically controlled water bath for 20 min. They observed that heat treatment at 42 ºC triggered the greatest damage to the testicles, such as testicular atrophy, presence of vacuolization and perforations in the seminiferous tubule epithelium, germ cell death, and fracture of the sterile junction of Sertoli cells. However, the treatment at 39 ºC had no significant impact on testicular histology and germ cell morphology, which suggests the existence of a threshold of the testicular response to thermal stress at this temperature.

The degeneration of ovarian tissue and reduction in folliculogenesis and oogenesis were also observed as responses to thermal injury in females (Zheng et al., 2019). In prepubertal female Wistar rats, Zheng et al. (2019) observed the ovarian function of these animals in the face of thermal stress at different temperatures (38, 40, and 42 °C for 2 h/ day). The plasma levels of LH and triglycerides and the expression of LH receptors FSH and estradiol-17β in the ovaries were significantly lower at higher temperatures (especially at 42 °C) than in the control (26 °C). Liver metabolic function was significantly reduced in rats heated to 42 °C, as caspase-3 and NK-κB gene expression were higher at higher temperatures. These results indicate that high temperatures may suppress ovarian function, decreasing the expression of steroidogenic enzymes, estrogen receptors, and gonadotropin in the ovary (Figure 1).

It is important to note, however, that depending on the species, the higher temperature will not always negatively affect reproduction. For example, when studying free-living Saudi Arabian rats (*A. imidiatus*), Sarli et al. (2016) found that during the months with the highest average air temperature (spring and summer), the best reproductive parameter results in the testes and ovaries occurred, along with higher values of testosterone and plasma progesterone in males and females, respectively. This was influenced by another element closely associated with the period of the year, rainfall, as previously mentioned. It is therefore clear that sometimes one isolated abiotic factor is not sufficient to influence reproductive performance of some species, but instead a sum of various factors that characterize the habitat in which the individuals live.

Habitat

The climate of a region is established by a series of variable atmospheric conditions throughout the year, such as rainfall, photoperiod, humidity, and air temperature (Bronson and Heideman, 1994). In each biome, these meteorological variables (Figure 1) directly or indirectly affect the fauna, flora, and relief. These geophysical interactions cause, in addition to the biotic factors, fluctuations in food and water availability during the year, by the environment (Stapp, 1997). This defines the survival of certain animal populations in a given habitat, and restricts or eliminates the reproduction and presence of those most sensitive to fluctuations in these resources (Wube et al., 2009; Muteka et al., 2006c, 2018). Habitats are chosen by animals for various reasons, mainly the availability of food, sexual partners, intra- and inter-specific competition and predation.

According to Stapp (1997), there are several survival criteria for the choice of habitat. For instance, insectivorous rodents that inhabit the state of Colorado, United States, choose microhabitats based on the availability of prey, according to seasonal and spatial variations and other resources such as water (Stapp, 1997), as illustrated in Figure 1. In contrast, pacas (*C. paca*) from the Atlantic Forest in southern Brazil chose their microhabitat based on good water availability and the presence of a dense forest cover (Goulart et al., 2009). According to these researchers, information on habitat selection allows more efforts to be directed to the habitat associated with focal species, and indicates the importance of environmental heterogeneity, which makes species coexistence possible.

In a continental country such as Brazil, small mammals may be subjected to different habitats and respond accordingly. In the Atlantic Forest, a region on the Brazilian east coast, where there is a predominance of dense forests, wetlands, and great diversity of fauna, Gentile

and Fernandez (1999) observed that different species of Sigmodontinae rodents (*Nectomys squamipes*, *Akodon cursor*, and *Oligoryzomys nigripes*) respond to different aspects of the microhabitat, thus presenting significant oscillations in their populations throughout the year. In the Cerrado biome, in midwestern Brazil, Santos-Filho et al. (2012) found no relationship between microhabitat, richness variables, and abundance of small rodent populations. In Pantanal (another Brazilian biome), Santos-Filho et al. (2008) analyzed 13 rodent species captured in forest fragments of the region and found no significant variation in the total richness and abundance of these animals between the dry and rainy periods of the year.

It is necessary to highlight, however, that sometimes, even among animals that coexist in the same habitat, large differences occur between genetically close rodent species. These aspects were clearly described by Dubost et al. (2005) in their study on rainforest rodents from French Guiana (*M. exilis, C. paca,* and *D. leporina*), which showed reproductive differences among them, regarding sexual behavior, puberty, and reproductive seasonality. It is therefore evident that the survival of a species is a consequence of the combination of its ability to successfully reproduce and minimize the loss of offspring through adaptation in its own microenvironment, which eventually has a direct influence on the choice of most suitable habitat for each species.

Reproductive seasonality

Reproductive seasonality is the phenomenon by which some species decrease or cease sexual activity during a certain period of the year, usually caused by climatic factors, especially temperature, rainfall, or photoperiod (Henry and Dubost, 2012; Maia and Silva, 2016). It is known that the effects of seasonality can cause changes in both the morphophysiology and biochemistry of sexual gonads during the reproductive cycle (Aguilera-Merlo et al., 2009).

Female rodents may exhibit one or more series of estrous cycles during the reproductive season, directing the parturition to the most favorable period for reproduction (Bronson and Heideman, 1994; Henry and Dubost, 2012; Muteka et al., 2006a, b, c). In contrast, some species may present a non-seasonal reproduction as observed for female black agoutis (*Dasyprocta fuliginosa*) that present a non-seasonal polyestrous cycle and can reproduce throughout the year (Mayor et al., 2011).

Male rodents can increase the testicular volume and seminiferous tubule diameter, thus improving spermatogenesis efficiency during reproductive seasons (Muteka et al., 2018). This variation in the activity of the gonads as a response to different seasonal periods suggests a possible evolutionary strategy for improved opportunistic breeding (Bronson and Heideman, 1994; Muteka et al., 2006c, 2018), which focuses on the availability of resources such as food, water, and favorable environmental conditions.

To exemplify the role of seasonality, we highlight the study on Namaqua rock rats (*A. namaquensis*) conducted by Muteka et al. (2006b) in South Africa. The researchers observed the occurrence of pregnant and lactating females only during spring and summer, while no lactating females were registered during autumn and winter. Gonadal activity was determined by the evaluation of ovarian follicles, corpora lutea, progesterone, and 17b-estradiol plasma concentrations, and was significantly higher during spring and summer, compared with autumn and winter. In males, plasma testosterone concentration, testicular volume, and seminiferous tubule diameter increased significantly during spring and summer, while low to no spermatogenesis or presence of sperm in the epididymis was observed in autumn and winter.

The effects of rainfall and fruit diet on the male and female reproductive characteristics were also observed for the acouchi (*M. exilis*), paca (*C. paca*), agouti (*D. leporina*), and spiny rat (*Proechimys cuvieri*), raised in the French Guiana rainforest. All the species presented slightly pronounced reproductive seasonality and synchronism regarding birth, directing them to the rainiest months with a greater variety of fruit (November to April). The researchers found that the more the diets of species were composed of fruits, the more seasonal were the births (Dubost and Henry, 2017).

It is worth noting that the reproductive seasonality of wild rodents is modulated by environmental variables and their joint interactions. In regions where rainfall is scarce, such as in deserts or semi-arid climates, high temperatures and low air humidity are observed throughout the year (Sarli et al., 2016; Salman et al., 2017), which can promote serious energy and water restrictions that may affect the reproduction of small mammals. Despite this, some rodent species as free-living Saudi Arabian rats (*A. imidiatus*) are largely acclimated to arid conditions by adapting their reproductive cycle to environmental conditions (Sarli et al., 2016).

When studying male viscachas (*L. maximus*) in Argentina, Cruceño et al. (2013) aimed to relate the morphological changes in epidydimal sperm evaluated through scanning electron microscopy (SEM) with the period of the year of full (summer-autumn) or reduced (winter) gonadal activity. The analysis revealed that the sperm from the epidydimal corpus and cauda had great seasonal variations in structural parameters. In general, a greater number of morphological defects were observed in the period of decreased gonadal activity (33.8%) than in the activity period (7.8%). These outcomes confirm that the morphological characteristics of *Lagostomus* sperm undergo significant changes during their reproductive cycle under a seasonal influence.

Final considerations

Myomorphic and hystricomorphic rodents show physiological changes in response to abiotic factors present in the environment in which they live. Temperature, rainfall, and photoperiod represent the main environmental agents that regulate, interfere, or contribute to their reproductive capacity. Temperature is the main element that can impair reproduction in most species, but the combined effect of other climate variables cannot be disassociated from temperature, which can increase reproduction losses or mitigate the damage caused by it. Some species of wild rodents suffer less effects caused by a specific climate element, while others, even belonging to the same habitat, show great changes in their reproductive parameters due to variations of this element throughout the year. These differences fluctuate according to the supply and competition for food and water resources, sexual partners, and predation, with aim to achieve success in species survival and opportunistic breeding.

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Author contributions

MRD, JBFSJ, TSC and AEAL: Conceptualization, Data curation, Formal analysis, Methodology, Writing – original draft, Writing – review & editing; ARS: Conceptualization, Methodology, Supervision, Writing – original draft, Writing – review & editing.

CAPÍTULO II

Morphological, morphometric, ultrastructural, and functional evaluation of red-rumped agouti (*Dasyprocta leporina*) sperm during epididymal transit

Avaliação morfológica, morfométrica, ultraestrutural e funcional do espermatozoides de cutia (*Dasyprocta leporina*) durante o trânsito epididimário

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Running Head: Sperm maturation in agouti

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ABSTRACT

The red-rumped agouti (*Dasyprocta leporina*) is a hystricognath rodent with reproductive anatomical peculiarities presenting as an intra-abdominal testes-epididymis complex. This study was carried out to describe, for the first time, details related to the morphological and functional changes in sperm along the epididymal transit in agoutis. The testes-epididymal complexes were sampled from seven sexually mature agoutis. Sperm from different epididymal regions (caput, corpus, and cauda) were collected using the floating technique, and their morphology, morphometry, ultrastructure, mitochondrial activity, membrane structural integrity, and kinetic parameters were determined. The number of sperm collected (823.5 x 10^6 sperm) was higher in the epididymis cauda. No significant differences in normal sperm morphology among the different epididymal regions (caput, 82.42%; corpus, 86.71%; and cauda, 88.86%) were observed. The mean head length, head width, and tail length were highest in the caput (5.15 μm, 3.44 μm, and 32.04 μm, respectively), decreasing along the epididymal transit. Ultrastructure by scanning electron microscopy (SEM) revealed agglomeration of spermatozoa from caput and corpus, thus, enabling analysis of the gametes from only the epididymal cauda with clarity. Sperm from epididymis cauda showed the greatest proportion of membrane integrity and mitochondrial activity, followed by those from corpus and caput (79.71%, 58.9%, 47.7%, respectively). Significant increase in total motility, progressive motility, velocity average pathway -VAP, velocity straightline – VSL, velocity curvilinear – VCL, and rapid sperm in the caput-corpus-cauda direction were observed. These novel data contribute to the knowledge of sperm maturation in the red-rumped agouti.

Keywords: Wildlife, Rodents, Epididymis, Sperm physiology

1. Introduction

Agoutis (*Dasyprocta spp.*) is a wild hystricognath rodent that is distributed throughout Neotropical America. They are important seed dispersers and play an essential role in ecological balance (Silva et al., 2013). There are 13 species catalogued in the genus *Dasyprocta*, some of which are endangered such as *D. ruantanica* (Schipper et al., 2016) and *D. mexicana* (Váquez et al., 2008). In this sense, non-endangered species such as the red-rumped agouti (*D. leporina* Linnaeus, 1758) have been highlighted as experimental models for the development of strategies for agoutis conservation (Silva et al., 2010). Moreover, non-endangered species can easily adapt to captive breeding conditions under which they have good prolificacy, precocity and a relatively short gestation period (Hosken and Silveira, 2001). For Latin American communities, agoutis represent an important source of protein for human consumption (Lopes et al., 2004) and for obtaining skin, leather, and bristles (Silva et al., 2010).

Arroyo et al. (2014) described the structure of sperm pathways, especially the epididymis, during sexual development in red-rumped agouti. Interestingly, the testis-epididymis complex in agoutis is located in the abdomen, and its physiological regulatory mechanisms remain to be elucidated. Moreover, knowledge related to sperm is limited to the characteristics of cells obtained from the epididymal cauda (Ferraz et al., 2011). Thus, details about the modifications that agouti sperm undergo during epididymal transit are still unknown.

In various mammals, epididymis has been scientifically investigated because of its importance in determining the fertilizing capacity and motility of sperm during its modifications, which is collectively called sperm maturation (Sullivan and Mieusset, 2016; Dantas, 2022). In laboratory murine rodents, this phenomenon occurs when male gametes transit through different regions of epididymis: the caput region present between the initial segment and corpus; the middle portion of corpus; and the distal region of cauda connected to vas deferens (Gervasi and Visconti, 2017). During epididymal transit in mice, various sequential biochemical changes related to the release and absorption of fluids, ions, antioxidants, and exosomes occur (Reilly et al., 2006; Trig et al., 2019). Consequently, there are modifications in sperm size and morphology, mainly reflected by a reduction in the proportion of sperm defects, as observed in rats (Trasler et al., 1988). Changes include ultrastructural modifications in the cells, such as an increase in chromatin condensation, as observed in hamsters (Olson et al., 2003). Functional modifications that occur in parallel with morphological changes, include an increase in sperm kinetic patterns of forward motility, as

described in rats (Soler et al., 1994). Moreover, mitochondria are condensed and arranged helically around the midpiece of the sperm tail, and become metabolically efficient during epididymal transit (Park and Pang, 2021).

In view of both the conservation and proper reproductive management of wild animals in captivity, the development of effective strategies depends on detailed knowledge of the reproductive morphology and physiology of the species (Silva et al., 2010; Comizzoli, 2015). Therefore, the present study is the first to describe details related to the structural and functional parameters of agouti sperm in different regions of epididymis.

2. Methodology

2.1. Animals

The present study was approved by the UFERSA Animal Use Ethics Committee (CEUA: Opinion 11/2019) and Chico Mendes Institute for Biodiversity Conservation (no. 66618-3). All agoutis (*Dasyprocta leporina*) belonged to the Center of Multiplication of Wild Animals (CEMAS/UFERSA, Mossoró, RN, Brazil, 5°10S, 37°10W) and were registered at the Brazilian Institute of Environment and Renewable Natural Resources (IBAMA; Opinion no. 1478912). Programmed slaughter is conducted every year for population control, and the animals are allocated for use in different experiments. In the present study, seven sexually mature agouti males aged approximately 12 months were used. Before euthanasia, the animals were kept in captivity and fed fruits, corn, and commercial rabbit feed, once a day. Water was provided *ad libitum*. The experiments were conducted in October and November 2019 during the dry season of the Caatinga biome.

2.2. Obtaining epididymal sperm

Before the experimental procedures, the animals were fasted for 12 h. They were then physically restrained using a capture net and pre-medicated with xylazine (1 mg/kg; Rompun, Bayer, São Paulo, SP, Brazil) and ketamine (15 mg/kg, Ketalar, Pfizer, São Paulo, SP, Brazil). After 15 min, anesthesia was induced with 50 mg/kg IM sodium thiopental (Thiopentax; Cristalia, São Paulo, SP, Brazil) administered intravenously. After anesthesia induction, the animals were euthanized with 1 mL/kg potassium chloride IV (Equiplex, Goiânia, GO, Brazil) (Castelo et al., 2015).

Immediately after euthanasia, abdominal cavity was opened, and testes-epididymis paired complex was recovered, examined for any pathology, and then washed in a salt solution. Samples were placed in a beaker containing gauze soaked in phosphate-buffered saline (PBS; Sigma-Aldrich, Burlington, MA, USA), stored in a polystyrene insulated box at 4 °C, and then transported to the laboratory for processing (Figure 1). The testes-epididymis complexes were placed in Petri dishes containing 5 mL of PBS solution heated to 37 °C, and sperm from different epididymal regions were recovered using the flotation technique (Silva et al., 2016). This technique involves using a scalpel to initially separate the epididymis from the vas deferens and testes, followed by slicing different regions of the epididymis. During dissection, unlike in rats and mice, the so-called initial segment could not be identified in epididymis. Therefore, slicing was performed to separate the three regions of the epididymis (caput, corpus, and cauda). The left and right epididymis fragments were mixed and then placed in different beakers for each region of the epididymis, containing 2 mL of PBS solution heated at 37 °C, followed by slicing. After allowed to stand for 5 min, the tissues were removed using tweezers. The sperm suspension was collected through a pipette and the total volume was transferred to Eppendorfs tubes for measurement and evaluation (Silva et al., 2016).

2.3. Quantitative analysis

To determine the number of sperm collected ($\times 10^6$ sperm), the total volume (uL) collected from each epididymis region was determined and multiplied by the respective sperm concentration ($\times 10^6$ sperm/mL) (Silva et al., 2016). This was ascertained through an aliquot of 10 µL of semen diluted in formalized solution (10%) with added buffer (1mL), and subsequently observed in a Neubauer chamber (Silva et al., 2011).

2.4. Sperm morphology

To analyse sperm morphology, 5 µL of sperm samples from different regions of epididymis were fixed and stained with a formaldehyde-Bengal rose solution (45 µL – distilled water 20 mL; sodium citrate 0.58 g; formaldehyde 0.8 mL; Bengal Rose 0.3 g; CAQ - Casa da Química, São Paulo-SP, Brazil) on glass slides (Silva et al., 2011). Subsequently, the slides were observed under a light microscope (Nikon Eclipse E200, Nikon Instrument, Tokyo, Japan) at 100× magnification, where 200 sperm per sample were considered to calculate the

proportions of normal sperm, and identify and characterize the defects in acrosome, head, midpiece, and tail between the regions of the epididymis. In rare sperm presenting two or more defects, all these alterations were noted.

2.5. Sperm morphometry

After morphological analysis, all the slides stained with Bengal Rose were used for morphometric analysis (Silva et al., 2016). Images from random fields were obtained for each sample, totaling 200 sperm per sample (magnification 400×). Cell metrics were obtained using image analysis software (ImageJ Software, Wayne Rasband, National Institute of Health, Maryland, United States). The following sperm measurements were obtained: head length (measured from the apex of the acrosome till the base of the head), head width (measured from the transverse axis of the largest diameter), length of the midpiece (measured from the starting point of the insertion of the base of the head till the region of the Jensen ring), length of the tail (measured from the beginning of the midpiece till the end of the caudal portion), and total length (measured from the apex of the acrosome till the end of the caudal part) (Silva et al., 2015).

2.6. Ultrastructural analysis through scanning electron microscopy (SEM)

For scanning electron microscopy (SEM), 5 μL aliquots of each sample were deposited in Eppendorf tubes, resulting in grouped samples of sperm for each region of the epididymis. The samples were then fixed with 2.5% glutaraldehyde solution and stored in a refrigerator at 4 °C (Bezerra et al., 2018). A day before microscopic analysis, the samples were washed three times in 0.1 M phosphate-buffered saline (PBS; Sigma-Aldrich, Burlington, MA, USA) at pH 7.4, for 5 min each. The supernatant was discarded and the pellet was kept. Subsequently, a series of increasing dehydration steps with alcohol (50%, 70%, 90%, and 100%) was carried out for 10 min each, discarding all the alcohol supernatant (Bezerra et al., 2018). After dehydration, the material was immediately deposited on the surface of the sample holders (stubs) and allowed to dry at room temperature (27–29 °C) for at least 2 h. Finally, the material was coated with gold by sputtering for observation under a scanning electron microscope (Tescan®, Type VEGA 3 LMU, Nº VG13671479, 50/60 Hz; Brno, Czech Republic). The samples were then processed for evaluation by SEM in the laboratory of the Center for Research in Plant Sciences of the Semiarid, UFERSA.

2.7. Mitochondrial activity and structural membrane integrity

To evaluate the mitochondrial activity and the structural integrity of the sperm membrane, a semen aliquot (10 µL) was incubated at 34 °C for 10 min in a solution containing the combination of the following fluorescent probes: 2µL of Propidium Iodide (PI, Thermo Fisher Scientific, Whaltam, MA, USA) composed of 25 mg/mL stock solution [25 mg of PI + 1mL of dimethyl sulfoxide (DMSO, Sigma-Aldrich, Burlington, MA, USA)] diluted at 2 mg/mL [80µL of PI stock solution + 920 µL of phosphate-buffered saline (PBS, Sigma-Aldrich, Burlington, MA, USA)]; 0.5 mg/mL 0.9% NaCl; 5 µL 500 nM CMXRos (Mito Tracker Red®, F-7512, Molecular Probes, Eugene, OR, US) (50 µg dilution in 94 µL PBS), and 3 µL Hoechst 33342 (H342, Molecular Probes, Eugene, OR, USA) diluted at 25 mg/mL in DMSO, as described by Celeghini et al., (2007). The excitation (λ_{ex}) and emission (λ_{em}) properties of fluorochromes were: PI: 488 nm and 568 nm; H342: 340 nm and 510 nm; and Mito Tracker Red: 580 nm and 600 nm, respectively.

Samples were subjected to analysis using an epifluorescence microscope (Olympus BX51TF, Tokyo, Japan), with a 100 W mercury discharge burner (U-LH100HG) as the fluorescence light source, and observed under a yellow filter U-FYW (540-585 nm). The structural integrity of the membrane and the mitochondrial membrane potential were evaluated through the association of PI/H342 and CMXRos, respectively. We counted 200 sperm per sample, and the heads of the sperm marked in blue (H342) were considered to have an intact membrane in comparison to those totally or partially marked in red (PI). Sperm with the midpiece region marked in red were considered to have mitochondrial activity.

2.8. Sperm kinetics parameters

The epididymal sperm were analyzed to obtain kinetic parameters, using computer-assisted sperm analysis (CASA, IVOS 7.4 G; Hamilton-Thorne Research, MA, USA). For analysis, 3 µL of the sample was used and evaluated using the settings previously described for agoutis (Castelo et al., 2015): temperature, 37 °C; straightness threshold, 30%; minimum contrast, 45; low-velocity average pathway (VAP) cutoff of 10 m/s; and medium VAP cutoff of 30 m/s. Five independent and nonconsecutive microscopic fields were selected for scanning. The following parameters were analyzed using the data obtained: total motility (%), progressive

motility (%), velocity average pathway (VAP, 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, %). The sperm population was subdivided into four categories: rapid, medium, slow, and static (%).

2.9. Statistical analysis

Data are expressed as mean ± SEM. The following parametric assumptions were tested: normality of the residuals using Shapiro-Wilk test and homoscedasticity using Levene test. Potential differences in all reproductive parameters among different epididymis regions were verified by paired t-test (PROC TTEST), performed using Statistical Analysis Software version 8.0 (SAS Institute Inc., Cary, NC, USA).

When parametric assumptions were not met, non-parametric tests were performed. Friedman test was performed using SPSS software (version 22.0; SPSS Inc., Chicago, IL, USA) to evaluate sperm defects such as a heavily curled tail, double tail, folded midpiece, broken midpiece, thick midpiece, duplicate head, macrocephaly, microcephaly, detached head, and abaxial insertion. For all analyses, $P < 0.05$ was considered statistically significant.

3. Results

3.1. Sperm quantitative evaluation

While the highest sperm volume was obtained from epididymal caput ($P < 0.05$), the sperm concentration was higher in the cauda of epididymis ($1{,}230.1 \pm 75.4 \times 10^6$ sperm/ml) than in the other two regions (Table 1). Consequently, the same region presented the highest number of sperm collected ($823.47 \pm 137.9 \times 10^6$ sperm).

3.2. Sperm morphology, morphometry, and ultrastructure evaluation

No differences in normal morphology, and morphological defects of sperm were observed across the three epididymal regions (Table 2). However, a higher percentage of distal and proximal droplets was found in sperm from epididymal caput than in corpus and cauda ($P < 0.05$). Some images related to normal sperm and defects are presented in Figure 2.

The spermatozoa from caput and corpus of the epididymis showed agglomeration and sticking to the substances of epididymis; dissociation was not possible even after centrifugation and dilution (Figure 3A and 3B). The observation of such sperm under viable condition was not possible, since they were difficult to dissociate. However, we could obtain the details of agouti sperm from the cauda of epididymis. These sperm had a slightly flattened head with an oval shape (Figure 3C and 3D), which measured an average of 4.89 μm in length and 3.27 μm in width. The sperm head had an intact and uninterrupted surface without a clear demarcation of the post-acrosomal region. In addition, a midpiece measuring proximately 5.52 μm in length and a long tail measuring 31.19 μm in length were clearly identified using SEM.

Sperm morphometry (Table 3) revealed a significant decrease in sperm dimensions in the caput-corpus-cauda direction ($P < 0.05$). Such findings were mainly represented by averages of head length, head width, and sperm tail length from the epididymal caput (5.15 ± 0.02 μm, 3.44 ± 0.02 μm, and 32.04 ± 0.08, respectively) that were higher in comparison to those from epididymal cauda (4.89 μm ± 0.01, 3.27 μm ± 0.01, and 31.19 ± 0.07, respectively). On the contrary, the measurements concerning the midpiece length increased along the epididymal transit (caput = 5.40 ± 0.02 μm, corpus = 5.53 ± 0.04 μm, and cauda = 5.52 ± 0.02 μm).

3.3. Sperm functional parameters

With regard to functional parameters (Table 4), we verified an increase ($P < 0.05$) in the percentage of sperm presenting membrane integrity and mitochondrial activity during sperm transit from the epididymal caput (47.7 ± 13.3%) to the cauda (79.71 ± 4.6%).

Results from evaluation of kinetic parameters using CASA showed that sperm from all epididymal regions exhibited motility with straight trajectories (Table 5). There was a significant increase ($P < 0.05$) in the values of total and progressive motility, VAP, VSL, and VCL in the caput-corpus-cauda direction. The values for ALH, BCF, STR, and LIN showed no statistical differences across the epididymal regions. Among sperm subpopulations, highest prevalence was observed for rapid (19.0 ± 3.5%) and medium (54.3 ± 3.6%) velocity sperm in epidydimal cauda in comparison to other regions ($P < 0.05$), whereas static sperm (86.9 ± 3.7%) was more prevalent in the epididymal caput ($P < 0.05$).

4. Discussion

In red-rumped agouti, the epididymis is divided into three anatomical regions: caput, corpus, and cauda (Arroyo et al., 2014). However, in rodents such as rats and mice, there is an additional epididymal, called the initial segment, located between the efferent ducts and the caput epididymis (Belleannée et al., 2012). These morphological differences reinforce the importance of investigating particular details of the morphology and physiology of wild rodents, such as hystricognath species, since there are marked differences from those postulated for well-studied laboratory murines.

The main functions of epididymis include receiving immature spermatozoa from testis, their transportation and maturation, and storage of functional sperm prior to their release in vas deferens during ejaculation (Briz et al., 1995, Cooper and Yeung, 2003). In the present study, this last function was evident in agouti, since the highest concentration and total number of sperm were obtained from epididymal cauda in comparison to other regions of the organ. The secretions from epididymal cauda provide an adequate environment for the preservation of sperm fertility when stored for several days (Jones and Murdoch, 1996). The genes regulating these epididymal secretions are dependent on adequate control of temperature, which is the reason why testes-epididymis complex is generally located in the scrotum (Regalado et al., 1993). However, in agoutis, these organs are located in the abdomen (Arroyo et al., 2014), and the thermoregulatory system has not been investigated for this species.

Normal sperm morphology is essential for sperm motility, helping normal fertilization processes (Gu et al., 2019). In agouti, no differences related to sperm morphology were found across different epididymal regions, except for the presence of cytoplasmic droplets, which were reduced from the epididymal caput to the cauda. Such droplets are cytoplasmic remnants, most of which are extruded as residual bodies that are phagocytized by Sertoli cells when the sperm leaves the seminiferous epithelium to become a free testicular sperm (Cooper, 2011). As observed in other rodents (Akbarsha et al., 2000; Krishnamurthy et al., 2000; Goyal et al., 2001), progressive migration of cytoplasmic droplets, as well as induction of motility, occurs initially in the caput region and increases along the epididymal transit in agoutis.

Using SEM analysis, we verified that the red-rumped agouti sperm had a slightly oval head shape, tending toward a more rounded morphology. Gu et al. (2019) reported similar results for rabbit (*Oryctolagus cuniculus*) sperm. However, agouti sperm diverged from the sperm of other rodents such as that of Spix's yellow-toothed cavy (*Galea spixii*), which has fusiform-shaped heads (Santos et al., 2013); guinea pigs (*Cavia porecellus*) with spatulate-shaped heads; and golden hamster (*Mesocricetus auratus*)s and rat (*Rattus norvegicus*) with

falciform-shaped heads (Gu et al., 2019). This finding suggests that the head shape of the red-rumped agouti sperm is uncommon in rodent species belonging to the superfamily Muroidea and infraorder Hystricognathi, but is similar to species of other groups such as Lagomorphs. According to Schmehl and Graham (1989), the different morphologies of sperm heads suggest a morphophysiological strategy to prevent the penetration of oocyte by morphologically compromised sperm.

A decrease in total sperm morphometry was observed from the epididymal caput till cauda in agoutis; however, an increase in midpiece length was verified in parallel. This is an expected morphophysiological phenomenon in rodents, as chromatin condensation causes a decrease in sperm head diameter as it matures along the epididymal transit (Manfredi Romanini et al., 1986; Pogany and Linder, 1993). Likewise, an increase in midpiece length is expected when mitochondrial sheath develops proportionally to the sperm maturation, giving motility potential parallel to this (Pogany and Linder, 1993; Gu et al., 2019).

Along the epididymal transit, morphological changes reflect functional changes in the sperm. In fact, sperm maturation causes physiological changes in the membrane and cytoplasmic organelles related to motility, such as mitochondria. Yuan et al. (2013) reported that during sperm maturation in mouse epididymis, a proportional relationship between mitochondrial function and sperm motility was observed. This morphophysiological process occurs due to the increase and maturation of mitochondrial sheath along the spermatic transit, which is associated with an increase in the assembly of mitochondrial respiratory chain complex, as well as development of crests (Gu et al., 2019; Park and Pang, 2021). At the same time, sperm plasma membrane remodeling occurs, which is carefully processed in different regions of epididymis, indirectly affecting intracellular membranes, organelles, and nucleoproteins, proportional to the acquisition of motility and fertilization capacity (Jones, 1998). These changes are mainly due to the absorption and repositioning of glycoproteins and lipid components in different membrane domains during epididymal transit (Moore et al., 1989). This pattern is expected in most species, which were also observed for agouti sperm maturation, because the mitochondrial activity and membrane structural integrity were much higher in sperm from cauda than in corpus and caput of epididymis.

In fact, CASA analysis showed a notable increase in agouti sperm motility as incidated by VAP, VSL, VCL, in rapid subpopulations in the caput-corpus-cauda direction. These observations showed clear trends in the positive development of sperm motility in the epididymal transit, as well as in other characteristics related to their movement in different

regions of the epididymis, similar to that observed in mice (Soler et al., 1994). This mobility is reflected in maturational changes in terms of morphophysiology, associated with functional motility and fertility (Soler et al., 1994, 2017).

5. Conclusion

This work addressed, for the first time, the sperm maturation of a wild hystricognath rodent species (*Dasyprocta leporine*), and analyzed and described the parameters inherent to its morphophysiology and kinetics. Despite the various anatomical peculiarities related to the position and structure of the red-rumped agouti epididymis, the pattern of morphological and functional changes in spermatozoa during epididymal transit appears to be similar to that postulated for murine rodents.

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Declaration of Competing Interest

The authors declare that there are no conflicts of interest

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Author contribution statement

All authors contributed to the work's conception and design. Material preparation and data collection were performed by M. R. T. Dantas, A. M. Silva, L. G. P. Bezerra, A. G. Pereira, N. R. N. Luz, M. F. de Oliveira, and A. R. Silva. The analysis of the samples was carried out by M. R. T. Dantas, L. G. P. Bezerra, A. G. Pereira, N. R. N. Luz, and J. B. F. Souza-Junior. The

first draft of the manuscript was written by Maiko Roberto Tavares Dantas. The author A. R. Silva commented and reviewed previous versions of the manuscript. All authors approved the final manuscript.

Data availability statement

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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FIGURES

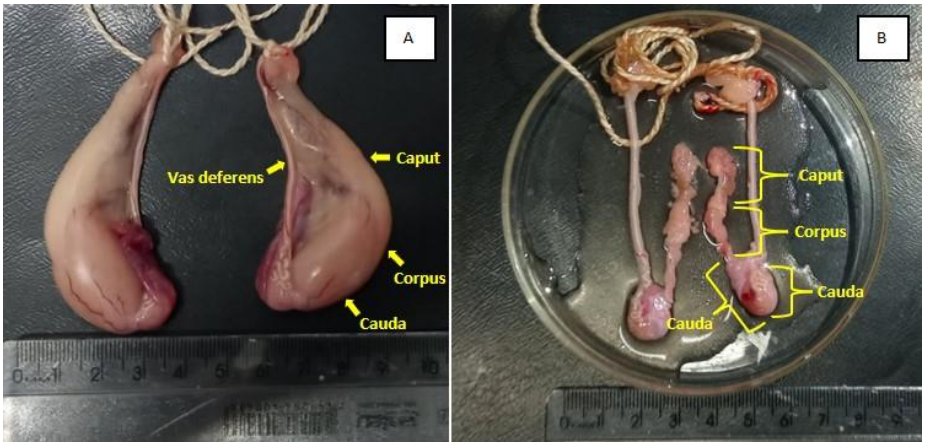

Fig. 1. A - Agouti testes-epididymis complex (*Dasyprocta leporina*). B - agouti epididymis (*Dasyprocta leporina*).

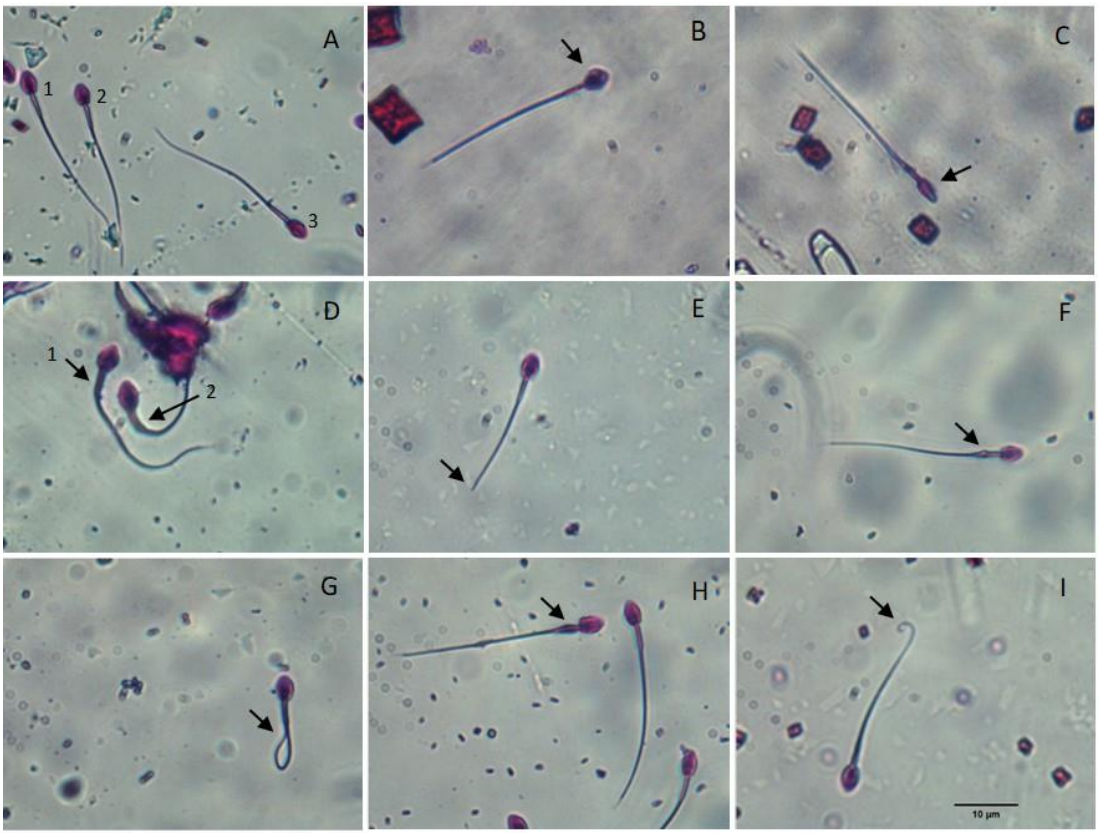

Fig. 2. Morphology of agouti epididymal spermatozoa (*Dasyprocta leporina*) increased 400x. (A) 1, 2, and 3 - Normal sperm. (B) Macro head. (C) Micro head. (D) 1 and 2 - Thick midpiece. (E) Broken tail. (F) Distal cytoplasmic droplet. (G) Heavily curled tail. (H) Proximal cytoplasmic drop. (I) Curled tail.

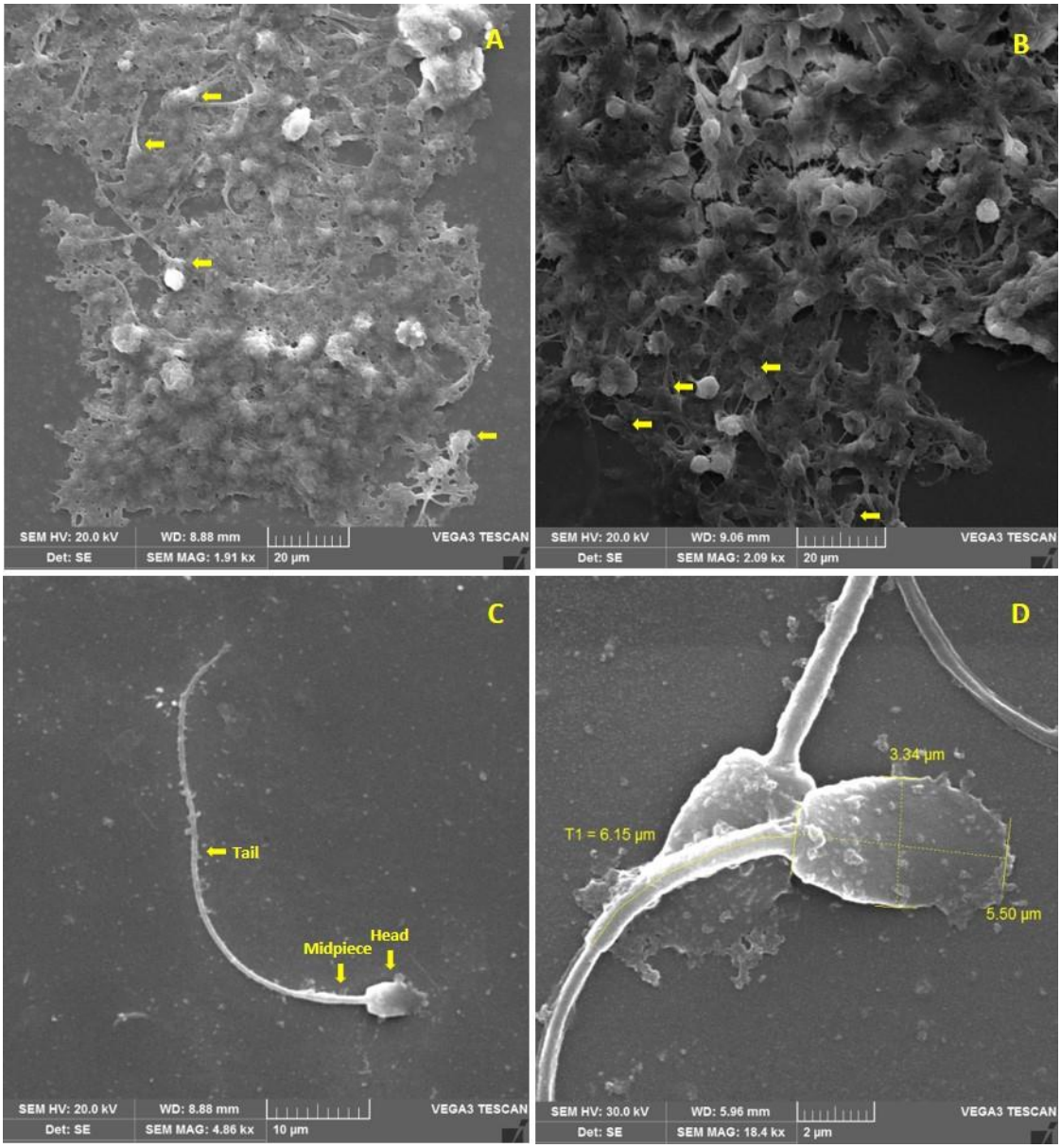

Fig. 3. Electron microphotograph of sperm morphology in red-rumped agouti (*Dasyprocta leporina*); A - Agglomerated sperm coming from the caput of the epididymis (Arrows highlight sperm). B - Agglomerated sperm from the epididymis corpus (Arrows highlight sperm); C and D - Sperm from the epididymis cauda.

TABLES

Table 1. Mean values (± SEM) for volume, concentration, and number of sperms collected from the different epididymis regions of red-rumped agoutis (*Dasyprocta leporina*; n = 7).

Region of epididymis	Volume (uL)	Concentration (sperm/mL x 10^6)	Number of sperms collected (sperm x 10^6)
Caput	1392.9 ± 34.0^A	108.3 ± 28.6^B	$150,85 \pm 43.3^B$
Corpus	821.4 ± 155.9^B	125.3 ± 37.1^A	$102,92 \pm 34.2^B$
Cauda	685.7 ± 207.1^B	1230.1 ± 75.4^A	823.47 ± 137.9^A

Values with different letters differ statistically for the same observed variable and between other epididymis regions (P <0.05).

Table 2. Mean values (± SEM) of the morphology of sperms collected from the different epididymis regions of red-rumped agoutis (*Dasyprocta leporina*; n = 7).

Sperm morphology (%)	Region of epididymis					
	Caput		Corpus		Cauda	
	Mean	Range	Mean	Range	Mean	Range
Normal sperms	82,42 ± 1,89	73 - 86	86,71 ± 1,57	82 - 93	88,86 ± 2,24	82 - 98
Acrosome defects	1,28 ± 0,61	0 - 4	1,57 ± 0,69	0 - 5	1,43 ± 0,69	0 - 4
Head defects						
Duplicate head	0 ± 0,00	0	0,1 ± 0,1	0 - 1	0,4 ± 0,3	0 - 2
Macrocephalic	0,1 ± 0,1	0 - 1	0,3 ± 0,2	0 - 1	0,1 ± 0,1	0 - 1
Microcephalic	1,3 ± 0,8	0 - 6	0,9 ± 0,4	0 - 3	1 ± 0,4	0 - 3
Abnormal head shape	1,1 ± 0,4	0 - 3	1,6 ± 0,7	0 - 5	1 ± 0,6	0 - 4
Detached head	0,6 ± 0,2	0 - 1	0,4 ± 0,3	0 - 2	0,7 ± 0,4	0 - 3
Abaxial head insertion	0,3 ± 0,2	0 - 1	0,3 ± 0,2	0 - 1	0,1 ± 0,1	0 - 1
Midpiece and droplet defects						
Folded midpiece	2,9 ± 1,2	0 - 8	0,6 ± 0,2	0 - 1	0,7 ± 0,4	0 - 3
Broken midpiece	0,3 ± 0,2	0 - 1	0,4 ± 0,3	0 - 2	0,4 ± 0,2	0 - 1
Thick midpiece	0,4 ± 0,3	0 - 2	0,6 ± 0,3	0 - 2	0,4 ± 0,2	0 - 1
Distal cytoplasmatic droplet	$2 \pm 0,4^A$	1,4	$1,3 \pm 0,4^B$	0 - 3	$0,9 \pm 0,5^B$	0 - 3
Proximal cytoplasmatic droplet	$2,4 \pm 0,7^A$	0 - 5	$0,9 \pm 0,4^B$	0 - 3	$0,6 \pm 0,3^B$	0 - 2
Tail defects						
Curled tail	2,4 ± 0,4	1,4	2,9 ± 0,9	0 - 6	2,6 ± 0,6	0 - 5
Heavily curled tail	0,4 ± 0,2	0 - 1	0,3 ± 0,2	0 - 1	0,3 ± 0,2	0 - 1
Double tail	0,6 ± 0,3	0 - 2	0 ± 0,00	0 - 0	0 ± 0,00	0 - 0
Broken tail	1,6 ± 0,5	0 - 4	1,3 ± 0,5	0 - 3	0,4 ± 0,3	0 - 2

Values with different letters differ statistically for the same observed variable and between other epididymis regions (P <0.05).

Table 3. Mean values (± SEM) of the morphometric parameters of sperms collected from the different epididymis regions of red-rumped agoutis (*Dasyprocta leporina*; n = 7).

Sperm regions	Caput	Corpus	Cauda
Head length (μm)	4.35 ± 0.02^{A}	4.25 ± 0.01^{B}	4.13 ± 0.01^{C}
Head width (μm)	2.91 ± 0.02^{A}	2.77 ± 0.01^{B}	2.76 ± 0.01^{B}
Midpiece length (μm)	4.56 ± 0.02^{B}	4.67 ± 0.04^{A}	4.66 ± 0.02^{A}
Tail length (μm)	27.06 ± 0.08^{A}	26.86 ± 0.09^{A}	26.34 ± 0.07^{B}
Total length (μm)	35.97 ± 0.09^{A}	35.63 ± 0.14^{B}	35.14 ± 0.07^{C}

Values with different letters differ statistically for the same observed variable and between other epididymis regions (P <0.05).

Table 4. Mean values (± SEM) of the kinetic parameters of sperms collected from the different epididymis regions of red-rumped agoutis (*Dasyprocta leporina*; n = 7).

kinetic parameters	Caput	Corpus	Cauda
Total motility (%)	12.6 ± 12.6^{C}	41.6 ± 8.4^{B}	73.3 ± 6.4^{A}
Progressive motility (%)	1.3 ± 1.3^{B}	6.4 ± 2.1^{B}	13.3 ± 2.4^{A}
Average path velocity (VAP; μm/s)	23.4 ± 5.1^{B}	34.4 ± 3.4^{AB}	39.0 ± 2.1^{A}
Straight line velocity (VSL; μm/s)	16.1 ± 3.9^{B}	23.2 ± 2.6^{AB}	27.5 ± 1.9^{A}
Curvilinear velocity (VCL; μm/s)	44.0 ± 8.8^{B}	63.9 ± 4.7^{B}	69.1 ± 3.3^{A}
Amplitude of lateral head displacement (ALH; μm)	4.3 ± 0.8	5.2 ± 0.4	5.8 ± 0.3
Beat cross frequency (BCF; Hz)	32.6 ± 5.7	41.8 ± 0.6	39.9 ± 0.7
Straightness (STR; %)	53.9 ± 9.3	60.9 ± 1.0	65.0 ± 1.1
Linearity (LIN; %)	28.9 ± 5.2	33.4 ± 1.1	53.9 ± 1.2
Subpopulations			
% of Rapid sperm	1.6 ± 0.6^{B}	9.7 ± 3.6^{B}	19.0 ± 3.5^{A}
% of Medium velocity sperm	10.9 ± 3.7^{C}	31.7 ± 5.1^{B}	54.3 ± 3.6^{A}
% of Slow sperm	0.6 ± 0.2	0.7 ± 0.3	0.3 ± 0.2
% of Static sperm	86.9 ± 3.7^{A}	57.9 ± 8.3^{B}	26.3 ± 6.4^{C}

Values with different letters differ statistically for the same observed variable and between other epididymis regions (P <0.05).

Table 5. Mean values (± SEM) of the membrane structure viability and mitochondrial activity of sperms collected from the different epididymis regions of red-rumped agoutis (*Dasyprocta leporina*; n = 7).

Membrane structure viability and mitochondrial activity of sperm (%)	Region of epididymis		
	Caput	Corpus	Cauda
Membrane integrity with mitochondrial activity (I, A)	47.7 ± 13.3^{B}	58.9 ± 10.8^{AB}	79.7 ± 4.6^{A}
Membrane integrity with unable mitochondrial activity (I, uA)	29.9 ± 11.7^{A}	15.0 ± 5.6^{AB}	3.3 ± 3.0^{A}
Membrane not functional with mitochondrial activity (nF, A)	1.6 ± 0.6^{A}	4.0 ± 1.7^{A}	2.1 ± 0.9^{A}
Membrane not functional with unable mitochondrial activity (uF, nA)	20.9 ± 4.6^{A}	21.9 ± 5.0^{A}	14.9 ± 2.8^{A}

Values with different letters differ statistically for the same observed variable and between other epididymis regions (P < 0.05).

CAPÍTULO III

Evaluation of sperm membrane functionality during epididymal transit in red-rumped agouti (*Dasyprocta leporina*)

Avaliação da funcionalidade da membrana espermática durante o trânsito epididimário em cutias (*Dasyprocta leporina*)

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Abstract: We studied the sperm membrane functionality through the epididymal transit by comparing different hypoosmotic solutions to assess the best osmotic response and verifying possible associations among osmotic response and functional parameters of sperm in red-rumped agouti (*Dasyprocta leporina*). For this purpose, seven sexually mature male agoutis were anesthetized, euthanized, and their epididymal sperm collected via flotation. Then, analyses of sperm parameters and HOS tests in solutions of different osmolarities (0 mOsm/l, 50 mOsm/l, and 200 mOsm/l) in different regions of the epididymis (caput, corpus, and cauda) were performed. Data were expressed as mean ± SEM and evaluated by Two Way ANOVA, followed by the Tukey test ($P < 0.05$) for comparisons between hypoosmotic solutions and epididymal regions. Correlations between sperm parameters and different hypoosmotic solutions were analyzed by Spearman's correlation test ($P < 0.05$). The 50 mOsm/l solution (83.3 ± 3.9%) was the one with the highest percentage of reactive sperm, superior to 0 mOsm/l (42.5 ± 3.5%) and 200 mOsm/l (61.8 ± 3.9%), with the main morphofunctional parameters being found in spermatozoa from the cauda of the epididymis: volume 1285.2 ± 319.3 µL, concentration 1285.2 ± 319.3 sperm/mL x 10^6, number of recovered sperm 1375.0 ± 244.1 sperm x10^6, normal sperm morphology 87.3 ± 1.9%, membrane structural integrity 83.5 ± 3.4%, and mitochondrial activity 85.8 ± 3.1%. Also, regarding the main kinetic parameters observed, the highlights were the total motility of 73.29 ± 6.36 % and progressive motility of 13.29 ± 2.39%. There were no significant correlations regards sperm parameters from the cauda region in any of the values for reactive sperm for the solutions hypoosmotic, different from caput and corpus. In summary, we suggest the use of a 50 mOsm/l hypoosmotic solution for the evaluation of the functional integrity of the sperm membrane in red-rumped agouti during the hypoosmotic swelling test.

Keywords: epididymal sperm, fertility, HOS test, sperm membrane, maturation, wildlife.

1. Introduction

The red-rumped agouti (*Dasyprocta leporina*) is a South American wild hystricognath rodent that has ecological importance as a seed disperser and as prey for carnivores (Hosken and Silveira, 2001; Jones et al., 2019). Despite declining in some biomes, its populations are stable, and the species is globally classified as least concerned (Emmons and Reid, 2016). For this reason, this agouti species has been currently considered as a model for the development of conservative strategies to be applied to close-related endangered rodents as *Dasyprocta*

ruatanique (Schipper et al., 2016), *Dasyprocta coibae* (Roach and Naylor, 2019), and *Dasyprocta mexicana* (Vázquez et al., 2008).

Unlike what is commonly observed for laboratory rodents, agoutis have reproductive organs (testes and epididymis) located in the abdomen, which may result in regulatory mechanisms not yet known (Arroyo et al., 2014). In this sense, various efforts have been conducted for the establishment of protocols for the recovery (Ferraz et al., 2011; Castelo et al., 2015b) and the preservation (Silva et al., 2012; Castelo et al., 2015a) of agouti male gamete, but some basic aspects of its sperm physiology, as the modifications of the sperm along the epididymal transit, remain unknown. In addition to species-specific osmotic characteristics, membrane functionality undergoes changes during sperm maturation, as a wide variety of functional, structural, and biochemical changes in sperm occur during epididymal transit (Olson et al., 2003). Such modifications affect the repositioning of protein, lipid, and glycoprotein components in different specific regions of the intracellular membranes and plasma membrane, remodeling it along the epididymal transit, conferring motility and fertilization capacity (Jones 1998; Soler et al., 2017).

The hypoosmotic swelling test (HOS test) has been proposed for the evaluation of sperm membrane functionality in various domestic species such as dogs (Kumi-Diaka, 1993), goats (Fonseca et al., 2001), pigs (Lechniak et al., 2002), and cattle (Martins et al., 2011). For wildlife, however, the use of the test is limited, mainly because the osmotic response of the sperm is unknown for various species. Thus, the test was validated for only a few species, such as lion (Malo et al., 2004), six-banded armadillo (Santos et al., 2011), brown bear (Pérez-Garnelo et al., 2013), collared peccary (Santos et al., 2013), and white-lipped peccary (Barros et al., 2019). The HOS test evaluates the capability of the sperm membrane to adapt to the influx of fluids into the cell in order to provide an osmotic balance between extra and intracellular fluids (Jeyendran et al., 1984). Therefore, the test is based on a simple physiological concept: living cells swell when exposed to a hypoosmotic environment, and cells with damaged membranes do not (Zubair et al., 2015). Despite the relative simplicity of the HOS test, there are some points that need to be clarified to obtain greater reliability, as the ideal osmolarity of the hypoosmotic solution (Melo et al., 2003), since each species can present a different sperm osmotic response (Matson et al., 2009).

To the best of our knowledge, the use of the HOS test throughout sperm maturation was validated neither for agoutis nor for any other hystricognath rodent. Thus, the relationship between functional membrane integrity and other sperm morphophysiological parameters of

this species during the epididymal transit remains known. Since electroejaculation remains a low effective technique for sperm obtaining in agoutis (Castelo et al., 2015b), the use of epidydimal sperm is proposed as an effective alternative for the establishment of protocols for sperm processing or evaluation for the species (Ferraz et al., 2011). Therefore, we aimed to investigate the osmotic response of agouti sperm along epididymal transit and in solutions of different osmolarities containing distilled water combined with sodium citrate, to access sperm membrane functionality and verifying the existence of possible associations among membrane functionality and other sperm metrics.

2. Material and methods

2.1. Animals

Six sexually mature male agoutis, aged approximately 12 months, were used for the experiment, as approved by the Ethics Committee on the Use of Animals at UFERSA (CEUA – Opinion nº 11/2019) and Instituto Chico Mendes de Biodiversidade (ICMBIO – Opinion nº 66618-1). Animals were originated from the Wild Animal Multiplication Center (CEMAS) of the Federal Rural University of the Semi-arid (UFERSA), which is a scientific breeding center authorized by the Brazilian Institute of Renewable Resources (IBAMA – Opinion nº66618-1), which conducts scheduled slaughter of animals destined for scientific experimentation as those used in the present study. The animals were isolated from females for six months before the start of the study and maintained under a 12-h natural photoperiod. They were allocated on a covered paddock (4 × 5 m) for their grouping and maintenance. Feed for the agoutis was a commercial rabbit ration, with 13% crude protein, 35% ether extract, 16% fiber, and 13% minerals. Fresh drinking water was available *ad libitum*.

2.2. Sperm collection

The animals were mechanically restrained using a hand net and premedicated with intramuscular administration of 1 mg/kg of ketamine (Ketalar; Pfizer, São Paulo, Brazil) and xylazine hydrochloride (Rompun; Bayer, São Paulo, Brazil). An intracardiac administration of 1 mg/kg potassium chloride was performed for euthanasia (Castelo et al., 2015b). Finally, the pairs of epididymis were collected and transferred to a beaker containing gauze moistened with

phosphate-buffered saline - PBS (287 mOsm/kg) at 37 ºC, then stored in a polystyrene insulated box at 4 ºC to be transported to the laboratory for processing. Subsequently, sperm were recovered from the epididymis by the slicing and floating method, as described by Silva et al. (2016). Briefly, slicing was performed to separate three distinct regions of the epididymis: caput, corpus, and cauda. After that, each fragment was placed in individual beakers containing 2 ml of PBS solution heated to 37°C, followed by slicing each one. After 5 min, the epididymis tissues were removed, and the sperm suspension was recovered and deposited in plastic tubes to obtain the volume (ul) collected per animal and subjected to evaluation.

2.3. Sperm evaluations

During evaluations, the samples were placed in a water bath 37°C. Sperm concentration (sperm/mL x 10^6) was evaluated through a Neubauer counting chamber. The number of sperms collected (sperm x 10^6) was determined by multiplying the sperm concentration and the total volume (ul) collected (Silva et al. 2016). For the analysis of sperm normal morphology, a 10-ul aliquot of the sperm sample was used to produce a smear that was stained with Bengal rose, counting 200 cells under light microscopy (×1000) (Silva et al. 2011).

For the analysis of the sperm structural membrane integrity and mitochondrial activity, an aliquot (10μL) was incubated at 34 °C for 10 min in a solution containing a combination of fluorescent probes composed of 2μL of Propidium Iodide (PI), NaCl 0.5 mg/ml at 0.9%, 5μL CMXRos (Mito Tracker Red®, Molecular Probes, F-7512) at 500 nM (50 μg dilution in 94 μL DPBS), and 3μL Hoechst 342 (H342) (diluted at 25mg/mL in DMSO) (Celeghini et al. 2007). After, the samples were analyzed by an epifluorescence microscope (Leica, Kista, Sweden), counting 200 sperm per sample. Sperms presenting heads marked in blue (H342) were classified as presenting an intact membrane, while those marked totally or partially in red (PI) were classified as non-intact. Moreover, the sperm presenting the region of the midpiece marked in red were considered as having mitochondrial activity (Celeghini et al. 2007).

A 3-μl aliquot was used for the analysis of the sperm kinetic parameters using a computer-assisted sperm analysis system (CASA, IVOS 7.4 G; Hamilton-Thorne Research, Beverly, MA, USA), following the settings previously established for the species: temperature, 37°C; straightness threshold, 30%; minimum contrast, 45; low-speed mid-lane cutoff (VAP), 10 μm/s; average VAP cutoff, 30 μm/s (Castelo et al. 2015b). Therefore, five independent and non-consecutive microscopic fields were selected for the scan. The parameters analyzed were

total motility (%), progressive motility (%), velocity average pathway (VAP, 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, %), linearity (LIN, %), and elongation (%). The total sperm population was subdivided into four categories: rapid, medium, slow and static (%).

2.4. Hypoosmotic swelling (HOS) test

For the evaluation of sperm membrane functionality, hypoosmotic solutions composed of only distilled water (0 mOsm/l) or a combination of distilled water (0 mOsm/l) with sodium citrate and fructose solutions at different osmolarities (50 and 200 mOsm/l) were used. Aliquots of 5 μL containing epididymal sperm plus 45 μL of hypoosmotic solutions were incubated in a dry bath for 40 min at 37º C. Then, 10 μL of each treatment were aliquoted and evaluated in a phase-contrast light microscope (×400). There were 200 sperms examined; we considered those with a swollen, coiled tail as having a functional membrane (Fonseca et al., 2005).

2.5. Statistical analysis

The data were analyzed by the SigmaPlot (Systat Software Inc, Version 14) and expressed as mean ± SEM. Data were checked for normality using the Shapiro-Wilk test and for homoscedasticity using the Brown-Forsythe test. Then, the sperm functional membrane integrity was evaluated by two-way repeated-measures ANOVA (Two Factor Repetition), followed by Bonferroni t-test for the pairwise multiple comparison procedures among the epididymal regions (caput, corpus, and cauda) and different hypoosmotic solutions ($P < 0.05$), as well as to compare the other sperm parameters among the regions of the epididymis. The relationship between sperm parameters and functional membrane integrity at different hypoosmotic solutions and regions of the epididymis was analyzed by Spearman's correlation test ($P < 0.05$).

3. Results

3.1. Sperm parameters in different epididymal regions

Regarding sperm parameters (Table 1), fresh sperm samples recovered from the agouti epididymis cauda presented the highest values ($P < 0.05$) related to volume concentration ($1285.2 \pm 319.3 \times 10^6$ sperm/mL) and total number of sperm recovered ($1375.0 \pm 244.1 \times 10^6$ sperm) when compared to other epididymal regions. Additionally, various sperm kinetic parameters presented higher values in epididymal cauda in comparison to other regions.

3.2. Sperm functional membrane integrity during epididymal transit

The HOS test revealed significant differences ($P < 0.05$) among the hypoosmotic solutions evaluated, being the most significant percentage of reactive sperm found at the use of a 50 mOsm/l solution in all regions of the epididymis assessed (Table 2). Additionally, there were no differences in sperm membrane functionality for any solution evaluated among different epidydimal regions (caput, corpus, and cauda).

3.3. Correlations among functional membrane integrity and other sperm parameters

There were significant correlations among spermatozoa reactive to the use of different hypoosmotic solutions and various sperm kinetic parameters for the caput and corpus regions, but none for the cauda (Table 3).

4. Discussion

Much more than just an indication of the structural integrity of the sperm membrane, the HOS test evaluates the functional capacity of the sperm. Through this test, we demonstrated that red-rumped agouti spermatozoa have a very functional membrane during the entire epididymal transit. This was especially evidenced with the use of a hypoosmotic solution of 50 mOsm/L, which revealed values around 70% of spermatozoa with functional membrane independent of the epididymal region; however, the number of spermatozoa reactive to the test varied according to the use of different hypoosmotic solutions.

As soon as the spermatozoa are released from the seminiferous tubules, the sperm membrane is subjected to continuous remodeling upon their transit in the epididymis (Kuo et al., 2016). Given that the osmotic pressure of the epididymal fluid, especially in the tail, is considerably higher than that of seminal plasma or blood (Sahin et al., 2009), the

approach in which a hypoosmotic challenge is used to monitor sperm membrane function along epididymal transit is very valuable. During the process of sperm maturation, the sperm membrane undergoes physiological changes along the epididymal transit that reflect on its functionality and consequently on the acquisition of motility and fecundating capacity (Jones, 1998). At this sense, Druart et al. (2009) observed that there is a progressive decrease in the hypotonic resistance of boar spermatozoa during their transit from the caput to the cauda of the epididymis, which could be associated with changes in lipid and protein compositions, resulting in different physical properties of the membrane. In agoutis, however, we did not observe differences related to sperm osmotic response during epididymal transit, similarly to what was described for cattle using a hypotonic challenge for sperm from the caput and the cauda of the epididymis (Sahin et al., 2009). Of course, differences in the composition of the plasma membrane of the spermatozoa of agoutis must also occur along the epidydimal transit, but the HOS test probably does not have the necessary sensitivity to express such detailed changes (Gervasi and Visconti, 2018).

Independently from the epididymal region, the agouti sperm showed the greatest osmotic response at the presence of an intermediate osmolarity (50 mOsm/l) in comparison to the extreme ones (0 mOsm/l and 200 mOsm/l). However, in murine rodents such as mice, the use of the "water test" at 0 mOsm/L appears to be well established for the analysis of sperm membrane function (Sliwa, 1993). When first described in 1984, the hypoosmotic test was used to assess human semen by using solutions with osmolarities varying from 50 to 300 mOsm/l, and the best sperm reactive rates were achieved at the use of a 150 mOsm/l solution (Jeyendran et al. 1984). Since then, the HOS test has been applied to several species and differences in sperm osmotic response have been noted even between phylogenetically close species (Zubair et al., 2015). Generally speaking, the ideal hypoosmotic solution would be one that exerts osmotic stress large enough to provide an observable increase in volume, but at the same time small enough that membrane lysis does not occur (Jeyendran et al., 1984). Therefore, due to the specific characteristics of the sperm membrane for each species, it is expected that there will be variation in the number of spermatozoa reactive to the HOS test.

In addition to the functionality of the sperm membrane, we observed an improvement in the morphofunctional parameters during the maturation of agouti sperm, especially those related to kinetics, similarly as described for other rodents as murines (Soler et al., 1994) and hamsters (Olsen et al., 2003). Although no differences were found in the osmotic response of spermatozoa between regions of the epididymis, the functionality of the agouti sperm

membrane seems to be intrinsically related to the acquisition of sperm movement considering the direction from the caput to the cauda region of the epididymis. This fact is supported by the observation of numerous correlations between the osmotic response and the kinetic parameters of spermatozoa obtained from the caput, when compared to those obtained from the epididymal cauda. Although it is known that there are specific biochemical interactions between secretions from the male reproductive tract and the germ cells along the epididymal transit, the cauda and corpus regions are described as mainly responsible for sperm maturation, while the cauda acts mainly as a sperm reservoir (Toshimori, 2003; Gervasi and Visconti, 2018).

In studies with spermatozoa from cats (Comercio et al., 2013), drones (Nur et al., 2011) and humans (Van den Saffele et al., 1992), positive correlations were found between the HOS test and sperm motility. According to the authors, it is expected that there will be correlations between the parameters regarding motility and spermatozoa reactive to the HOS test since it is known that sperm motility depends in part on sperm membrane functionality, as well as biochemical and metabolic processes (Jeyendran et al. 1984). On the other hand, in our study, no correlations were identified between spermatozoa from the epididymal cauda of agouti. These results were similar to those described for spermatozoa ejaculated from goats (Martins et al., 2006; Oliveira et al., 2013), horses (Snoeck et al., 2007) and six-banded armadillos (Santos et al., 2011). According to these authors, the reason for this lack of correlations between the HOS test and sperm morphofunctional parameters is a consequence of the different specificities and purposes between these analyses. In fact, while spermatic swelling evidences the functional integrity of sperm membranes in the HOS test, sperm motility and viability depend not only on the transport of substances that pass through the membranes, but also on several other biochemical functions that modulate the sperm metabolism, as well as the microtubular activity of the fibers present in the region of the sperm tail.

5. Conclusions

In summary, we provided novel data related to the function of the sperm membrane along the epididymal transit, which presents significant correlations with some sperm kinetic parameters in the red-rumped agouti (*Dasyprocta leporina*), especially in the epidydimal caput. Moreover, we recommended the use of a 50 mOsm/l hypoosmotic solution for the analysis of this parameter through the hypoosmotic swelling test.

Conflict of interest statement

The authors state that there are no conflicts of interest

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Data availability statement

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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TABLES

Table 1. Mean values (± SEM) for the sperm parameters of red-rumped agouti (*Dasyprocta leporina*; n = 6) recovered from different regions of the epididymis.

Sperm parameters	Epididymal region		
	Caput	Corpus	Cauda
Sperm concentration (x 10^6 sperm/mL)	105.0 ± 23.8 ^{B}	116.3 ± 42.8 ^{B}	1285.2 ± 319.3 ^{A}
Number of sperm recovered (x 10^6 sperm)	691.7 ± 39.6 ^{B}	691.7 ± 20.1 ^{B}	1375.0 ± 244.1 ^{A}
Normal morphology (%)	84.0 ± 1.2	85.7 ± 1.4	87.3 ± 1.9
Membrane structural integrity (%)	80.7 ± 4.0	75.2 ± 6.8	83.5 ± 3.4
Mitochondrial activity (%)	73.8 ± 7.2	45.0 ± 12.5	85.8 ± 3.1
Total motility (%)	12.2 ± 4.2 ^{C}	37.7 ± 8.8 ^{B}	76.8 ± 6.2 ^{A}
Progressive motility (%)	0.8 ± 0.3 ^{B}	5.3 ± 2.2 ^{B}	13.7 ± 2.8 ^{A}
Average path velocity (VAP; μm/s)	19.9 ± 4.2 ^{B}	33.1 ± 3.8 ^{AB}	38.9 ± 2.6 ^{A}
Straight line velocity (VSL; μm/s)	13.2 ± 3.0 ^{B}	22.2 ± 2.9 ^{B}	27.2 ± 2.2 ^{A}
Curvilinear velocity (VCL; μm/s)	38.9 ± 8.4 ^{B}	62.0 ± 5.1 ^{B}	69.4 ± 3.9 ^{A}
Amplitude of lateral head displacement (ALH; μm)	4.2 ± 1.0	5.0 ± 0.4	5.7 ± 0.3
Beat cross frequency (BCF; Hz)	31.0 ± 6.4	41.9 ± 0.7	39.5 ± 0.7
Straightness (STR; %)	51.7 ± 10.7	60.5 ± 1.2	65.2 ± 1.3
Linearity (LIN; %)	27.0 ± 5.7	33.0 ± 1.2	36.3 ± 1.5
Elongation (%)	40.3 ± 8.3	49.5 ± 1.9	58.8 ± 2.1
Subpopulations			
% of Rapid sperm	1.0 ± 0.4 ^{B}	8.2 ± 3.8 ^{B}	19.8 ± 4.0 ^{A}
% of Medium velocity sperm	11.0 ± 4.0 ^{C}	29.3 ± 5.3 ^{B}	57.0 ± 2.8 ^{A}
% of Slow sperm	0.5 ± 0.2	0.7 ± 0.3	0.2 ± 0.2
% of Static sperm	87.3 ± 4.3 ^{A}	61.7 ± 8.7 ^{B}	22.7 ± 6.2 ^{C}

^{ABC} Different uppercase letters indicate significant differences among the sperm parameters and the epididymal region ($P < 0.05$).

Table 2. Values for (means ± SEM) osmotic response of red-rumped agouti (*Dasyprocta leporina*; n = 6) sperm to different hypoosmotic solutions in different regions of the epididymis.

Epididymal region*	Osmolarity of solutions		
	0 mOsm/l	50 mOsm/l	200 mOsm/l
Caput	37.3 ± 10.6 ^{B}	68.2 ± 10.3 ^{A}	49.7 ± 9.3 ^{AB}
Corpus	35.0 ± 11.8 ^{B}	75.8 ± 7.8 ^{A}	54.8 ± 12.0 ^{AB}
Cauda	42.5 ± 3.5 ^{B}	83.3 ± 3.9 ^{A}	61.8 ± 3.9 ^{B}

^{ABC} Different uppercase letters indicate significant differences among osmolarities within the same region of the epididymis ($P < 0.05$). *There were no significant differences among the different regions of the epididymis with the same osmolarity ($P > 0.05$).

Table 3. Values for Spearman correlations (ρ) among the osmotic response to different hypoosmotic solutions and the sperm parameters in different regions of the agouti (*Dasyprocta leporina*; n = 6) epididymis.

Kinetic parameters*	Osmolarity of solutions	Epididymal region			
		Caput	Corpus	Cauda**	
Straight line velocity (VSL; μm/s)	0 mOsm/l	0.886	-0,836	-	ρ
		0.033	0.033	-	*P*
Subpopulation of slow sperm (%)		0.878	-	-	P
		0.033	-	-	*P*
Average path velocity (VAP; μm/s)	50 mOsm/l	0.841	0,886	-	ρ
		0.033	0.033	-	*P*
Subpopulation of rapid sperm (%)	200 mOsm/l	0.956	-	-	ρ
		0.003	-	-	*P*
Elongation (%)		-0.943	-	-	ρ
		0.017	-	-	*P*

* Only the sperm parameters that showed significant correlations were expressed in the table ($P < 0.05$).
** There were no significant correlations among osmotic responses in the epididymal cauda region and other sperm parameters ($P > 0.05$).

CAPÍTULO IV

Weather changes between seasons impact the epididymal sperm characteristics of red-rumped agouti (*Dasyprocta leporina*) reared in a semiarid region

Mudanças ambientais entre as estações impactam nas características do espermatozoide epididimário de cutia (*Dasyprocta leporina*) criada em uma região semiárida

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Abstract: In view of the worrying recent climate changes that can directly impact the reproduction of rodent species, understanding the seasonal influence on sperm quality of agoutis is essential for the conservation and management of this species. The present study aimed to evaluate the impacts of weather changes derived from the dry and rainy seasons of a semiarid region on the sperm characteristics of red-rumped agouti (*Dasyprocta leporina*). A total of 14 agoutis reared in captivity were used in the experiment, being the sperm from the epididymal cauda of seven individuals collected per season (dry and rainy) through the floating technique. Samples were evaluated for kinetic parameters, membrane structural and functional integrity, mitochondrial activity, morphology, morphometry. To characterize the seasons, the environment variables were measured by a nearby meteorological station. The maximum air temperature (ºC), relative humidity (%), wind speed (m/s), and the total rainfall (mm) for dry and rainy seasons were, respectively: 36.2 and 34.1 ºC, 66.8 and 80.1%, 4.0 and 1.9 m/s, 0.2 and 517.7 mm). Sperm concentration and number of sperm collected were higher in the dry season (1028.7 sperm/mL x 10^6 and 1361.2 x 10^6 sperm) compared to the rainy season (758.9 sperm/mL x 10^6 and 714.6 sperm x 10^6). Acrosome defects showed a much lower percentage of defects during the rainy (0.1%) than during the dry season (1.43%). The averages of head length, head width, and midpiece length were higher during the rainy season (5.42 μm, 3.61 μm, and 5.78 μm, respectively) compared to dry season (4.90 μm, 3.23 μm, and 5.50 μm, respectively). The sperm during the rainy season showed greater membrane structural integrity with mitochondrial activity (85.7 %), but it does not differ for functional integrity between seasons. Regarding motility patterns, the increases during the rainy season stand out in terms of total and progressive motility, VAP, VSL, VCL, and subpopulations of rapid velocity. In summary, weather changes related to the rainy season of a semiarid region positively influenced the sperm quality in red-rumped agouti. Furthermore, the largest amount of male gametes was obtained during the dry season, probably due to compensatory mechanisms.

Keywords: biotechniques, changes climate, environmental influence, morphophysiology, wild animal.

1. Introduction

The Caatinga is the largest tropical dry forest in South America and the only biome exclusively Brazilian (Silva et al., 2017). It is an ecosystem with great biological diversity, and that is seriously threatened due to anthropic action. Deforestation and predatory hunting alone have destroyed half of this biome over the decades and threatened other Brazilian areas (Brasil, 2018). Although hunting wild species and their by-products participate in the traditional life of native communities in the Caatinga, as in the Brazilian semiarid (Mendonça et al., 2016), many of these animals participate in the food chain as a regular diet of carnivores (Alves et al., 2009), requiring control over the preservation of wild species. It is known that in tropical regions, the rise in atmospheric temperature, as well as the reduction in annual rainfall, prolong periods of drought, which affects the entire ecosystem (Parry et al., 2007). Due to global climate change, several wild species have increased their state of vulnerability (Nobre, 2009; Bronson, 2009). These changes reveal the urgency of improving biodiversity conservation strategies (Brooke, 2008; Hansen et al., 2010). Furthermore, heat stress is widely known as one factors that most significantly affect animal performance. (Veléz-Terranova et al., 2021; del Río Avilés et al., 2021).

The agouti is a wild rodent belonging to the Dasyproctidae family, which are cataloged eleven species in the genus *Dasyprocta*. Within these, seven species live naturally in Brazilian ecosystems (Woods; Kilpatrick, 2005), with *D. leporina* and *D. prymnolopha* being the species with the most frequent populations in Northeastern Brazil, in the Caatinga biome. Although predatory hunting occurs, *D. leporina* is classified as an animal in a state of little concern (Emmons; Reid, 2016), according to IUCN (International Union for Conservation of Nature) criteria. However, due to the constant deforestation of the Caatinga and the gradual loss of its natural habitat, the rearing in captivity is advised for the conservation of the species since this practice makes possible the management and confers its preservation and ex-situ multiplication of the animals, increasing the accumulated knowledge about them (Hosken; Silveira, 2001).

It is known that the reproductive characteristics of wild rodents are affected by environmental factors, such as photoperiod, precipitation, humidity, and temperature (Dantas et al., 2021). These factors vary throughout the year, and, as the semiarid region of the Caatinga biome has only two distinct seasons, one dry and the other rainy, it is to be expected a large variation in humidity, rainfall, and temperature between these periods (Sobrinho et al., 2011). Thus, it is essential to assess and understand how these environmental factors affect the

spermatic characteristics of the agouti so that it is possible to establish strategies that can mitigate the harmful effects on reproductive activity (Rezende and Bozinovic, 2019; Dantas, 2022).

Given what was exposed, in view of the worrying climate changes, understanding the seasonal influence on the reproductive physiology of agouti sperm is essential for the conservation of this species. Thus, the present study aimed to evaluate the seasonal impacts caused during the dry and rainy seasons of a semiarid region on the spermatic characteristics of agouti (*D. leporina*) reared in captivity.

2. Methodology

2.1. Animals

This study used sexually mature male agoutis (*Dasyprocta leporina*) at approximately 12 months of age. The animals belonged to the Center of Multiplication of Wild Animals (CEMAS/UFERSA, Mossoró, RN, Brazil), which is registered at the Brazilian Institute of Environment and Renewable Natural Resources (IBAMA) as a scientific breeding ground (no. 1478912). The study was authorized and approved by the Ethics Committee for the Use of Animals at UFERSA (CEUA: Opinion 11/2019) and by the Chico Mendes Institute for Biodiversity Conservation (no. 66618-3). The animals were obtained from an on-site scheduled slaughter that is carried out every year for population control.

In the present study, seven agoutis were used in the dry period (September and November 2019) and seven in the rainy period (February to May 2021), submitted to the same rearing conditions, both in facilities and in a diet. The animals were placed separately in paddocks measuring 2.25 m^2 and 1.5 m in height, covered with ceramic tiles. The sides of the pickets are made of wire mesh for the free circulation of the wind. The animals were all kept in captivity, fed with fruits, corn, and commercial rabbit food, given once a day. Water was offered ad libitum

2.2. Environmental characterization of the study site

The municipality of Mossoró (latitude 5°11'S, longitude 37°20'W, 18 m above sea level) is located in the northeastern Brazilian hinterland, in the Caatinga biome. According to the

Köppen-Geiger climate classification, the climate is semiarid tropical (BSh type), typically presenting eight to nine dry months and three to four rainy months (Sobrinho et al., 2011). The natural photoperiod has few variations, around 12 hours throughout the year (Sobrinho et al., 2011).

The rainfall index (mm) and solar radiation (W/m^2) were measured by the National Meteorological Institute station for each season. In addition, air temperature (ºC), relative humidity (%), and wind speed (m/s) were measured with a thermo-hygrometer-anemometer (INSTRUTERM-HT300; São Paulo, Brazil) installed next to the animal paddocks. These last parameters were measured every 30 min throughout the 24 hours of the day, obtaining the average, minimum, and maximum values per season.

During the experiment, the total rainfall during the dry season (0.2 mm) was significantly lower ($P < 0.05$) than during the rainy season (517.7 mm). The average relative humidity during the rainy season was higher than in the dry season ($p < 0.05$). Also, the solar radiation and maximum air temperature were significantly higher in the dry season ($p < 0.05$). The values of the other environmental parameters measured during the seasons are presented in Table 1.

Table 1: Values of the climatic variables observed during the dry (September, October, and November 2019) and rainy (February, March, April, and May 2021) periods in the semiarid equatorial region.

Seasons	Air temperature (ºC)			Solar radiation (W/m^2)	Relative humidity (%)	Wind velocity (m/s)	Rainfall (mm)
	Average	Minimum	Maximum	Average	Average	Average	Total
Dry	27.3 ± 0.1	23.1 ± 0.2	36.2 ± 0.1^{A}	527.3 ± 51.1	66.8 ± 0.3^{B}	4.0 ± 0.1^{A}	0.2^{B}
Rainy	27.5 ± 0.1	23.2 ± 0.1	34.1 ± 0.2^{B}	441.8 ± 44.3	80.1 ± 0.4^{A}	1.9 ± 0.1^{B}	517.7^{A}

^{A,B}Values with different letters on the same column differ statistically for the same observed variable ($P < 0.05$).

2.3. Epididymal sperm collection

The animals were submitted to food and water fasting for 12 hours before the experimental procedures. After being physically contained by a capture net, the animals were premedicated with an association of xylazine (1 mg/kg, Rompun, Bayer, São Paulo, SP, Brazil) and ketamine (15 mg/kg, Ketalar, Pfizer, São Paulo, SP, Brazil), given IM. After 15 min, 50 mg/kg IM sodium thiopental (Thiopentax; Cristalia, São Paulo, SP, Brazil) was given IV. After

anesthetic induction, the animals were sacrificed with 1 mL/kg of potassium chloride IV (Castelo et al., 2015).

After euthanasia, the abdominal cavity was opened, and the pairs of testis-epididymis complexes were retrieved, examined for any pathology. After, they were washed in a salt solution, placed in a beaker containing gauze soaked with phosphate-buffered saline (PBS), stored in an insulated polystyrene box at 4 ºC, and transported to the laboratory for processing. Subsequently, the testis-epididymis complexes were placed in Petri dishes containing 5 ml of PBS solution heated to 37 °C, where sperm from the epididymis tail were recovered through the flotation technique, using a scalpel (Silva et al., 2016).

2.4. Sperm evaluation

To determine the number of sperm collected (sperm x 10^6) (Silva et al., 2016), the total volume (ul) collected through the fluctuation technique was multiplied by the sperm concentration (sperm x 10^6/mL). As for sperm concentration, it was measured using an aliquot of 10µL of semen diluted in formolized solution (10%) buffered (1mL), which was subsequently observed in a Neubauer chamber (Silva et al., 2016).

2.4.1. Sperm kinetics parameters

The analysis of sperm kinetic parameters was performed using a Computer-Assisted Sperm Analysis (CASA, IVOS 7.4 G; Hamilton-Thorne Research, Beverly, MA, USA). For this, 3 µl samples were evaluated using specific adjustments previously described for agouti (Castelo et al., 2015): straightness threshold, 30%; minimum contrast, 45; low-velocity average pathway (VAP) cutoff, 10 m/s; medium VAP cutoff, 30 m/s; temperature, 37 °C. Scanning proceeded by analyzing five independent and non-consecutive microscopic fields. Thus, the following parameters were evaluated: total motility (%), progressive motility (%), velocity average pathway (VAP, 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, %). Furthermore, the total sperm population was subdivided into four categories: rapid, medium, slow and static (%).

2.4.2. Sperm membrane functionality

To assess the membrane functionality of the agouti sperm, the HOS test was employed, as proposed by Dantas et al. (2022). Therefore, 5 µL of the sample containing epididymal sperm was used, added to 45 µL of a hypoosmotic solution containing a combination of distilled water with sodium citrate and fructose (50 mOsm/L). The aliquots were placed to incubate in a dry bath for 40 min at 37º C, and then 10 µL of each sample was deposited on slides to be evaluated under a phase-contrast light microscope (x40). Finally, 100 sperm were counted, classifying as reactive to HOS test those that presented their tail curled (Fonseca et al., 2005).

2.4.3. Sperm membrane integrity and mitochondrial activity

The mitochondrial activity and the structural integrity of the sperm membrane were evaluated according to the methodology described by Celeghini et al. (2007). For this, an aliquot of semen (10µL) was incubated at 34 °C for 10 min in a solution containing the combination of fluorescent probes, whose composition was 2µL of propidium iodide (PI), NaCl 0.5 mg/ml at 0 .9%, 5µL of CMXRos (Mito Tracker Red®, Molecular Probes, F-7512) at 500 nM (50 µg dilution in 94 µL of DPBS) and 3µL of Hoechst 342 (H342) (diluted to 25 mg/mL in DMSO). Afterward, the samples were evaluated in an epifluorescence microscope (EFA Fluorescent Attachment "EFA" halogen lamp set, Leica, Kista, Sweden), observing 200 spermatozoa per sample. The structural integrity of the membrane and the potential of the mitochondrial membrane were evaluated by the association PI/H342 and CMXRos, respectively. Thus, the heads of spermatozoa marked in blue (H342) were considered to have an intact membrane, and those totally or partially marked with red (PI) were considered non-functional membranes. The sperm in which the midpiece region was marked in red were considered to have mitochondrial activity.

2.4.4. Sperm morphology and morphometry

As for sperm morphology (Silva et al., 2011), the analysis proceeded using 5 µL aliquots of sperm samples, fixed with 45 µL of Rose Bengal (20 mL distilled water; 0.58 g sodium citrate; 0.8 formaldehyde mL; Rose Bengal 0.3 g; CAQ - Casa da Química, São Paulo-SP, Brazil), deposited between slides and glass coverslips. After that, the slides were observed for normal sperm and with sperm defects, using a light microscope with a magnification of 100x,

counting 200 sperm for each sample. Thus, the percentages of normal sperm and defects in the acrosome, head, midpiece, and tail of the sperm were identified.

As for the morphometric analysis (Silva et al., 2016), the Rose Bengal stained slides used for the morphological analysis were used. For that, a light microscope with 400x magnification was used, where images were obtained in random fields for each sample, counting 200 spermatozoa per sample. To obtain the sperm metrics, image analysis software (ImageJ Software, Wayne Rasband - National Institute of Health, Maryland, United States) was used. The following sperm measurements were obtained: head length (measured from the apex of the acrosome to the base of the head), head width (measured from the transverse axis with the largest diameter), length of the intermediate piece (measured from the base insertion from the head to the Jensen ring region), tail length (measured from the beginning of the intermediate piece to the end of the tail) and total length (measured from the apex of the acrosome to the end of the tail) (Silva et al., 2015).

2.5. Data analysis

All analyzes were performed using the Statistical Analysis Software version 8.0 (SAS Institute Inc., Cary, NC, USA). Data were expressed as the mean ± SEM, and parametric assumptions were tested for all analyzed variables (from environment and animals): normality of the residuals using the Shapiro-Wilk test and homoscedasticity using the Levene test. A One-Way ANOVA (PROC GLM; *F*-test) was conducted to assess potential differences between seasons (rainy and dry), which were considered significant when $P < 0.05$. The Spearman correlation test was performed to verify the relationship between the reproductive parameters and environmental variables averages in the dry and rainy periods.

3. Results

3.1. Sperm quantitative evaluation

The sperm concentration was higher during the dry period (1028.7 ± 147.4 sperm/mL x 10^6) than in the rainy period (758.9 ± 136.8 sperm/mL x 10^6) (Table 2). As a result, the number of sperm collected was significantly higher ($P < 0.05$) during the dry season (1361.2 ± 190.2 sperm x 10^6) than those collected during the rainy season (714.6 ± 147.4 sperm x 10^6).

Table 2: Mean values (± SEM) for volume, sperm concentration, and number of sperm collected from agouti (*Dasyprocta leporina*), obtained in the dry and rainy seasons of an equatorial semiarid region.

Parameters	Seasons	
	Dry	Rainy
Volume (uL)	1392.9 ± 207.1	940.0 ± 115.3
Concentration (sperm/mL x 10^6)	1028.7 ± 147.4	758.9 ± 136.8
Number of sperm collected (sperm x 10^6)	1361.2 ± 190.2^A	714.6 ± 147.4^B

^{A,B}Values with different letters on the same column differ statistically for the same observed variable ($P < 0.05$).

3.2. Sperm kinetic parameters

When observing the kinetic parameters assessed by CASA, sperm collected during the rainy season showed a clear increase in motility with straight trajectories (Table 3). Most of these parameters showed a significant increase ($P < 0.05$) during the rainy season than during the dry season, highlighting those with greater contrast, respectively: total motility (93.28 ± 0.8 and 73.3 ± 6.4%), progressive motility (63.6 ± 2.3 and 13.3 ± 2.4%), VAP (98.4 ± 4.6 and 39.0 ± 2.1 μm/s), VSL (86.0 ± 4.2 and 27.5 ± 1.9 μm/s), VCL (128.7 ± 5.0 and 69.1 ± 3.3 μm/s), and subpopulations of rapid (74.7 ± 3.2 and 19.0 ± 3.5%) and static velocities (6.3 ± 0.6 and 26.3 ± 6.4%). ALH, BCF, STR, and LIN also showed significant differences between periods.

Table 3: Mean values (± SEM) of the kinetic parameters of sperm agouti (*Dasyprocta leporina*), obtained in the dry and rainy seasons of an equatorial semiarid.

Kinetic parameters	Seasons	
	Dry	Rainy
Total motility (%)	73.3 ± 6.4^B	93.28 ± 0.8^A
Progressive motility (%)	13.3 ± 2.4^B	63.6 ± 2.3^A
Average path velocity (VAP; μm/s)	39.0 ± 2.1^B	98.4 ± 4.6^A
Straight line velocity (VSL; μm/s)	27.5 ± 1.9^B	86.0 ± 4.2^A
Curvilinear velocity (VCL; μm/s)	69.1 ± 3.3^B	128.7 ± 5.0^A
Amplitude of lateral head displacement (ALH; μm)	5.8 ± 0.3^B	4.8 ± 0.2^A
Beat cross frequency (BCF; Hz)	39.9 ± 0.7^A	32.7 ± 1.0^B
Straightness (STR; %)	65.0 ± 1.1^B	83.0 ± 0.8^A
Linearity (LIN; %)	36.4 ± 1.2^B	62.3 ± 1.6^A
Subpopulations		
% of Rapid sperm	19.0 ± 3.5^B	74.7 ± 3.2^A
% of Medium velocity sperm	54.3 ± 3.6^A	18.6 ± 2.9^B
% of Slow sperm	0.3 ± 0.2	0 ± 0
% of Static sperm	26.3 ± 6.4^A	6.3 ± 0.6^B

^{A,B}Values with different letters on the same line differ statistically for the same observed variable ($P < 0.05$).

3.3 Mitochondrial activity and sperm membrane structure functionality and viability

As for the evaluation of the functional integrity of the membrane, performed by the HOS test, there was no significant difference ($P > 0.05$) between the seasons where sperm were collected (Table 4). The values obtained between the dry and rainy periods were very similar (83.3 ± 3.3 and 82.7 ± 6.4%, respectively). Regarding the parameters of membrane structural integrity and mitochondrial activity, it is observed that there were no significant differences between any of these variables ($P > 0.05$) between seasons (Table 4).

Table 4: Mean values (± SEM) of mitochondrial activity and membrane structure functionality and viability of sperm agouti (*Dasyprocta leporina*), obtained in the dry and rainy seasons of an equatorial semiarid.

Sperm parameters (%)	Seasons*	
	Dry	Rainy
Membrane functional integrity	83.3 ± 3.3	82.7 ± 6.4
Membrane integrity with mitochondrial activity	79.7 ± 4.6	85.7 ± 3.0
Membrane integrity with unable mitochondrial activity	0.6 ± 0.4	0.1 ± 0.1
Membrane not functional with mitochondrial activity	2.1 ± 0.9	0.8 ± 0.5
Membrane not functional with unable mitochondrial activity	14.9 ± 0.8	13.6 ± 2.5

*There was no statistical difference between sperm parameters and seasons ($P > 0.05$).

3.4. Sperm morphology and morphometry

As for normal sperm morphology and sperm defects from different regions of the sperm (Table 5), only acrosome defects showed a significant difference, obtaining a much lower percentage of defects during the rainy season (0.1 ± 0.1%), when compared to the period dry (1.43 ± 0.69%) ($P < 0.05$).

Regarding sperm morphometry (Table 6), there is a significant increase in sperm dimensions for head length (5.42 ± 0.03 μm), head width (3.61 ± 0.03 μm), and midpiece length (5.78 ± 0.05 μm) during the rainy season, then the same metrics found during the dry season (4.90 ± 0.01 μm, 3.23 ± 0.01 μm, and 5.50 ± 0.02 μm, respectively) ($P < 0.05$). The tail length and total length showed no significant difference between periods.

Table 5: Mean values (± SEM) for the morphology parameters of sperm agouti (*Dasyprocta leporina*), obtained in the dry and rainy seasons of an equatorial semiarid.

Sperm morphology (%)	Seasons			
	Dry		Rainy	
	Mean	Range	Mean	Range
Normal sperm	88.9 ± 2.2	82 - 98	91.4 ± 1.1	87 - 97
Acrosome defects	1.4 ± 0.7^{A}	0 - 4	0.1 ± 0.1^{B}	0 - 1
Head defects				
Double head	0.4 ± 0.3	0 - 2	0 ± 0	0
Macrocephalic	0.1 ± 0.1	0 - 1	0 ± 0	0
Microcephalic	1 ± 0.4	0 - 3	0.3 ± 0.2	0 - 2
Abnormal head shape	1 ± 0.6	0 - 4	1.2 ± 0.6	0 - 4
Detached head	0.7 ± 0.4	0 - 3	2.1 ± 0.8	0 - 6
Total head defects	3.8 ± 1.1	0 - 4	3.6 ± 1.2	0 - 9
Midpiece and droplet defects				
Folded midpiece	0.7 ± 0.4	0 - 3	0.9 ± 0.4	0 - 4
Broken midpiece	0.4 ± 0.2	0 - 1	0.1 ± 0.1	0 - 1
Thick midpiece	0.4 ± 0.2	0 - 1	0.3 ± 0.2	0 - 1
Abaxial midpece insertion	0.1 ± 0.1	0 - 1	0.3 ± 0.2	0 - 3
Distal cytoplasmatic droplet	0.9 ± 0.5	0 - 3	0.3 ± 0.2	0 - 1
Proximal cytoplasmatic droplet	0.6 ± 0.3	0 - 2	0.1 ± 0.1	0 - 1
Total midpiece defects	3.1 ± 0,9	0 - 3	2.0 ± 0.4	0 - 4
Tail defects				
Curled tail	2.6 ± 0.6	0 - 5	1.5 ± 0.3	0 - 3
Heavily curled tail	0.3 ± 0.2	0 - 1	1.0 ± 0.4	0 - 3
Double tail	0 ± 0	0	0 ± 0	0
Broken tail	0.4 ± 0.3	0 - 2	0.2 ± 0.1	0 - 1
Total tail defects	3.3 ± 0.7	0 - 5	2.7 ± 0.6	0 - 5

^{A,B}Values with different letters on the same line differ statistically for the same observed variable ($P < 0.05$).

Table 6: Mean values (± SEM) of the morphometric parameters of sperm agouti (*Dasyprocta leporina*), obtained in the dry and rainy seasons of an equatorial semiarid.

Sperm regions (μm)	Seasons	
	Dry	Rainy
Head length	4.90 ± 0.01^{B}	5.42 ± 0.03^{A}
Head width	3.23 ± 0.01^{B}	3.61 ± 0.03^{A}
Midpiece length	5.50 ± 0.02^{B}	5.78 ± 0.05^{A}
Tail length	31.01 ± 0.06	30.63 ± 0.18
Total length	41.40 ± 0.07	41.83 ± 0.18

^{A,B}Values with different letters on the same line differ statistically for the same observed variable ($P < 0.05$).

3.5. Correlations

The existing correlations between climatic variables during dry and rainy seasons with the sperm parameters are presented in table 7.

Table 7: Correlations (ρ) between meteorological variables and sperm parameters in agouti (*Dasyprocta leporina*).

Sperm parameters*	Meteorological variables							
	Rainfall	Solar radiation	Average air temperature	Maximum air temperature	Minimum air temperature	Relative humidity	Wind velocity	
Total Motility	0.6691	-0.6691	0.6691	-0.6691	0.6691	0.6691	-0.6691	ρ
	0.0089	0.0089	0.0089	0.0089	0.0089	0.0089	0.0089	P
Progressive motility	0.9746	-0.9746	0.9746	-0.9746	0.9746	0.9746	-0.9746	ρ
	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	P
% of Rapid sperm	0.9588	-0.9588	-0.9588	-0.9588	0.9588	0.9588	-0.9588	ρ
	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	P
% of Static sperm	-0.6687	0.6687	-0.6687	0.6687	-0.6687	-0.6687	0.6687	ρ
	0.0089	0.0089	0.0089	0.0089	0.0089	0.0089	0.0089	P
Average path velocity (VAP)	0.9588	-0.9588	0.9588	-0.9588	0.9588	0.9588	-0.9588	ρ
	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	P
Straight line velocity (VSL)	0.9647	-0.9647	0.9647	-0.9647	0.9647	0.9647	-0.9647	ρ
	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	P
Curvilinear velocity (VCL)	0.9445	-0.9445	0.9445	-0.9445	0.9445	0.9445	-0.9445	ρ
	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	P
Amplitude of lateral head displacement (ALH)	-0.6682	0.6682	-0.6682	0.6682	-0.6682	-0.6682	0.6682	ρ
	0.0090	0.0090	0.0090	0.0090	0.0090	0.0090	0.0090	P
Beat cross frequency (BCF)	-0.8691	0.8691	-0.8691	0.8691	-0.8691	-0.8691	0.8691	ρ
	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	P
Straightness (STR)	0.9672	-0.9672	0.9672	-0.9672	0.9672	0.9672	-0.9672	ρ
	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	P
Linearity (LIN)	0.9641	-0.9641	0.9641	-0.9641	0.9641	0.9641	-0.9641	ρ
	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	P
Number of sperm collected	-0.5561	0.5561	-0.5561	0.5561	-0.5561	-0.5561	0.5561	ρ
	0.0389	0.0389	0.0389	0.0389	0.0389	0.0389	0.0389	P
Membrane integrity with unable mitochondrial activity	-0.5852	0.5852	-0.5852	0.5852	-0.5852	-0.5852	0.5852	ρ
	0.0279	0.0279	0.0279	0.0279	0.0279	0.0279	0.0279	P
Head length	0.4549	-0.4549	0.4549	-0.4549	0.4549	0.4549	-0.4549	ρ
	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	P
Head width	0.3562	-0.3562	0.3562	-0.3562	0.3562	0.3562	-0.3562	ρ
	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	P

*Only the sperm parameters that present a significant correlation were expressed in the table ($P < 0.05$).

Discussion

Depending on the species in question, the seasonal characteristics of a given region influence gonadal activity in males and females, which directs pregnancies and offspring births to the most favorable periods of the year for survival (Dubost et al., 2005; Bronson, 2009; Đuričić et al., 2021;). Several species of mammals, as well as rodents, have their reproductive

characteristics affected by environmental variables due to seasonal variations in each region (Dubost et al., 2005; Dantas et al., 2021; Mylostyvyi and Izhboldina, 2021). The present study showed that for the red-rumped agouti (*D. leporina*), the sperm characteristics of sexually mature males suffer the greatest impacts due to the climatic conditions of the dry period in the semiarid equatorial region, especially those related to the acquisition of motility. As well established by some authors, heat stress can trigger damage to the seminiferous epithelium of rodents, also with deleterious effects on semen quality (Salman et al., 2017; Muteka et al., 2018; Dantas and Souza-Junior, 2021). The severity of the damage depends on the time and temperature of exposure, but even a 1 or 2 °C increase in testicular temperature for eight hours can cause significant changes in spermatogenesis (Entwistle, 1992; Setchell, 2006).

Although the rainy season of the study presents a total rainfall of 517.7 mm, the average and minimum air temperatures of the rainy season were similar to those of the dry season of 0.2 mm. However, it is possible to verify differences in thermal conditions between seasons when observing the solar radiation (441.8 and 527.3 W/m^2), maximum air temperatures (36.2 and 34.1 ºC), and relative humidity (66.8 and 80.1%) of the dry and rainy periods, respectively. These data are similar to those found by Maia et al. (2021). The authors found slight variations between the minimum (27.1 and 26.7 ºC) and maximum (36.0 and 37.2 ºC) air temperatures in the dry and rainy seasons, respectively, in the same region of the present study. This fact occurs because the rainy season in the study region occurs during the months (February - May) that comprise summer and autumn in the southern hemisphere, comprising the period in which there is higher incidence of solar radiation and consequently higher levels of air temperature. Thus, it was expected that due to the water conditions of the rainy season and the consequent high relative humidity, the air temperature during the hottest times of the day was milder than in the dry season. Most of the agouti sperm characteristics in this study were qualitatively superior to those found during the dry period.

Notably, the physiology of sperm maturation in *D. leporina* is associated with seasonal conditions in semiarid region, as shown in Table 7. It is noticed that there is a strong correlation ($\rho > 0.9$ and $\rho < -0.9$) with some sperm parameters depending on the environmental conditions of the year, mainly those related to the acquisition of sperm mobility, such as progressive motility, rapid sperm subpopulation, average path velocity, straight line velocity, curvilinear velocity, beat cross frequency, straightness, and linearity. These parameters showed better values during the rainy season, positively correlated with rainfall, minimum air temperature, and relative humidity. On the other hand, the same parameters are negatively correlated with

solar radiation, maximum air temperature, and wind speed, which were higher during the dry period, typical of the semiarid region.

Regarding the quantitative characteristics, it is noted that the concentration and number of sperm collected were higher during the dry season when compared to the rainy season, going against the trend observed in most species of mammals (Bronson and Heideman, 1994). Likewise, in the present study, Prastowo et al. (2018), studying the sperm of Bali cattle bulls (*Bos javanicus*) collected in the rainy season (November - May) and dry season (June - October) from Indonesia, found an increase in sperm concentration in the dry period (from 936.5 to 1036.2 x 10^6 sperm/ml), where mean air temperatures were higher. Furthermore, Luther et al. (2020), evaluating semen from African elephants (*Loxodonta africana*), observed that the sperm concentration (976.4 x 10^6 sperm/mL) was higher during the very dry season than during the rainy season in the region (862.1 x 10^6 sperm/ml). Thus, although the climatic conditions of the dry period cause harm in the agouti's sperm as to morphofunctional characteristics, the increase in sperm concentration can act as a compensatory physiological mechanism for the animal to supply the low sperm quality during the dry periods. Thus, this physiological strategy may compensate for the low quality of the kinetic parameters observed in the present study, also allowing the animals to reproduce during the dry period of the semiarid region. This hypothesis is corroborated by the studies of Pinheiro et al. (1989) and Diniz et al. (2019), which evaluated the reproductive activity of agouti (*D. Prymnoptra*) reared in captivity in equatorial semiarid and equatorial sub-humid warm climates, respectively, observed that the animals reproduced in both the dry and wet seasons. Furthermore, it is noteworthy that the sperm observed in this study came from the epididymal collection, which may have differences in sperm concentrations than if they were obtained from the ejaculate. In the studies by Ferraz et al. (2011) and Castelo et al. (2015), both evaluating agouti sperm (*D. leporine*), it can be seen that the quantitative and qualitative sperm parameters vary when sperm are obtained via epididymis or by electroejaculation, respectively.

Values for sperm kinetic parameters were those with the greatest variation between the two study periods. The agouti spermatozoa collected during the rainy season in the semiarid region exhibited better kinetic characteristics than those collected during the dry season, showing better mobility patterns and straight trajectory. It was observed mainly by the parameters of total and progressive motility, VAP, VSL, VCL, and rapid velocity subpopulation. Likewise, other studies that evaluated sperm kinetic parameters in different seasons of the year identified that the climatic conditions of the hottest season of the year caused

more harmful to sperm quality in different species, such as birds (Santiago-Moreno et al., 2012), elephants (Luther et al., 2020), and collared peccaries (Maia et al., 2018). Several authors have pointed out that sperm motility is commonly applied to assess sperm quality, being the main parameter that indirectly indicates the metabolic activity and integrity of the sperm membrane (Mortimer, 2000; Berlinguer et al., 2009; Bronson, 2009). Since prolonged heat stress, intensified during the hottest season of the year, promotes increased oxidative stress by increasing ROS and consequently lipid peroxidation of sperm membranes (Zhu et al., 2019; Luther et al., 2020), it is expected that during this period, the parameters directly or indirectly related to sperm mobility will be reduced.

In the present study, it was found that during the rainy season, the percentage of sperm with membrane structural integrity and mitochondrial activity was higher when compared to the dry period. This is possibly caused by prolonged exposure to higher temperatures and lower humidity during the hottest hours of the day in the dry season (Llamas-Luceño et al., 2020; Queiroz et al., 2019). Among the main cell damage caused by higher air temperatures, one of the most common is lipid peroxidation of cell membranes caused by increased production of reactive oxygen species (ROS) (Sabés-Alsina et al., 2019; Zhu et al., 2019). Several reports in studies with different species demonstrate that the low mitochondrial potential of sperm is directly related to increased ROS production (Thuwanut et al., 2010; Zakerabasali et al., 2013; Ghafarizadeh et al., 2020). This is due to the fact that ROS causes damage to the plasma membranes and mitochondria due to oxidative stress, which directly impairs the membrane's viability and structure and reduces the mitochondrial potential (Amaral et al., 2013). Regarding the functional integrity of the sperm membrane, the osmotic response was similar for both seasons. This fact demonstrates that, although the structural integrity of the membrane was negatively affected by the climatic conditions of the dry period, its functionality, reflected in the osmotic response, remained unchanged despite this. Maia et al. (2018), studying peccaries (*Pecari tajacu*) raised in captivity in the semiarid climate, observed that the osmotic response did not vary significantly during the dry and rainy periods.

The normal sperm morphology of agouti (*D. leporina*) was little affected by seasonal variations, increasing by 1.5% during the rainy season. Similarly, regarding the normal morphology of collared peccaries reared in the Brazilian semiarid region, there was no significant variation between the dry and rainy periods (Maia et al., 2018). However, cruceño et al. (2013), studying the wild rodent viscachas (*Lagostomus maximus maximus*) in a natural habitat in Argentina, observed that the period of greatest reproductive activity could be

observed during the summer and early autumn, the rainy season in the region. It was found that the number of sperm defects was 28% higher in the dry period. In the present study, there was only a 2.5% increase in the number of normal sperm during the rainy season, which suggests that the climatic conditions of the dry period in the semiarid region, mainly due to the higher maximum temperature during this period, can cause more spermatic defects in the agouti. Sailer et al. (1997) observed, under laboratory conditions, that mouse caudal epididymal sperm submitted to a temperature of 38 ºC showed greater sperm abnormalities induced by the heat stress. Similarly, Aguiar et al. (2018) observed that the percentual of normal sperm from goats reared in the semiarid climate increased 3.9% during the rainy season. In our study, the most striking percentage of sperm defects was in the acrosome, which was much higher during the dry season than the rainy season (from 0.1 to 1.43%), demonstrating that it is the spermatic region most impacted by climatic conditions. The same result was observed with the sperm of African elephants (*L. Africana*), where the percentage of defects in the head and acrosome were 2.5 times higher in the dry period than in the rainy period (Luther et al., 2020). It directly affects the fertilizing potential of sperm since the acrosome reaction plays an essential role during fertilization, enabling sperm to penetrate the zone and fuse with the plasma membrane of the egg (Crozet, 1994).

In our study, there were increases in length head, width head, and length midpiece metrics of agouti sperm (*D. leporina*) during the rainy period of the semiarid region. Studies have long shown that the variation in morphometric measurements acts as a sensitive biomarker related to fertilizing capacity and abnormal sperm chromatin structure (Sailer et al., 1996; Saacke, 2008). In the study of Luther et al. (2020) with African elephant sperm (*L. Africana*), it was verified that the width and length of the head are slightly greater during the rainy season than in the dry season. For the present study, the decrease in the metrics of the head and midpiece of sperm agouti during the dry period is possibly due to the impact caused by the climatic conditions over this period, which affected the mitochondria and membrane and chromatin structures. Sailer et al. (1997) exposed the mouse caudal epididymal sperm to high temperatures and observed that at 40.0°C, there was marked DNA denaturation and, when exposed to 38.0°C, the sperm showed greater abnormalities in chromatin, impairing sperm functions. Also, due to the increase in the length of the midpiece of agouti sperm during the rainy period, caused by the greater development of the mitochondrial sheath, is reflected in the remarkable increase in sperm kinetic parameters in this period (Zhu et al., 2019).

Although the present study shows that the climatic conditions of the rainy periods of the semiarid equatorial favor the sperm quality of the agoutis, this rodent can reproduce in captivity in both seasons in the semiarid climate (Pinheiro et al., 1989; Diniz et al., 2019). It is known that the animal reproduction of wild rodents in the equatorial zone can be linked to several factors, as well as the supply of food and water, the photoperiod, sexual competition, predation, among others (Dubost et al., 2005; Dantas et al., 2021). Thus, it is not appropriate to assume that only seasonal climatic conditions can determine the breeding seasons in rodents. Even in unfavorable environmental conditions, some animals can reproduce throughout the year because they have behavioral, autonomous, and adaptive mechanisms aimed at thermoregulation and eventual maintenance of reproduction despite the climate conditions (Queiroz et al., 2019; Dantas and Souza-Junior, 2021; Mota-Rojas et al., 2021).

Diniz et al. (2019) studied the reproductive activity of agouti (*D. prymnolopha*) reared in captivity in northeastern Brazil with a warm sub-humid tropical climate. This region had a short rainy season of up to five months, from January to May, with a photoperiod duration of approximately 12 hours throughout the year. The authors found that the agouti's reproductive activity is associated with environmental conditions. The increase in birth rates coincides with the hottest periods of the year and with less rainfall (September to December), correlating positively with the maximum temperature and average, and negatively with the relative humidity. The authors hypothesized that, despite the climate conditions in the dry season being more severe than in the rainy season, reproduction is not affected due to the good supply of food throughout the year. This demonstrates that, like *D. leporina* reared in the semiarid, *D. prymnolopha* is well adapted to captive breeding in its region.

Conclusions

This work showed the seasonal impact on sperm characteristics in agouti (*D. leporina*) reared in captivity in a semiarid region. It is evident that the climatic conditions of the dry period, mainly due to the absence of rain and consequent low humidity and high maximum temperature, cause damage to the morphofunctionality of agouti sperm. In general, the parameters regarding the membrane structure and spermatic functionality showed a pattern of better results during the rainy period than the dry period, while the variables concerning the amount of sperm were better during the dry season.

Thus, this study demonstrates the need to establish reproductive strategies and conservation protocols aiming to mitigate the damage caused to agouti sperm due to the climatic conditions of the dry period in the semiarid region. In addition, it was found that, for the collection of germplasm from these animals, the rainy season is the most suitable, as it presented greater fertile potential.

Conflict of interest statement

The authors state that there are no conflicts of interest

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CONCLUSÕES

Nossos resultados mostraram, pela primeira vez, a maturação espermática de uma espécie da família dasyproctidae, analisando e descrevendo os parâmetros inerentes a morfofisiologia e cinética do espermatozoide de cutia (*Dasyprocta leporina*). Nota-se que o padrão de alterações morfológicas e funcionais decorridas do processo de maturação ao longo do trânsito epididimário apresenta-se semelhante ao postulado para roedores murinos, apesar de as cutias apresentarem epidídimos e testículos intra-abdominais..

A avaliação da integridade funcional da membrana espermática da cutia pode ser realizada por meio de teste hiposmótico, com solução à base de citrato de sódio e frutose e com osmolaridade de 50 mOsm /L. Foram observados os parâmetros relacionados à função da membrana espermática ao longo do trânsito epididimário, os quais apresentam correlações significativas com alguns parâmetros cinéticos espermáticos.

Foi evidenciado o impacto sazonal sobre características espermáticas de cutias criadas em cativeiro em uma região semiárida, ao identificar os danos à morfofuncionalidade dos espermatozoides. De maneira geral, qualitativa e quantitativamente, os espermatozoides apresentaram melhores resultados durante o período chuvoso da região. Além disso, verificou-se que, para a coleta de germoplasma desses animais, o período chuvoso é o mais adequado, pois apresentou maior potencial fértil.

É importante ressaltar que o uso de diferentes testes específicos para avaliação dos parâmetros estruturais ou morfofuncionais dos espermatozoides permitem a seleção de melhores reprodutores e o estabelecimento e aprimoramento de protocolos de conservação seminal. Assim, os resultados obtidos por estas análises somam-se e devem ser vistos como exames andrológicos complementares.

PERSPECTIVAS

As informações a respeito da fisiologia da maturação espermática da cutia (*Dasyprocta leporina*) aqui presentes, fornecem dados que podem ser usados para estabelecer estratégias e melhorar as biotécnicas já estabelecidas a fim de conservar não só desta espécie, mas também outras espécies filogeneticamente próximas.

O teste hiposmótico de água destilada com osmolaridade 0 mOsm/L, que outrora era utilizado para avaliar a funcionalidade de membrana espermática da *D. leporina*, deve ser substituído pela solução à base de citrato de sódio e frutose e de osmolaridade 50 mOsm /L, com melhor resposta osmótica.

Em se tratando da coleta de espermatozoides epididimários da *D. leporina* para formação de banco de germoplasma, indica-se que a colheita seja procedida durante o período chuvoso da região semiárida.

Nesse contexto, dado as descobertas aqui apresentadas, e como continuidade desta pesquisa, os próximos passos seriam a observação das correlações entre as variáveis climáticas e as características espermáticas de cutia. Além disso, pesquisas acerca da avaliação bioquímica dos constituintes orgânicos e inorgânicos do plasma seminal, bem como a proteômica do espermatozoide epididimário, podem fornecer informações que expliquem com maior fidedignidade os efeitos das condições climáticas durante as estações seca e chuvosa sobre os parâmetros espermáticos.

ANEXOS

Resumos desenvolvidos a partir de dados oriundos da tese.

Todos os resumos foram aprovados e apresentados em seus respectivos congressos.

Área do conhecimento: Ciências Agrárias

AVALIAÇÃO DA APLICABILIDADE DA TERMOGRAFIA INFRAVERMELHA NA AFERIÇÃO DA TEMPERATURA SUPERFICIAL DE CUTIAS (*Dasyprocta leporina Linneaus*, 1753) E SUA RELAÇÃO COM O AMBIENTE TÉRMICO

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A termorregulação é um conjunto de processos que ocorre pelo equilíbrio dos mecanismos de produção e de dissipação de calor entre o corpo e o ambiente, por meio da utilização de mecanismos autonômicos e comportamentais. Objetivou-se avaliar a relação da temperatura superficial de cutias com o ambiente térmico do semi-árido brasileiro, considerando as variáveis ambientais estudadas - radiação solar (W/m^2), temperatura do ar (T_A, ° C), umidade relativa do ar (R_H, %), velocidade do vento ($V\ v$, ms^{-1}) - e a temperatura superficial de regiões específicas de cutias, como extremidades distais dos membros paralelos anterior e posterior, tronco, cabeça, orelha, olho e vibrissas. As coletas de dados foram realizadas às 7h00min. e 14h00min, no decorrer dos meses de fevereiro, março e abril de 2019. Utilizaram-se quatorze animais, machos, com uma média (± EP) de idade e peso corporal de 13 ± 0,5 meses e 2,6 ± 0,4 kg, respectivamente. Utilizaram-se para as coletas uma câmera termográfica de infravermelho (*FLIR-B60)*. Ao momento da fotografia, os animais estiveram sempre em estação, a uma distância de 1,5m do avaliador e nunca estiveram expostos diretamente ao sol. Posteriormente, as imagens obtidas foram avaliadas no *software ThermaCAM Researcher*. Os efeitos da hora do dia foram determinados por meio de uma ANOVA e a relação entre as temperaturas corporais e as variáveis ambientais por meio da correlação de *Pearson*. Os resultados mostram que houve diferença significativa (P<0,001) para as temperaturas superficiais de todas as regiões corporais analisadas entre a hora do dia, sendo que as maiores médias foram encontradas às 14h00min. As maiores diferenças entre as temperaturas superficiais aferidas às 7h00min. e 14h00min. foram encontradas nos membros posteriores (7h00min. 30,94 ºC e às 14h00min. 33,28 ºC) e anteriores (7h00min. 31,17 ºC e 14h00min. 33,28 ºC). Os maiores valores de correlação entre as temperaturas corporais e as variáveis ambientais foram encontrados às 14h00min. A temperatura do ar e a radiação solar foram os componentes ambientais que apresentaram a maior influência sobre a temperatura superficial com coeficientes de correlação entre 0,68 e 0,73 (P<0,001). Constatou-se, portanto, que o grau de ligação entre as variáveis ambientais analisadas e as regiões do corpo desses roedores oscila de acordo com o horário do dia. Assim, pode-se concluir que tais regiões do corpo desses roedores sofrem influência ambiental de forma isolada, provavelmente para facilitar a troca de calor, auxiliando, assim, na termorregulação do seu corpo.

Palavras-chave: *Dasyprocta leporina*, Temperatura superficial, Termografia infravermelha, Termorregulação.
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Área temática: Ciências Agrárias

Descrição das características bioquímicas do plasma seminal de cutias (*Dasyprocta leporina*, LINEAUS 1758) coletadas durante o período seco de uma região semiárida

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Em mamíferos, alguns constituintes bioquímicos do plasma seminal são responsáveis por lhe conferirem ação antimicrobiana, além de participarem da ativação da motilidade espermática, neutralização dos metabólitos espermáticos e fornecerem proteção contra a acrosina por meio de inibidores de proteases. Entretanto, pouco se sabe a respeito da composição do plasma seminal de cutias (*Dasyprocta leporina*), um roedor histricognato silvestre, típico da fauna brasileira. O objetivo do presente trabalho foi identificar os componentes bioquímicos presentes no plasma seminal de cutias coletadas durante o período seco no bioma da Caatinga. Os procedimentos experimentais foram desenvolvidos conforme aprovação do CEUA/UFERSA (Parecer nº 11/2019). Para tanto, foram utilizados seis machos adultos de cutias provenientes do Centro de Multiplicação de Animais Silvestres (CEMAS) da UFERSA. Os animais foram coletados no período de setembro a novembro de 2019, caracterizando o pique do período seco na região. As coletas foram realizadas por meio de eletroejaculação, em um protocolo de estimulação serial com 3 ciclos de estimulação em intervalos de 5 minutos entre eles. Para se conhecer os constituintes bioquímicos produzidos pelas glândulas anexas, sem influência do produto epididimário, apenas os ejaculados aspérmicos foram utilizados. Assim, as amostras foram coletadas em tubos e analisadas sob microscopia de luz para confirmar a ausência de espermatozoides. Em seguida, foram centrifugadas a 800 g durante 10 minutos e refrigeradas a -20ºC. As análises bioquímicas foram realizadas utilizando-se kits comerciais e as absorbâncias foram lidas em espectrofotômetro de acordo com o comprimento de onda estabelecido em cada kit. Os resultados foram descritos em média e erro padrão. O protocolo de eletroejaculação proporcionou a obtenção de 1091,66 ± 205,49 µL de plasma, variando entre 500 e 1850 µL. Neste período, foram identificados os seguintes constituintes bioquímicos no plasma seminal das cutias: albumina (6.64 ± 2.31 g/dL), colesterol (125.16 ± 34.35 mg/dL), cloretos (283.66 ± 104.11 mEq/L), magnésio (4.24 ± 0.38 mg/dL), proteínas totais (1.9 ± 0.62 g/dL), triglicérides (282.04 ± 83.58 mg/dL), fósforo (3.67 ± 0.59 mg/dL), cálcio (12.47 ± 1.85 mg/dL), ferro (620.63 ± 266.33 µg/dL) e fosfatase alcalina (86.37 ± 32.32 U/L). Salienta-se que esta é a primeira descrição da composição bioquímica do plasma seminal na espécie Dasyprocta leporina. Estas informações serão úteis para o aperfeiçoamento de protocolos de conservação espermática na espécie.

Palavras-chave: conservação, semiárido, cutias, bioquímica, reprodução.

Agência financiadora: Bolsista IC PIBIC - CNPq.

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ANÁLISE BIOQUÍMICA DOS CONSTITUINTES ORGÂNICOS E INORGÂNICOS DO PLASMA SEMINAL DE CUTIAS

(*Biochemical analysis of the organic and inorganic constituents of the agouti seminal plasma)*

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ABSTRACT

The objective of the present study was to identify the biochemical components of the agoutis' seminal plasma. For this purpose, six adult males were collected by means of electroejaculation. Biochemical analysis was performed using commercial kits and the absorbances were read on a spectrophotometer. The results were described as mean and standard error. The following organic constituents were identified in the seminal plasma: albumin (6.64 ±2.31g/dL), total proteins (1.9±0.62g/dL), glucose (26.27±9.84mg/dL), fructose (26.92±8.08mg/dL), citric acid (408.28±227.92mg/dL)), triglycerides (282.04±83.58mg/dL) and cholesterol (125.16±34.35mg/dL). Regarding inorganic components, chlorides (283.66±104,11mEq/L), magnesium (4.24±0.38mg/dL), phosphorus (3.67±0.59mg/dL), calcium (12.47±1.85mg/dL), and iron (620.63±266.33µg/dL). It should be noted that this is the first description of the biochemical composition of seminal plasma in the species *Dasyprocta leporina*. This information will be useful for the improvement of sperm conservation protocols for the species.

Key words: Conservation, Semiarid, Rodents, Reproduction.

INTRODUÇÃO

A cutia (*Dasyprocta leoporina*) é um roedor histricognato silvestre, típico da fauna brasileira, que desempenha um papel de fundamental importância na natureza, pois possui o hábito de cavar tocas, contribuindo para a aerificação do solo e se configura em importante elo na cadeia alimentar (RODRIGUES *et al*., 2003). A criação destes animais em cativeiro é uma alternativa para a conservação da espécie, que pode ser ameaçada pela pressão ambiental imposta pelo homem, por meio da caça predatória e destruição de seus habitats (CASTELO, 2015). No intuito de ampliar as estratégias reprodutivas da espécie, necessita-se conhecer a sua fisiologia reprodutiva, visando a aplicação de biotécnicas como a tecnologia de sêmen.

O plasma seminal é uma mistura de secreções dos testículos, epidídimos e, principalmente, das glândulas acessórias que serve como meio para os espermatozoides. Em mamíferos, alguns constituintes bioquímicos do plasma seminal são responsáveis por lhe conferirem ação antimicrobiana, além de participarem da ativação da motilidade espermática (ELZANATY *et al*., 2002). Entretanto, pouco se sabe a respeito da composição do plasma seminal de cutias, o que seria um conhecimento importante para o aperfeiçoamento de protocolos de conservação espermática. Neste sentido, objetivou-se identificar os componentes bioquímicos orgânicos e inorgânicos presentes no plasma seminal de cutias.

MATERIAL E MÉTODOS

Foram utilizados seis machos adultos provenientes do Centro de Multiplicação de Animais Silvestres (CEMAS/UFERSA), que está registrado no IBAMA como criadouro científico sob a numeração 1478912. Os procedimentos experimentais foram desenvolvidos conforme aprovação do CEUA/UFERSA (Parecer nº 11/2019) e Instituto Chico Mendes de Biodiversidade (SISBIO nº 66618-1).

As coletas foram realizadas por meio de eletroejaculação, utilizando-se um protocolo de estimulação serial com 03 ciclos de estimulação e intervalos de 5 minutos entre cada ciclo (CASTELO et al., 2015). Para evitar a influência de constituintes testiculares ou epididimários, apenas os ejaculados azoospérmicos foram utilizados no experimento. Assim, após a coleta em tubos plásticos, mensurou-se o volume coletado e a ausência de espermatozoides foi confirmada em microscopia de luz (x400). Posteriormente, as amostras foram centrifugadas (700g) e refrigeradas a – 20 ºC até a ocasião da análise bioquímica.

As análises bioquímicas foram feitas por meio de kits bioquímicos comerciais (Labtest Diagnóstica SA, Lagoa Santa, MG, Brazil) e Espermoteste® (InVitro Diagnostic S/A, Itabira-MG, Brazil). Para determinação e quantificação dos constituintes orgânicos e inorgânicos do plasma seminal, as absorbâncias das amostras foram analisadas sob espectrofotometria (Biospectro modelo SP-22. Curitiba, PR - Brasil), utilizando-se comprimentos de ondas especificados em cada kit. Os resultados foram expressos na forma de média e erro padrão.

RESULTADOS E DISCUSSÃO

O protocolo de eletroejaculação mostrou-se eficiente na obtenção do plasma seminal de cutias, proporcionando a obtenção de um volume médio de 1091,66±205,498µL, comprovadamente azoospérmico (Tab. 01).

Tabela 01: Valores médios (±erro padrão) para os constituintes orgânicos encontrados no plasma seminal de cutias *Dasyprocta leporina* (n=6).

Constituintes	**Média (±erro padrão)**	**Variação (Mínimo – Máximo)**
Proteínas Totais (g/dL)	1,9±0,62	0,44 – 4,43
Albumina (g/dL)	6,64±2,31	1,19 – 15,66
Colesterol (mg/dL)	125,16±34,35	38,12 – 270,58
Triglicérides (mg/dL)	282,04±83,58	33,01 – 556,92
Ácido cítrico (mg/dL)	408,28±227,92	17,44 - 1300
Frutose (mg/dL)	26,92±8,08	4,16 – 57,35
Glicose (mg/dL)	26,27±9,84	2,02 – 60

Os constituintes orgânicos encontrados no plasma seminal de cutias (Tab. 01) são similares aos já descritos no plasma seminal de outros mamíferos, tais como coelhos (ALVAREZ e STOREY, 1983), bovinos (LAPOINTE *et al*., 1996), ovinos (ABDEL-RAHMAN et al., 2000) e suínos (RODRIGUEZ *et al*. 2013). Murase *et al*. (2007) relataram que a albumina é uma proteína associada à proteção espermática. Já a presença dos

triglicerídeos no plasma seminal pode estar relacionada com a qualidade espermática e a fertilidade do macho (KOZIOROWSKA-GILUN *et al*., 2011). Em adição, o colesterol exerce importante função de proteção das células contra o choque térmico resultante de alterações na temperatura (SOFIKITIS e MIYAGAWA 1991).

O ácido cítrico atua de modo positivo sobre a motilidade espermática (MANN, 1948). A frutose é utilizada pelas células para produção de ATP, sendo convertida em energia utilizada durante o movimento do espermatozoide (FORD, 2001). No entanto, algumas espécies podem utilizar a glicose como fonte de energia para a movimentação espermática. Nas cutias, entretanto, evidenciou-se que ambos os açúcares estão presentes em quantidades similares, sendo ainda necessária uma elucidação a respeito do metabolismo energético para o espermatozoide da espécie.

Tab. 02. Valores médios (±erro padrão) para os constituintes inorgânicos encontrados no plasma seminal de cutias *Dasyprocta leporina* (n=6).

Constituinte	**Média (± erro padrão)**	**Variação (Mínimo – Máximo)**
Fósforo (mg/dL)	3,67±0,59	1,77 – 5,52
Magnésio (mg/dL)	4,24±0,38	3,68 – 5,98
Cálcio (mg/dL)	12,47±1,85	6,73 – 18,28
Ferro (mg/dL)	620,63±266,33	126 – 1902,35
Cloretos (mEq/L)	283,66±104,11	99,07 – 777,17

No que diz respeito aos constituintes inorgânicos, os íons cálcio, magnésio, fósforo, ferro e cloreto foram encontrados no plasma seminal de cutias (Tab. 02). O cálcio é importante por permitir a regulação fisiológica das células espermáticas (WONG et al., 2001). O magnésio é um cátion que atua como cofator em mais de 300 reações enzimáticas, tendo importante atuação sobre a motilidade espermática (ABOU-SHAKRA et al., 1989). O fósforo participa de processos envolvendo o metabolismo energético, ativação e inativação enzimática (CORTEZ, 2003). O ferro tem função fundamental na síntese de DNA e transporte de elétrons e oxigênio (FERREIRA, 2009). No que diz respeito ao cloreto, Martins et al. (1999) verificaram que baixas concentrações de cloreto na fração espermática implicam em um mecanismo de proteção que independe da espécie e raça.

CONCLUSÃO

Ao nosso conhecimento, esta é a primeira descrição da composição bioquímica do plasma seminal na espécie *Dasyprocta leporina*. Estas informações serão úteis para o aperfeiçoamento de protocolos de conservação espermática na espécie.

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AVALIAÇÃO DA INTEGRIDADE DE MEMBRANA ESPERMÁTICA DE CUTIA (*Dasyprocta leporina*) ATRAVÉS DO TESTE HIPOSMÓTICO: TRABALHO DE PESQUISA

(EVALUATION OF SPERM MEMBRANE INTEGRITY OF THE AGOUTI (*Dasyprocta leporina*) THROUGH HYPOSMOTIC TEST)

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A cutia (*Dasyprocta leporina*) é um roedor silvestre adaptado a região semiárida que possui importância ecológica como dispersor de sementes e presa de carnívoros. Desta forma, justifica-se o estabelecimento de protocolos de análise espermática, visando a conservação desta espécie através da formação de bancos de germoplasma. Neste sentido, objetivou-se avaliar a integridade funcional de membrana do espermatozoide epididimário de cutia através do teste hiposmótico, comparando-se soluções com diferentes osmolaridades. Para tanto, foram coletados sete pares de epidídimos de cutias machos, sexualmente maduros e eutanasiados, conforme aprovação pela Comissão de Ética no Uso de Animais da UFERSA (CEUA: Parecer 11/2019). Em seguida, os epidídimos foram subdivididos em cabeça, corpo e cauda, e os espermatozoides de cada região foram recuperados pelo método de flutuação. Posteriormente, foi realizado o teste hiposmótico utilizando-se água destilada (0 mOsm/L), bem como com soluções de citrato de sódio e frutose com diferentes osmolaridades (50 e 200 mOsm/L). Este teste tem por finalidade avaliar o influxo de fluidos para o interior do espermatozoide, através da membrana plasmática, até que haja o equilíbrio entre os meios. O mesmo pode ser usado como indicativo de que o transporte de água através da membrana se deu normalmente. Considerou-se os espermatozoides que se mostraram com caudas enroladas, àqueles com plena funcionalidade de membrana. Utilizou-se o teste Holm-Sidak para os procedimentos de comparação múltipla entre as regiões e as diferentes osmolaridades. Constatou-se que a solução de 50 mOsm/L obteve a maior média de espermatozoides funcionais para as 3 regiões do epidídimo (cabeça 69,57 %; corpo 77,29%; cauda 82,71%), diferindo estatisticamente das demais soluções 0 mOsm/L e 200 mOsm/L ($P < 0,05$). No entanto, não houve diferença estatística entre as diferentes regiões analisadas ($P < 0,05$). Desta feita, recomenda-se que o teste hiposmótico a ser utilizado para avaliação da integridade funcional da membrana espermática nesta espécie, seja a solução de 50 mOsm/L contendo citrato de sódio e frutose.

PALAVRAS-CHAVE: Conservação; Espermatozoide; Epidídimo; Osmolaridade; Silvestre.

DESCRIÇÃO MORFOLÓGICA DO ESPERMATOZOIDE EPIDIDIMÁRIO DE CUTIA (*Dasyprocta leporina*): TRABALHO DE PESQUISA

(MORPHOLOGICAL DESCRIPTION OF AGOUTI EPIDIDIMARY SPERM (*Dasyprocta leporina*)

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Roedores silvestres histricognatos como a cutia (*Dasyprocta leporina*), apresentam importância ecológica como dispersores de sementes, além de participação na cadeia alimentar de carnívoros, sendo peças vitais na manutenção de florestas tropicais. No entanto, é uma espécie pouco estudada quanto as suas características espermáticas, sendo necessários estudos para melhor compreensão de sua fisiologia reprodutiva. Assim, este trabalho objetivou descrever a morfologia do espermatozoide de cutias oriundo de três regiões distintas do epidídimo: cabeça, corpo e cauda. Para tanto, foram utilizados sete pares de epidídimos de cutias machos, sexualmente maduros e eutanasiados, conforme aprovação pela Comissão de Ética no Uso de Animais da UFERSA (CEUA: Parecer 11/2019). Posteriormente, estes foram colocados em placas petri contendo solução de PBS, onde os espermatozoides das diferentes regiões epididimárias foram recuperados por meio da técnica de flutuação. Em seguida, as células espermáticas foram coradas com Rosa de Bengala e dispostas em lâminas para análise morfológica por microscopia de luz (100x), observadas 200 células e classificadas como espermatozoides morfologicamente normais ou anormais, procedendo-se a identificação dos defeitos de acrossoma (ac), cabeça (cb), peça intermediária (pi) e cauda (cd). Para comparação das diferentes regiões epididimárias no tocante ao número de espermatozoides morfologicamente anormais, utilizou-se o teste de Tukey ($P < 0,05$). Os espermatozoides das três regiões do epidídimo não apresentaram diferença significativa quanto a morfologia normal (cabeça = 83,42%; corpo = 88,00%; cauda = 88,57%) ou quanto aos defeitos espermáticos encontrados. Em cada uma das regiões do epidídimo, o defeito espermático significativamente menos predominante foi aquele relativo ao acrossoma ($P < 0,05$). De modo geral, na cabeça do epidídimo, observaram-se as seguintes proporções de defeitos espermáticos: ac = 2,57%, cb = 6,86%, pi = 14,57% e cd = 10,86%; já no corpo do epidídimo, observou-se: ac = 3,14%, cb = 6,56%, pi = 6,57% e cd = 8,86%; finalmente, na cauda epididimária, verificaram-se: ac = 3,14%, cb = 8,55%, pi = 6,29% e cd = 6,86%. Os resultados sugerem que, ao longo do trânsito epididimário em cutias, não ocorrem maiores alterações quanto à morfologia dos espermatozoides mediante avaliação sob microscopia de luz. Esta informação contribui para uma melhor compreensão do processo de maturação espermática na espécie.

PALAVRAS-CHAVE: Animal silvestre; Maturação espermática; Epidídimo.

Environmental conditions affect the sperm quality of agoutis (*Dasyprocta leporina*) during the dry period of a semiarid region

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Abstract: We evaluate the relationships among some environmental elements and the kinematic parameters of agouti (*Dasyprocta leporina*) epididymal sperm, during dry season in a semiarid region. The sperm was recovered from the epididymis cauda of 07 individuals. The evaluations of kinematic parameters were assessed by computerized analysis (CASA). The most significant correlations were related to the means of the 42 days before the day in which sperm collection was conducted: sperm motility was negatively correlated to the temperature (ρ = -0.78) and solar radiation (ρ = -0.78); static subpopulation was correlated to the temperature (ρ = 0.78) and solar radiation (ρ = 0.78); while wind speed was negatively correlated to the rapid subpopulation (ρ = - 0.78) and sperm curvilinear velocity (ρ = -0.76). During the dry period, some environmental elements of a semiarid region presented a strong negative relationship with the quality of sperm parameters of captive-bred agoutis.

Keywords: Caatinga; germplasm; wildlife.

Introduction - Understanding the characteristics of the reproductive physiology of wild rodents, can result in the preservation of ecosystems, minimizing impacts caused by anthropic action or natural causes, such as global warming. Since wildlife breeding is strongly influenced by environmental factors, some climatic elements may impair or contribute to the quality of rodent reproductive parameters [1, 2]. Thus, it is necessary to know these interactions in order to establish and improve protocols for more efficient management or application of biotechniques to the conservation of rodents' genetic material. The agouti (*Dasyprocta leporina* Linnaeus, 1758) is a medium-sized wild rodent adapted to the semiarid region that has ecological importance as a seed disperser, prey for carnivores, and as a source of protein for human consumption, which justifies the significance of this study. Therefore, we aimed to evaluate the relationships among environmental elements and the kinematic parameters of agouti (*D. leporina*) epididymal sperm during the dry season in a semiarid region.

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Material and methods - The study was conducted at the Center for Wildlife Multiplication, located at the Universidade Federal Rural do Semi-Árido, Mossoró, Brazil (5° 11 S, 37° 22 W, 18 m above sea level). The epididymis were obtained from 7 sexually mature males agouti, euthanized during the dry season (September and October 2019), as approved by the UFERSA Animal Use Ethics Committee (CEUA: Opinion 11/2019) and by the Instituto Chico Mendes de Biodiversidade (ICMBio: Opinion 66618-3). The sperm was recovered from the epididymis cauda using the flotation technique [3]. The evaluations of kinematic parameters were assessed by computerized analysis (CASA; IVOS 12.0, Hamilton-Thorne), using 5 µl of the semen sample [4]. There were analyzed: total motility (%), progressive motility (%), velocity average pathway (VAP, 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, %). Also, the total sperm population was subdivided into four categories: rapid, medium, slow, and static (%). The environmental elements observed were Temperature (T, °C), Humidity (H, %), Wind Speed (W, m/s), and Solar Radiation (SR, W/m2), obtained from a nearby meteorological station installed on the enclosure of the animals [5]. Once data presented non-linear relations, the Spearman's correlation test was applied using the PROC CORR of SAS software to evaluate the correlation of the sperm parameters with the environmental variables from specific days priors to the collection. Therefore, we consider the previous day (Day 1st), the seventh day (Day 7th), the 21st (Day 21st), and the 42nd day (Day 42nd) priors to the day of sperm collection. For each of these days, the means of the environmental variables were calculated based on measurements every 15 minutes throughout the 24 hours by the meteorological station. Besides, the "Mean 42 Days" was observed, which referred to the means of the environmental variables among all the forty-two days prior to the day of sperm collection. We chose 42 days for the most prolonged period because this is the time corresponding to agouti spermatogenesis. The correlations were considered significant when $P < 0.05$.

Results and Discussion - For Day 1st and Day 7th before sperm collection, no significant correlation among evaluated parameters was found. Likewise, no significant relation was found between humidity and sperm parameters at any time. Because of this, this environmental element, and those days (Days 1st and 7th) are not shown in table 1. Regarding the day 21st, 42nd, and the "mean of 42 days", correlations are presented at the Table 1.

Table 1. Correlations among kinematic parameters of agouti (*Dasyprocta leporina*) epididymal sperm and climatic elements evaluated at the 21st and 42nd days, as well as at the mean of all the 42 days, before sperm collection, in the dry period of the Brazilian semiarid region.

Climatic elements	Day 21st	Day 42nd	Mean 42 Days
Temperature (T)	-	-	Motility: ρ = -0,78 (P = 0.037) Static subpopulation: ρ = 0.78 (P = 0.037)
Wind speed (W)	Rapid subpopulation:ρ = -0.78 (P = 0.037) VCL: ρ = -0.76 (P =0.045)	LIN: ρ = -0.77 (P = 0.041)	Rapid subpopulation: ρ = -0.78 (P = 0.037) VCL: ρ = -0.76 (P = 0.045)
Solar radiation (SR)	-	BCF: ρ = 0.87 (P = 0.01)	Motility: ρ = -0.78 (P = 0.037) Rapid subpopulation: ρ = 0.78 (P = 0.037)

Since the reproduction of rodents is strongly influenced by environmental factors, it was expected that during the dry period of a semiarid region, the high average of temperature and solar radiation, as well as the scarcity of rainfall, would harm the reproductive parameters, thus decreasing the sperm quality [1, 5, 6]. In the present study, it was verified that the decrease in sperm quality was increasingly greater and more significant as the more days the animals suffered the effects of environmental elements in the dry period [1, 7]. When observing the sperm characteristics of rodents throughout the year, the worst average values for sperm quality are found during the driest period [2]. This showed that high temperatures and long-term solar radiation cause deleterious effects during rodent's spermatogenesis, especially those related to sperm motility and speed [2, 5]. Thus, this study shows that it is possible to predict the motility quality of agouti's sperm, using the mean values of temperature, wind speed, and solar radiation from the mean of 42 days preceding the collection day.

Final considerations - During the dry period in the semiarid region, the climatic elements temperature, wind speed, and solar radiation have a strong negative relationship with the quality of sperm parameters of captive-bred agoutis. Knowing this information is important to establish conservation strategies for the species.

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Área temática: Ciências Agrárias

Caracterização de espermatozoides epididimários de cutias (*Dasyprocta leporina*) em região semiárida

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A cutia (*Dasyprocta leporina*) é um mamífero roedor distribuído na Américas Latina, e que pode ser criado em cativeiro como fonte de proteína animal alternativa na alimentação humana. Embora não seja uma espécie ameaçada de extinção, a perda progressiva de habitats e a caça predatória tem levado ao declínio considerável de algumas populações em determinadas regiões. Assim, faz-se necessário o melhoramento e aplicação de biotécnicas reprodutivas, tanto para o manejo em cativeiro visando a produção zootécnica, como para a multiplicação e conservação da espécie. Este trabalho objetivou estudar a influência dos períodos climáticos sobre os parâmetros espermáticos de cutias mantidas em cativeiro em uma região semiárida. O trabalho foi submetido ao comitê de ética CEUA/UFERSA (Parecer nº 11/2019). Foram utilizados 10 cutias machos sexualmente maduros, em cada período climático (seco e chuvoso). Os animais foram anestesiados e eutanasiados para posterior remoção dos epidídimos. Em laboratório, os espermatozoides da cauda do epidídimo foram recuperados utilizando-se a técnica de flutuação. Depois, procedeu-se as análises quanto ao volume (ul), a concentração espermática (10^6 espermatozoides/mL) através de câmera de Neubauer, e os parâmetros cinéticos em um equipamento de análise espermática computadorizada (CASA). Os dados foram expressos em média e erro padrão utilizando o efeito de período climático (seco; chuvoso) e analisados por ANOVA, utilizando o teste de Tukey ($P < 0,05$). Durante o período seco, foi obtido um volume de 685,7 (± 207,14) µL e concentração de 1230,1 (± 275,36) x 10^6 espermatozoides/mL, resultando em um total de 823,47 (± 137,81) x 10^6 espermatozoides recuperados. Tais valores foram superiores ($P < 0,05$) aos obtidos durante o período chuvoso, no qual obtiveram-se 940,00 (± 115,29) mL para volume e 758,89 (± 136,83) x 10^6 espermatozoides/mL de concentração espermática, obtendo um total de 714,58 (± 147,36) x 10^6 espermatozoides recuperados. Em contrapartida, os valores indicativos de qualidade espermática, relativos à motilidade total e progressiva foram superiores ($P < 0,05$) nas amostras obtidas durante o período chuvoso [93,33 (± 0,95%); 63,67 (± 2,74%)] do que no período seco (73,29 ± 6.36%; 13,29 ± 2,39%). Com base nos parâmetros avaliados, apesar dos animais terem apresentado maior número de espermatozoides recuperados durante o período seco, os resultados apontam para uma maior qualidade espermática no período chuvoso em função da melhor qualidade da motilidade espermática das cutias.

Palavras-chave: Animal silvestre, Conservação, Epidídimo, Clima, Flutuação.

Agência financiadora: PIVIC/UFERSA.

Área temática: Ciências Agrárias

Constituintes bioquímicos do plasma seminal de cutias (*Dasyprocta leporina* Linnaeus, 1758) – análise durante diferentes condições climáticas

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O conhecimento de como as variáveis ambientais podem influenciar os aspectos reprodutivos das espécies silvestres é de fundamental importância para o desenvolvimento de estratégias adequadas para o seu manejo e conservação, uma vez que as mudanças climáticas têm impactado fortemente a sua sobrevivência. Dentre os aspectos reprodutivos, a compreensão da composição bioquímica do plasma seminal auxilia no desenvolvimento de técnicas de reprodução assistida que salvaguardam os espermatozoides de uma determinada espécie, como a cutia (*Dasyprocta leporina* Linnaeus, 1758). Portanto, o objetivo deste trabalho foi descrever os constituintes bioquímicos orgânicos e inorgânicos presentes no plasma seminal de cutias, analisados durante o pico do período seco e chuvoso de uma região semiárida. Para tanto, foi coletado plasma seminal de seis machos adultos, por meio de eletroejaculação, durante os picos dos períodos seco (setembro, outubro e novembro de 2019) e chuvoso (fevereiro, março e abril de 2020) do bioma caatinga. O plasma seminal foi analisado quanto a presença de componentes bioquímicos orgânicos (proteínas totais, albumina, colesterol, triglicérides, frutose e glicose) e inorgânicos (fósforo, magnésio, cálcio, ferro, cloretos, sódio e potássio) e os valores obtidos foram correlacionados com as variáveis climáticas do ambiente estudado. Foram identificadas concentrações maiores de glicose no período seco (88.24 mg/dl em comparação com 26.27 mg/dl). Os valores de fósforo (66.40 mg/dl em comparação com 3.67 mg/dl) e potássio (92.67 mmol/L em comparação com 19.68 mmol/L) foram maiores no período seco do que no chuvoso. Entretanto, as concentrações de cloretos (43.04 mEq/L em comparação com 201.40 mEq/L) foram maiores no período chuvoso. Houve correlações ($P < 0,05$) entre as variáveis climáticas e os constituintes bioquímicos analisados. As concentrações de glicose correlacionaram-se negativamente com o volume de chuva total ($r = -0,69$) e com o nível de umidade ($r = -0.67$). No entanto, houveram correlações positivas com a radiação global ($r = 0.78$), temperatura do ar ($r = 0.74$) e velocidade do vento ($r = 0.72$). No que diz respeito aos cloretos, houveram correlações positivas entre as concentrações obtidas e o volume de chuva total ($r = 0.83$) e também a umidade ($r = 0.678$). Já com relação ao nível de radiação global ($r = -0.64$), temperatura do ar ($r = -0.54$) e velocidade do vento ($r = -0.71$), as concentrações de cloretos correlacionaram-se negativamente. Houveram correlações positivas entre as concentrações de fósforo e a radiação global ($r = 0.81$), temperatura do ar ($r = 0.80$) e velocidade do vento ($r = 0.77$). Em contrapartida, o volume de chuva total ($r = -0,82$) e o nível de umidade ($r = -0.72$) correlacionaram-se negativamente com as concentrações do componente. Houve correlação negativa entre as concentrações de potássio e volume de chuva total ($r = -0,79$). Esta é a primeira descrição da composição bioquímica do plasma seminal na espécie *Dasyprocta leporina*, indicando que há correlações importantes entre os constituintes bioquímicos do plasma seminal e o período climático em um ambiente semiárido. Estas informações serão úteis para o aperfeiçoamento de protocolos de conservação espermática na espécie.

Palavras-chave: Reprodução, Bioquímica, Semiárido, Conservação.

Agência financiadora: Bolsista IC PIBIC – CNPq.

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Organic and inorganic biochemical constituents of the seminal plasma of agouti (*Dasyprocta leporina*) collected during the dry and rainy period of a semiarid region.

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The objective of this work was to describe the organic and inorganic biochemical constituents present in the seminal plasma of agouti, analyzed during the dry and rainy period of a semiarid region. For this purpose, the seminal plasma of 6 adult males was collected, through electroejaculation, during the peaks of the dry (September, October and November) and rainy (February, March and April) periods of the caatinga biome. The samples obtained were analyzed under light microscopy to confirm the absence of sperm, since the purpose of the procedure was to obtain seminal plasma. Then they were centrifuged at 700 g speed, to separate solid residues, and the supernatant was refrigerated at -20 ºC until the occasion of the biochemical analysis. Seminal plasma was analyzed for the presence of organic (total protein, albumin, cholesterol, triglycerides, fructose and glucose) and inorganic (phosphorus, magnesium, calcium, iron, chloride, sodium and potassium) biochemical components using commercial biochemical kits and the values obtained were correlated with the climatic variables of the studied environment. To characterize the peak periods of the dry and rainy seasons of the semi-arid climate in the Caatinga, total precipitation data (in mm) were obtained for each period in the system from the National Institute of Meteorology (INMET) station, located in Mossoró, RN, Brazil. The climatic variants related to air temperature, wind speed, global radiation and humidity in each period were analyzed, measured by a meteorological station close to the location of the animals' pens. 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 and homoscedasticity using the Shapiro-Wilk test and Levene test, respectively. To assess potential seasonal differences on seminal plasma biochemical parameters and thermal environment, a one-way ANOVA was performed using the PROC GLM of SAS. Spearman's correlation test was applied using the PROC CORR of SAS to determine associations among studied variables. Higher glucose concentrations were identified in the dry period (88.24 mg/dl compared to 26.27 mg/dl). Phosphorus (66.40 mg/dl compared to 3.67 mg/dl) and potassium (92.67 mmol/L compared to 19.68 mmol/L) values were higher in the dry season than in the rainy season. However, chloride concentrations (43.04 mEq/L compared to 201.40 mEq/L) were higher in the rainy season. There were correlations ($P < 0.05$) between the climatic variables and the analyzed biochemical constituents. Glucose concentrations were negatively correlated with total rainfall volume ($r = -0.69$) and with moisture level ($r = -0.67$). However, there were positive correlations with global radiation ($r = 0.78$), air temperature ($r = 0.74$) and wind speed ($r = 0.72$). With regard to chlorides, there were positive correlations between the concentrations obtained and the total rainfall volume ($r = 0.83$) and also moisture ($r = 0.678$). Regarding global radiation level ($r = -0.64$), air temperature ($r = -0.54$) and wind speed ($r = -0.71$), chloride concentrations were negatively correlated. There were positive correlations between phosphorus concentrations and global radiation ($r = 0.81$), air temperature ($r = 0.80$) and wind speed ($r = 0.77$). On the other hand, the total rainfall volume ($r = -0.82$) and the humidity level ($r = -0.72$) were negatively correlated with the concentrations of the component. There was a negative correlation between potassium concentrations and total rainfall volume ($r = -0.79$). This is the first description of the biochemical composition of seminal plasma in the species Dasyprocta leporina, indicating that there are important correlations between the biochemical constituents of seminal plasma and the climatic period in a semiarid environment. This information will be useful for the improvement of sperm conservation protocols in the species.

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Seasonal variation of epididymal sperm parameters of agouti (*Dasyprocta leporina*) reared in the semiarid region

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The agouti (*Dasyprocta leporina*) is a forager rodent that has a habit of dispersing seeds with the intention of storing its food for later consumption. Due to this behavior, it ends up making seed dispersions, thus having great ecological importance, contributing to the diversity and maintenance of forests. Its natural habitat, however, has been suffering constant deforestation, causing a progressive decline in wild populations. Thus, attempts to obtain data related to its reproductive physiology contribute to the development of appropriate strategies for the management and conservation of the species. In this sense, we studied the influence of environmental factors on the epididymal sperm parameters of male agouti kept in captivity in different seasons of a semiarid region. To characterize the climatic seasons, the rainfall regime (mm) was obtained daily from the automatic station of the National Institute of Meteorology - INMET, located in Mossoró, RN, Brazil. Twelve animals were euthanized (according to CEUA/UFERSA recommendations – nº 11/2019), and the testicular-epididymis complexes were collected. Six of these collections were conducted during the peak of the dry season (November and October 2019) and six at the peak of the rainy season (March and May 2021). In the laboratory, epididymal sperm were obtained from the epidydimal cauda by using the slicing-floating technique. The washing containing sperm was evaluated for volume (µl) using micropipettes, and for sperm concentration (sperm/mL x 10^6), using a Newbauer counting chamber. With basis on volume and concentration, we calculated the total number of sperm obtained in each season. Total and progressive sperm motility were evaluated by a computerized system (CASA – IVOS 12.0, Hamilton-Thorne, Beverly, USA). Bengal Rose-stained smears were used for evaluating the sperm morphology, using light microscopy (1000×), counting 100 cells per slide. Data were presented as means and SEM and compared between seasons by variance analysis followed by Student's t test ($P < 0.05$). Dry season was characterized by an accumulated rainfall regime of only 1.2 mm, while a total of 568.1 mm was obtained during rainy season. Regarding sperm parameters, a significantly higher ($P < 0.05$) number of epidydimal sperm was recovered during the dry season (1911.6 ± 420.1 sperm) in comparison to those obtained during the rainy season (788.4 ± 184.2 sperm). On the contrary, rainy season provided higher values for total (93.3 ± 1% vs. 76.5 ± 6.5%) and progressive (63.7 ± 2.7% vs. 14.7 ± 2.3%) sperm motility than dry season. Regarding sperm normal morphology, similar values were obtained for dry (89.8 ± 2.4%) and rainy (90.8 ± 1.4%) seasons. In summary, our results showed that a better sperm quality of agouti reared in the semiarid region can be obtained during the rainy season when compared to the dry season. However, it is possible that there is some compensatory mechanism, since a greater number of sperm is obtained in drier periods, to the detriment of rainy periods. These are valuable data for the improvement of strategies for the reproductive management and conservation of the species.

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