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INFLUÊNCIA DAS VARIÁVEIS METEOROLÓGICAS EM UMA REGIÃO SEMIÁRIDA SOBRE A FUNÇÃO TESTICULAR E CONGELABILIDADE DO SÊMEN DE CATETOS (*Pecari tajacu* LINNEAUS, 1758)

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RESUMO

Maia, KMM. **Influência das variáveis meteorológicas em uma região semiárida sobre a função testicular e congelabilidade do sêmen de catetos (*Pecari tajacu* LINNEAUS, 1758)**. Universidade Federal Rural do Semi-Arido, UFERSA - Mossoró, RN 2018.

A presente tese teve por objetivo avaliar a influência das variáveis meteorológicas de uma região semiárida sobre a atividade reprodutiva e a congelabilidade de sêmen de catetos (*Pecari tajacu*). Para tanto, foram utilizados 11 machos adultos, criados em cativeiro, oriundos do Centro de Multiplicação de Animais Silvestres (CEMAS) da Universidade Federal Rural do Semi-Árido (UFERSA), cujo sêmen foi coletado por eletroejaculação e imediatamente avaliado. No primeiro experimento, objetivou-se caracterizar os parâmetros reprodutivos (qualidade espermática, morfometria testicular e perfil sérico de testosterona) de catetos e verificar a influência das variáveis meteorológicas do semiárido em diferentes índices pluviométricos sobre a reprodução de machos desta espécie. Os referidos parâmetros foram obtidos mensalmente, por 18 meses, de setembro de 2015 a fevereiro de 2018. Paralelamente, as variáveis meteorológicas (temperatura do ar, umidade relativa, velocidade do vento e precipitação pluviométrica) foram obtidas, nos dias de coleta de sêmen. O índice de precipitação foi utilizado para agrupar os meses em diferentes classes (classe 1 - sem chuva, classe 2 - até 50 mm e classe 3, com precipitação acima de 50 mm). Foi obtido um índice de precipitação total de 650 mm durante todo o período experimental. Houve diferença significativa ($P < 0,05$) na temperatura média do ar (° C), velocidade do vento (m/s) e umidade relativa (%), bem como valores de carga térmica radiante ao sol (W/m^2) entre as classes de precipitação, no entanto, não houve diferenças significativas nos parâmetros reprodutivos avaliados. Além disso, várias correlações moderadas significativas entre as variáveis meteorológicas e os parâmetros reprodutivos foram determinadas, evidenciando que as alterações drásticas causadas por modificações climáticas futuras poderiam interferir na reprodução da espécie. O segundo experimento, teve por objetivo avaliar a influência das variáveis meteorológicas de uma região semiárida sobre as características do sêmen fresco e descongelado desta espécie. O sêmen foi coletado durante os picos dos períodos seco (outubro, novembro e dezembro de 2015) e chuvoso (março, abril e maio de 2016), enquanto as variáveis meteorológicas foram medidas. Verificou-se que a umidade relativa foi significativamente maior durante o período chuvoso (74,6%) do que durante o período seco (66,8%). As amostras congeladas durante o período chuvoso apresentaram melhor integridade de membrana (28,6 ± 6%), motilidade (29,5 ± 7,7%) e espermatozoides rápidos (13,7 ± 6,2%) pós-descongelação do que em amostras congeladas durante o período seco (14,6 ± 4,1%, integridade de membrana; 14,6 ± 4,1%, motilidade e 4,1 ± 1,2 %, rápidos; $P < 0,05$). Assim, demonstrou-se que as variáveis meteorológicas do semiárido não afetaram a qualidade do sêmen fresco, mas podem influenciar a conservação dos espermatozoides de cateto. As informações aqui geradas podem ser aplicadas ao manejo da espécie, no intuito de desenvolver estratégias para sua conservação e reprodução.

Palavras chave: reprodução animal, estacionalidade reprodutiva, bioma Caatinga, cateto, criopreservação de sêmen

ABSTRACT

Maia, KMM**. Influence of the meteorological variables in a semiarid region on the testicular function and freezing of the collared peccary (*Pecari tajacu* LINNEAUS, 1758) semen**. Federal Rural University of the Semiarid, Mossoró, RN 2018.

The aim of the present thesis was to evaluate the influence of the meteorological variables of a semiarid region on the reproductive activity and the freezing of collared peccaries (Pecari tajacu) semen. For that, 11 adult males, bred in captivity, from the *Centro de Multiplicação de Animais Silvestres* (CEMAS) of the *Universidade Federal Rural do Semiárido* (UFERSA) were used, and their semen was collected by electro ejaculation and immediately evaluated. In the first experiment, it was aimed to characterize the reproductive parameters (sperm quality, testicular morphometry and serum testosterone profile) of collared peccaries and to verify the influence of the meteorological variables of the semiarid on different rainfall indexes on the reproduction of males of this species. These parameters were obtained monthly for 18 months, from September 2015 to February 2018. In parallel, the meteorological variables (air temperature, relative humidity, wind speed and rainfall) were obtained on the days of semen collection. The precipitation index was used to group the months into different classes (class 1 - without rain, class 2 - up to 50 mm and class 3, with precipitation above 50 mm). A total precipitation index of 650 mm^3 was obtained over the entire period. There was a significant difference ($P < 0.05$) in the average air temperature (°C), wind speed (m/s) and relative humidity (%), as well as radiant heat load values in the sun (w/m^2); among the rainfall classes, however, there were no significant differences in the reproductive parameters evaluated. In addition, several significant moderate relationships between environmental and reproductive parameters were determined, showing that drastic changes caused by future climate changes could interfere with species reproduction. The second experiment had as objective to evaluate the influence of meteorological variables of semiarid region on the characteristics of fresh and frozen / thawed semen of this species. Semen was collected during the dry (October, November and December 2015) and rainy season peaks (March, April and May 2016), while the meteorological variables were measured. It was verified that the relative humidity was significantly higher during the rainy period (74.6%) than during the dry period (66.8%). The samples frozen during the rainy season presented better membrane integrity (28.6 ± 6%), motility (29.5 ± 7.7%) and rapid spermatozoa (13.7 ± 6.2%) after thawing than the samples frozen during the dry season (14.6 ± 4.1%, membrane integrity, 14.6 ± 4.1%, motility and 4.1 ± 1.2%, rapid spermatozoa, respectively, $P < 0.05$). Thus, it was demonstrated that the meteorological variables of the semiarid region did not affect the quality of the fresh semen but may influence the conservation of the spermatozoa from the collared peccaries. The information generated here can be applied to the management of the species, in order to develop strategies for its conservation and reproduction.

Key words: animal reproduction, reproductive seasonality, Caatinga biome, collared peccaries, cryopreservation of semen.

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LISTA DE ABREVIATURAS E SIGLAS

µm	Micrômetro
AB	Azul de Bromofenol
ACP	Agua de coco em pó
ALH	Amplitude de Deslocamento Linear da cabeça
ANOVA	Análise de Variância
CAPES	Coordenação de Aperfeiçoamento de Pessoal de Nível Superior
CASA	Análise Computadorizada de Sêmen
CEMAS	Centro de Multiplicação de Animais Silvestres
CFDA	Diacetato de carboxifluoresceína
cm	Centímetro
CTR	Carga térmica radiante
DNA	Ácido Desoxirribonucleico
DHEA	Dehidroepiandrosterona
EP	Erro Padrão
EUA	Estados Unidos da América
g	Grama
h	Hora
HOST	Teste hipo-osmótico
Hz	Hertz
IA	Inseminação artificial
PI	Iodeto de Propídeo

IUCN	International Union for Conservation of Nature
IV	Intravenoso
Kg	Quilograma
LCGA	Laboratório de Conservação de Germoplasma Animal
LDL	Lipoproteína de Baixa densidade
LIN	Linearidade
m	Metro
m/s	Metro por segundo
mA	Microampere
Mg	miligrama
Min.	Minuto
mL	Mililitro
mm	Milímetro
mm^3	Milímetro cúbico
mOsm	Miliosmól
MPI	Integridade de Membrana Plasmática
ºC	Graus Celsius
s	Segundo
STR	Retilinearidade
Ta	Temperatura do ar
Tg	Temperatura de globo negro
TRIS	Tris-hydroxymethyl-aminomethane
TRM	Temperatura radiante média

Tu	Temperatura de bulbo úmido
U	Velocidade do Vento
UFERSA	Universidade Federal Rural do Semiárido
UR	Umidade Relativa
USTR	Ultrassonografia em tempo real
V	Volume
VAP	Velocidade Média da Trajetória
VCL	Velocidade Curvilínea
VSL	Velocidade Linear Progressiva
v	Volt
W/m^2	Watt por metro quadrado

LISTA DE SÍMBOLOS

±	Mais ou menos
$<$	Menor
≤	Menor igual
%	Porcentagem
®	Marca Registrada
10^6	Milhões

SUMÁRIO

1. INTRODUÇÃO

O *Pecari tajacu*, conhecido como cateto ou caititu, é um mamífero da família Tayassuidae e ordem Artiodactyla (CARTER et al., 2005). São animais de pequeno porte, exclusivos das Américas (BODMER et al., 2012). O estado de conservação desta espécie foi avaliado de acordo com os critérios da União Internacional para Conservação da Natureza - IUCN (GONGORRA et al., 2011), com base nos dados disponíveis até 2010, sendo classificado como menos preocupante (Least concern – LC). No entanto, a caça predatória, a perda de qualidade de habitat e a fragmentação têm levado a uma redução populacional significativa, na qual, se o cenário atual não for alterado, as populações tenderão a declinar futuramente, tornando a espécie vulnerável a extinção em algumas regiões (DESBIEZ et al., 2012).

Uma alternativa perante esse declínio da população de catetos seria sua criação em cativeiro, tanto para desenvolvimento de estratégias de conservação da espécie, como para fins comerciais (SANTOS et al., 2009, BIONDO et al., 2014). Devido à suas características reprodutivas e sua capacidade de adaptação a diversos biomas, os catetos podem ser explorados racionalmente por meio de um plano de manejo que favoreça sua sobrevivência em cativeiro (VENTURIERI; LE PENDU, 2006, NOGUEIRA, NOGUEIRA-FILHO, 2011).

Embora seja uma espécie de interesse comercial, haja vista a qualidade de sua carne e pele, sua manutenção em cativeiro depende do conhecimento de sua biologia reprodutiva (MAYOR et al., 2007; CASTELO et al., 2010). Este conhecimento seria também útil para permitir o emprego de técnicas de reprodução assistida como inseminação artificial (IA), fertilização "in vitro" (FIV), transferência de embriões (TE) e criopreservação de gametas. Estas técnicas são essenciais ao desenvolvimento de pesquisas básicas, melhoramento do desempenho reprodutivo e conservação da biodiversidade (GOBELLO; CORRADA, 2003).

A conservação de recursos genéticos é uma alternativa para reduzir a perda contínua dos animais devido à degradação ambiental (SILVA et al., 2012a; AGUILAR et al., 2014). A criação de bancos de germoplasma, que consiste na criopreservação de gametas, embriões e células somáticas em botijões criogênicos (DOMINGUES et al., 2011), surge como alternativa para manutenção da biodiversidade (HIEMSTRA et al., 2005). Para tanto faz-se necessário o estabelecimento de protocolos de coleta, avaliação e criopreservação do material genético, de acordo com as particularidades da espécie estudada (MAYOR et al., 2007).

Recentemente, alguns estudos têm discutido sobre as estratégias de conservação do germoplasma dos catetos (SILVA et al., 2013a; SOUZA et al., 2015, 2016). Contudo, os estudos com machos em condições controladas e a longo prazo ainda são escassos, havendo ainda uma carência de informações acerca de possíveis influências de características ambientais sobre os parâmetros resprodutivos nesta espécie (HELLGREEN et al., 1989; KAHWAGE et al., 2010). Aspectos básicos sobre a fisiologia reprodutiva e formas de conservação do sêmen destes animais devem ser estudados, uma vez que, estes podem sofrer alterações nas suas características em função dos diversos aspectos climáticos ao longo do ano (KAHWAGE et al., 2010).

Sabe-se que a influência do período e suas mudanças são determinantes para a qualidade do sêmen em diversas espécies, tais como em pequenos ruminantes (NUNES et al., 1982; MAIA, 2011; AGUIAR et al. 2013); búfalos (GARCIA, 2006) e até mesmo catetos (HELLGREN et al., 1989; KAHWAGE et al., 2010) mantidos em diferentes regiões. Porém, ressalta-se que o estudo específico da espécie *Pecari tajacu* sob clima semiárido torna-se relevante uma vez que este clima é caracterizado por uma longa estação seca e um curto período chuvoso, onde a temperatura é fator determinante do ritmo de vida, limitando a eficiência reprodutiva das diversas espécies animais que ali habitam (SILVA et al., 2011; TAKAHASHI, 2012). Neste sentido, o conhecimento de características testiculares e qualidade de sêmen fresco e criopreservado dos catetos, em observância à influência dos parâmentros ambientais do clima semiárido poderia potencializar o manejo da espécie tanto visando sua melhor exploração econômica como sua conservação.

2. REVISÃO DE LITERATURA

2.1 Aspectos reprodutivos do cateto

Os catetos (*Pecari tajacu*) são animais de pequeno porte, que quando adultos, medem de 75 a 100 cm de comprimento e aproximadamente 45 cm de altura, com o peso variando de 14 a 30 kg. Possuem pelagem longa e áspera geralmente de tonalidade cinza mesclado de preto, com uma faixa de pelos brancos ao redor do pescoço que dá o aspecto de um colar (figura 1) (NOWAK; PARADISO, 1983, CARTER et al., 2005, OLIVEIRA et al., 2016).

Figura 1: Cateto (*Pecari tajacu*) – Fonte: Arquivo pessoal.

Do ponto de vista anatômico, os catetos (*Pecari tajacu*) possuem a porção cranial do pênis de forma espiralada, semelhante ao do suíno, e a bolsa escrotal localizada entre a região inguinal e perineal, a qual apresenta alguns pêlos finos e curtos. Os testículos apresentam cerca de 2,8 cm de largura, 2,6 cm de espessura, 5,4 cm de comprimento e 22,5 cm^3 de volume, sendo simétricos (BELLATONI, 1991, SONNER et al., 2008).

Peixoto et al., (2012) relataram uma variação individual para biometria testicular nessa espécie. Esses autores obtiveram a morfometria testicular por ultrassonografia, mensurando o comprimento, altura e largura dos testículos direito (4,7 ± 0,3; 2,4 ± 0,3; 2,1 ± 0,3cm) e esquerdo (4,7 ± 0,4; 2,4 ± 0,3; 2,1 ± 0,3cm); respectivamente, calculando o volume testicular direito (17,0 ± 4,0 cm^3), esquerdo (16,7 ± 3,6 cm^3) e total (33,9 ± 6,2 cm^3).

A maturidade sexual nos machos é atingida por volta dos 11 meses e a das fêmeas entre 8 e 14 meses (SANTOS, 2007; SILVA et al., 2016). Não apresentam dimorfismo sexual aparente, com exceção da presença do saco escrotal, que pode ser observado a curta distância (SOWLS, 1997; MAYOR et al., 2007).

Nessa espécie, o ciclo do epitélio seminífero e o processo da espermatogênese apresentam duração aproximada de 12,3 ± 0,2 e 55,1 ± 0,7 dias respectivamente (COSTA et al., 2004). Sua espermatogênese é considerada muito semelhante à dos suínos, caracterizada pela alta eficiência na produção espermática, uma vez que os catetos possuem um dos mais baixos números de células de Sertoli por grama de testículo e uma das mais altas proporções volumétricas, em que 16% do parênquima é ocupado por compartimento intertubular e 84% por túbulos seminíferos (COSTA et al., 2004, GARCIA et al., 2009).

O sêmen de catetos possui três frações: uma clara, com predominância de secreção das glândulas acessórias e baixa concentração de espermatozoides; uma rica em espermatozoides e uma fração gel. Essas três frações podem ou não ocorrer nos ejaculados colhidos por eletroejaculação (HELLGREN et al., 1989; COSTA, PAULA, 2005). Em catetos, esse método foi eleito por vários autores, como sendo o mais indicado para obenção de sêmen (LOCHMILLER et al., 1985; HELLGREN et al., 1989, COSTA, PAULA, 2005), consistindo em aplicar estímulos elétricos nos nervos que suprem os órgãos reprodutores, após introdução de uma sonda via retal (GARCIA et al., 2009). Castelo et al., (2010), desenvolveram um protocolo com bons resultados para obtenção de sêmen em catetos. Realizando-se estímulos contínuos, iniciando com 10 repetições de 5 V, aumentando a intensidade em 1 V, gradativamente, até atingir 12 V, durante 10 minutos.

Nesse sentido, já foram realizadas com sucesso coletas de sêmen por esse método, utilizando-se diferentes protocolos anestésicos como a associação da tiletamina-zolazepam (COSTA; PAULA, 2005) e o uso do anestésico intravenoso Propofol, tendo este último apresentado maior eficiência para obtenção de espermatozoides viáveis (SOUZA et al., 2009).

Trabalhos de avaliação de sêmen desta espécie, obtido por eletroejaculação, foram realizados por diversos autores apresentando motilidade de 49,5 ± 15,8%, obtido por Lochmiller et al. (1985), no estado do Texas – EUA; 48,7 ± 31,5%, por Costa e Paula (2005), no estado de Minas Gerais - Brasil; 93,6 ± 1,6,0 %, por Castelo et al., (2010), no Nordeste Brasileiro; 52,8 ± 29,1, por Kahwage et al., (2010), na Amazônia Oriental. Esta variação nos resultados encontrados, provavelmente, deve-se à época do ano e/ou clima característico da região aos quais os animais habitam (Souza et al., 2016). Neste sentido, foram observadas diferenças nos parâmetros seminais, sobretudo no volume seminal, nas características do sêmen fresco, em trabalhos realizados na região semiárida do nordeste brasileiro, por Peixoto et al. (2012) e Silva et al., (2013a) (Tabela 1).

Quadro 1: Características do sêmen fresco de catetos (*Pecari tajacu*) obtido por eletroejaculação, por Peixoto et al., 2012 e Silva et al., 2013a.

Variáveis	**Peixoto et al., 2012**	**Silva et al., 2013a**
Volume (ml)	3,5 ± 3,8	6,8 ± 1,3
Concentração (sptz/ml)	800 ± 70,0 x 10^6	978,3 ± 118,7 x 10^6
Motilidade (%)	85,1 ± 10,7	91,3 ± 1,9
Vigor	4,2 ± 0,9	4,7 ± 0,1
Morfologia espermática (%)	79,9 ± 12,8	77 ± 4,7
Integridade de membrana (%)	86,3 ± 13,3	89,7 ±1,2
Funcionalidade da membrana (%)	76,9 ± 13,2	79,2 ± 3,4

Hellgren et al (1989), ao avaliarem a testosterona sérica e a biometria testicular em catetos radicados no sul do Texas, em regiões áridas (verão de 1982 – julho a agosto, e inverno de 1983 - fevereiro a março) verificaram que esta espécie permanece reprodutivamente fértil ao longo do ano, mas pode sofrer uma quiescência de verão, influenciada pela temperatura ambiente e fatores sociais. Kahwage et al., (2010), por sua vez, estudando a variação da qualidade de sêmen da espécie na Amazônia oriental, caracterizada pelo clima equatorial sub-úmido, no período de 2007 a 2009, atribuíram a redução nos parâmetros seminais, em determinado período, tanto em função de fatores ambientais como por fatores intrínsecos ao indivíduo. Já na região semiárida não há estudos a longo prazo avaliando uma possível estacionalidade reprodutiva desta espécie.

2.2. Características seminais

2.2.1 Avaliação Clássica de Sêmen

Logo após ser coletado, o sêmen deve ter sua qualidade avaliada com base em parâmetros macroscópicos, como aparência e volume (figura 2), e parâmetros microscópicos como motilidade, concentração, morfologia e integridade estrutural e funcional da membrana, entre outros.

Figura 2: Sêmen de cateto (*Pecari tajacu*) obtido por eletroejaculação - Fonte: Arquivo pessoal.

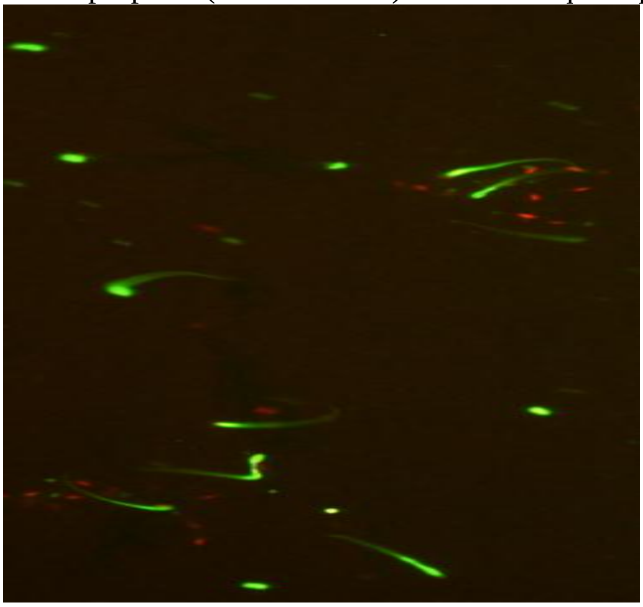
As relações entre as características seminais e a fertilidade têm sido investigadas em muitas espécies domésticas, com o objetivo de predizer a fertilidade (CAVALCANTE et al., 2005). Entre as diversas variáveis analisadas destaca-se a motilidade espermática. A avaliação da motilidade deve ser realizada imediatamente após a coleta do sêmen através da microscopia óptica, sendo definida como o percentual de espermatozoides móveis em uma amostra e sua intensidade de movimento (0-100%); simultaneamente, avalia-se o vigor, que é medido pela qualidade da motilidade exibida pelos espermatozoides móveis em uma escala de 0-5 (CASTELO et al., 2010; PEIXOTO et al., 2012).

Para avaliação das características morfológicas dos espermatozoides de catetos, utilizam-se esfregaços corados com Rosa Bengala, em microscópio de contraste de fase ou óptico, sob óleo de imersão (aumento de 1000 x), onde 200 células, em cinco campos selecionados para cada esfregaço, são classificadas quanto aos defeitos de forma e estrutura. Os defeitos morfológicos são classificados de acordo com a região do espermatozóide, como cabeça, peça intermédia ou defeitos da cauda (SOUSA et al., 2013). As anormalidades espermáticas podem reduzir a fertilidade por não permitir a chegada do espermatozoide ao local da fecundação (CHEMINEAU, 2005)

A membrana plasmática dos espermatozoides é responsável pela homeostase celular, por meio das trocas realizadas com o meio externo, formam uma barreira semipermeável para moléculas, e entre outras funções, protege a célula também de influências não fisiológicas, quando essas células são colocadas nos diluentes seminais (VARNER, 2008). Os espermatozoides funcionais mantêm um equilíbrio osmótico com o ambiente em que se encontram. Quando são expostos às soluções hiposmóticas, as células com membranas funcionais sofrem aumento de tamanho, com o influxo da água para o interior da célula, buscando equilíbrio osmótico, provocando edemaciação celular (SILVA et al., 2013a). Nesse sentido, com o intuito de avaliar a funcionalidade da membrana realiza-se o teste hiposmótico (HOST), no qual os espermatozóides por apresentarem morfologia singular, ao sofrerem o choque osmótico tendem a promover um dobramento de cauda, dando, portanto, um indicativo que apresentavam membrana funcional (QUINTELA et al., 2004). Santos et al. (2013) testaram soluções hiposmóticas com diferentes concentrações (0, 50, 100, 150, 200mOsm/L) para a avaliação funcional da membrana espermática de catetos e verificaram que a água destilada (0 mOsm/L), apresentando 71,8% de espermatozoides com reação osmótica, seria a melhor solução para realização do teste hiposmótico nesta espécie.

Paralelamente, a marcação com associação de sondas fluorescentes, como diacetato de carboxiflurosceína (CFDA) e o iodeto de propídio (IP), tem o objetivo avaliar a integridade estrutural da membrana plasmática da célula espermática. O CFDA é uma solução não fluorescente que penetra o ambiente celular e é, rapidamente, convertida em carboxifluorosceína pelas esterases intracelulares. A carboxifluorosceína age, por ser uma substância fluorescente não permeável que é mantida no meio intracelular na presença de uma membrana plasmática intacta, apresentando a coloração verde (SOUZA et al., 2003). Por outro lado, o PI tem a capacidade de marcar o DNA de células que estão mortas ou têm sua membrana danificada, emitindo fluorescência vermelha (SOUZA et al., 2003). De acordo com a fluorescência apresentada pelos espermatozoides, os mesmos são classificados com membrana plasmática íntegra, quando expressam fluorescência verde, e vermelha quando espermatozoides estão com membrana plasmática lesadas (SILVA et al.,2013a; SOUZA et al., 2016). Souza et al. (2013) valídararam a técnica de avaliação da integridade da membrana plasmática de espermatozoides de catetos pela associação de sondas fluorescentes (IP e CFDA) e estabeleceram sua relação com o teste de viabilidade utilizando o corante Azul de Bromofenol. Os autores verificaram uma relação significativa ($p = 0,005$) e positiva ($r = 0,75$) entre os dois métodos, demonstrando que o teste de integridade estrutural utilizando estas sondas fluorescentes pode ser utilizado de forma confiável para as avaliações da qualidade espermática de catetos (*Pecari tajacu*) (figura 3)

Figura 3: Teste de integridade estrutural da membrana plasmática de espermatozoides de catetos (*Pecari tajacu*) utilizando sondas fluorescentes: diacetato de carboxifluroscеína (CFDA - verde) e o iodeto de propídio (IP - vermelho) – Fonte: Arquivo pessoal.



2.2.2 Avaliação Computadorizada de Sêmen

Durante muito tempo a qualidade do ejaculado foi avaliada de forma subjetiva, o que tornava as análises com certo grau de variação, pois era descrita de acordo com a percepção de cada pesquisador. Com o advento da avaliação por computador, proposta por Dott e Foster em 1979, esse método passou a ser utilizado com êxito para várias espécies nos diversos centros andrológicos, Gtinzel et al. (1993) confirmaram em cães uma correlação significativa entre os parâmetros medidos subjetivamente e as obtidas pelos sistemas CASA (Computer Assisted Sperm Analysis), sendo demonstrado também para outras espécies de animais e humanos.

O CASA consiste em um sistema automático (hardware e software) utilizado para visualizar, digitalizar e analisar imagens sucessivas, fornecendo informações acuradas, precisas e significativas do movimento individual de cada célula, bem como de subpopulações de células espermáticas (AMANN e KATZ, 2004). As imagens obtidas permitem a análise de diversos parâmetros, incluindo concentração do sêmen, motilidade e alguns aspectos da morfologia, particularmente da cabeça do espermatozoide (VERSTEGEN et al., 2002). Esse instrumento é mais uma ferramenta a ser utilizada para analisar objetivamente, motilidade e morfometria espermática, padronizando os parâmetros seminais para espécie estudada, auxiliando na melhor aplicação das biotécnicas da reprodução. São avaliados motilidade total (%), VAP velocidade média de trajetória (μm/s), velocidade linear progressiva (VSL; μm /s), velocidade curvilinear (VCL; μm /s), amplitude de deslocamento lateral da cabeça (ALH; μm), frequência de batimento flagelar cruzado (BCF, Hz), índice de retidão (STR; %), e índice de progressão linear (LIN; %). Ainda, a população total espermática é subdividida em quatro categorias: rápida, média, lenta e estática (VERSTEGEN et al., 2002, SOUZA et al., 2015).

Souza et al. (2016) validaram as configurações do CASA para ser utilizado na análise computadorizada de sêmen de catetos (*Pecari tajacu*), à temperatura de 37 °C; 60 frames/s; contraste mínimo, 45; limiar de retidão, 30%; baixa velocidade média de trajetória (VAP), 10 m/s; e ponto médio de corte de VAP, 30 m/s. Cinco campos microscópicos independentes e não consecutivos são selecionados aleatoriamente e digitalizados. Quando necessário pode ser realizada a diluição de 1:1 em solução salina, para uma melhor avaliação no caso de amostras com concentração espermática alta.

2.2.3 Avaliações Complementares

Adicionalmente, o plasma seminal, que é uma mistura complexa de secreções provenientes dos testículos, epidídimo, e glândulas acessórias do sistema reprodutor, no qual os espermatozoides estão imersos, tem sido utilizada para complementar os estudos acerca da qualidade seminal. Estando bem estabelecido que o mesmo contém fatores que influenciam tanto espermatozoides quanto o transporte do esperma no trato genital feminino (CABALLERO et al., 2008). As proteínas do plasma seminal podem ser analisadas por eletroforese bidimensional, separando-as pelo ponto isoelétrico e massa, com alta resolução, identificando dessa forma, importantes marcadores ligados à fertilidade e congelabilidade do sêmen em diversas espécies (STRZEZEK et al., 2002)

Em catetos, Santos et al., (2014) relataram que muitos grupos de proteínas foram associados a função de proteção espermática, incluindo as chaperonas e as proteínas antioxidantes. Como chaperonas, o plasma seminal expressou Clusterina e T-Complex protein (TCP1), subunidade gama, enquanto que os antioxidantes, são as glutationa peroxidase, ceruloplasmina, albumina e transferrina.

2.3 Criopreservação de sêmen de cateto

A criopreservação de sêmen de catetos, foi relatada primeiramente por Castelo et al., (2010), em trabalho realizado com animais na região semiárida. O sêmen foi diluído em Tris e acrescido de frutose ou glicose. Por ocasião do experimento, foram testadas também duas curvas de descongelação (37°C por 1 min e 55°C por 7 s), não tendo sido observado diferença entre elas. Além disso, os autores testaram uma curva lenta de congelação, sendo o sêmen mantido em refrigerador programável a 27°C, durante 240 min, atingindo a temperatura de 5°C, posteriormente, o sêmen foi envasado, submetido ao vapor de nitrogênio, a uma altura de 3 cm, por 20 minutos e acondicionadas em botijão criobiológico.

Em seguida, Alves et al. (2012) testaram três concentrações de gema (5, 10 e 20%) e duas de glicerol (3 e 6%) como crioprotetores, associados ao diluente Tris, obtendo como melhores resultados a adição de 10 ou 20% de gema, com 3% de glicerol à solução de criopreservação do sêmen de catetos.

Adicionalmente, Silva et al., (2012b) avaliaram a eficácia de um diluente à base de água de coco em pó (ACP-116c), muito utilizado em caprinos, testando também diferentes concentrações de gema de ovo (10 e 20%) e glicerol (1,5 e 3%), como alternativa para a criopreservação de sêmen de catetos. Verificaram que melhor qualidade do sêmen descongelado foi obtido com o uso do ACP, adicionado de 20% de gema de ovo e 3% de glicerol.

Estudos posteriores realizados por Silva et al., (2013a) verificaram o efeito de diferentes curvas de congelação, tamanhos de palheta e descongelação sobre a criopreservação de sêmen de cateto. Avaliaram a curva de congelação rápida, na qual o sêmen era mantido em caixa térmica por 40 minutos, atingindo a temperatura de 15°C, seguido de mais 30 minutos, a 5°C em refrigerador programável, e então envasadas em palhetas de 0,25 ou 0,5 ml, submetidas ao vapor de nitrogênio, por 5 minutos e, por fim, mergulhadas em botijão criobiológico. Esses autores recomendaram o uso de uma curva de congelação rápida, que reduz o tempo gasto para o processo de criopreservação, o envase em palhetas de 0,25 ou 0,50 mL e a descongelação a 37 ° C / 1 min (SILVA et al., 2013a).

Outros trabalhos foram realizados por Souza et al., (2015) comparando as características de qualidade espermática de catetos (*Pecari tajacu*), após criopreservação em diluentes suplementados com gema de ovo inteira e diferentes concentrações de lipoproteínas de baixa densidade (LDL) (5, 10 e 20%), concluindo que o diluente a base de Tris, suplementado com 20% de LDL, fornece melhor qualidade do sêmen descongelado do que a gema de ovo inteira ou outras concentrações de LDL.

Recentemente, Souza et al., (2016) compararam o uso de Tris acrescido de gema de ovo a 20% ao extrato de Aloe vera (AV) (5, 10 ou 20%) e glicerol (3%) para criopreservação do sêmen nessa mesma espécie. Verificando-se que o extrato de AV, a uma concentração de 20%, poderia ser usado como um substituto alternativo a gema de ovo, na suplementação de diluentes a base de Tris. Se constituindo em uma alternativa de substituição de produtos de origem animal, em meios de conservação de sêmen, garantindo maior segurança sanitária.

Apesar do desenvolvimento de vários trabalhos que levaram ao avanço nas técnicas de criopreservação do sêmen de catetos (quadro 1), não há informação da influência das variáveis meteorológicas sobre a qualidade do sêmen descongelado desta espécie.

Quadro 2. Avanços obtidos na qualidade seminal no processo de criopreservação de sêmen de cateto (*Pecari tajacu*)

	Castelo et al., (2010)	**Silva et al., (2012b)**	**Silva et al., (2013a)**	**Souza et al., (2016)**
Motilidade espermática (%)	37,9 ± 4,2	48,3 ± 6,1	38,7 ± 6,8	46,4 ± 3,9
Integridade estrutural (%)	47,4 ± 2,6	45,3 ± 4,8	37,7 ± 5,6	27 ± 3,7
Integridade funcional (%)	41,7 ± 5,3	59,9 ± 5	27,0 ± 2,8	31,4 ± 5,6
Moroflogia normal (%)	48,7 ± 2,7	64,8 ± 5,6	64,4 ± 5,6	66,9 ± 4,2

2.4 Perfil sérico de testosterona

A testosterona, hormônio produzido pelas células de Leydig, também é necessária para a espermatogênese e, juntamente com outros andrógenos, tais como androstediona e dehidroepiandrosterona (DHEA), é responsável pela diferenciação e maturação dos órgãos reprodutivos masculinos, pelo desenvolvimento das características sexuais masculinas secundárias, e pelo comportamento de macho (THIÉRY et al., 2002).

A dosagem de testosterona sérica faz parte da rotina laboratorial há mais de duas décadas, com indicações e utilidades bem definidas. Desde a introdução do primeiro radioimunoensaio (RIE), no início da década de 1970, o que tornou a dosagem viável tecnicamente, as metodologias para sua medida passaram por várias transformações (FURUYAMA, et al., 1970). Como a testosterona encontrada no soro apresenta-se basicamente ligada a proteínas, sendo a fração livre (biologicamente ativa) da ordem de apenas 1% a 3%, a dosagem total se sujeita a flutuações induzidas pelas variações quantitativas das proteínas carregadoras, em especial a SHBG (Globulina de Ligação de Hormônios Sexuais). Os métodos indiretos, com base na dosagem de testosterona total e de SHBG, e aplicação de cálculos para a avaliação da fração livre apresentam boa correlação com o método de referência (VERMEULEN, 1999)

Existem vários métodos de mensuração de testosterona sérica, dentre eles, Lupi-Chen et al., (1999) validaram um método de radioimunoensaio direto para dosagem de esteroides em animais, com melhores resultados comparando a um método de ensaio indireto, nesse estudo, as doses de testosterona foram feitas em amostras de plasma de rato. Adicionalmente, Meunier (2005) avaliaram cálculos e dosagens de testosterona livre ou biodisponível, comparando diversos métodos, entre eles o micro-particle enzyme immunoassay (MEIA).

Macchi et al., (2010), utilizando o método de radioimunoensaio, verificaram que as concentrações de testosterona plasmática e fecal em javalis (*Sus scorfa*) foram mais elevados durante a estação de acasalamento, que é no outono e início do inverno de acordo com as influências do fotoperíodo.

Nos catetos radicados no sul do Texas, Hellgren (1989) utilizando este mesmo método, determinaram que as maiores concentrações de testosterona ocorreram no outono e inverno (1150 – 1400 pg/ml) e os valores mínimos, no verão (500 - 700 pg/ml), demonstrando uma tendência estacional. Os autores afirmaram que os valores máximos foram coincidentes com a época de maior número de acasalamentos da espécie nessa região, e que a relação entre as concentrações circulantes desse hormônio à estação do ano e temperatura ambiente sugere uma redução no desempenho reprodutivo dos machos durante o final do verão.

Adicionalmente, a técnica de quimioluminescência, com kits Rocha®, em aparelho Elecsys (sensibilidade do teste = 3 ng/dL) foi utilizada para dosagem de testosterona sérica e avaliação das características reprodutivas de bovinos da raça Nelore, sob clima tropical. Concluindo-se que as concentrações deste hormônio apresentam grande variação nos animais, demonstrando um perfil cíclico (Assumpção et al., (2013).

Outro método utilizado é a dosagem de testosterona de forma eficiente pelo ensaio de imunoabsorção enzimática (ELISA), utilizando-se kits comerciais (Testosterone, Human-Invitro, Itabira - MG), baseado em reações antígeno-anticorpo detectáveis por meio de reações enzimáticas. Este método possui alta sensibilidade e é um ensaio altamente específico, possibilitando, ainda, a realização de avaliação de várias amostras ao mesmo tempo. Além disso, tem uma realização rápida e de baixo custo, levando em comparação algumas outras técnicas de imunodiagnóstico. Esses autores relataram que fatores nutricionais, climáticos e estressantes também podem influenciar a secreção desse hormônio. Causando variações nos níveis de testosterona que podem vir a influenciar a eficiência reprodutiva dos animais Moura et al. (2009).

2.5 Ultrassonografia e biometria testicular

Dentre as técnicas de estudo a disposição da pesquisa em reprodução animal, a ultrassonografia em tempo real (USTR) tem se revelado uma técnica precisa, segura e de alta praticidade. É uma técnica de exame de estruturas onde a identificação fisiológica dos tecidos, assim como o diagnóstico de condições patológicas, é realizada de forma dinâmica através da reconstituição da anatomia seccional dos órgãos estudados (CRUZ; FREITAS, 2001).

O exame ultrassonográfico dos testículos é um método não invasivo e rápido, que, aliado aos dados de exame clínico, pode conduzir ao diagnóstico precoce de desordens desse órgão. A importância da determinação da diferença de ecogenicidades reside no fato de que as desordens testiculares, como tumores e processos inflamatórios são tipicamente representadas por mudanças na ecogenicidade e ecotexturas quais são visíveis em contraste com a ecogenicidade moderada dos testículos de animais adultos e essas alterações podem afetar diretamente os parâmetros reprodutivos (ABDEL-RAZEK et al., 2005).

Para a estimativa de dimensões ou volume testiculares, diversas técnicas foram utilizadas incluindo régua ou fita métrica (LAMBERT, 1951), paquímetro (TAKIHARA et al., 1983), e, ultrassonografia (DIAMOND et al., 2000). Sendo esta última, reconhecida como o mais preciso método de avaliação *in situ* do volume testicular em suínos domésticos (FORD, WISE, 2011) e em catetos (PEIXOTO et al., 2012)

Em catetos, foram realizados trabalhos recentes por Peixoto et al. (2012), os quais identificaram ao exame ultrassonográfico que o parênquima testicular dos adultos se apresentava ecogênico, com ecotextura homogênea e o mediastino testicular se apresentou como uma linha hiperecóica no centro do parênquima. Nesse estudo foram detectadas diferenças significativas nas medições testiculares entre os indivíduos. Além disso, o perímetro escrotal médio dos catetos foi de 19,4 ± 1,4 cm (figura 5). De posse dos dados fisiológicos a respeito dos parâmetros ultrassonográficos do parênquima testicular de catetos, esse método também pode ser empregado para a identificação de distúrbios que possam vir a afetar os parâmetros reprodutivos da espécie. Auxiliando assim na detecção precoce de fatores que possam interferir na qualidade seminal.

Figura 4: Circunferência escrotal em cateto (*Pecari tajacu*) – Fonte: Arquivo pessoal.

Com o animal sob sedação para coleta de sêmen é possível ainda averiguar aspectos biométricos da anatomia testicular, como textura, posição relativa entre testículos, conformação das estruturas reprodutivas, como indicadores da função sexual (ALMEIDA et al., 2010) (figura 6). A avaliação morfométrica dos testículos tem fundamental importância para a identificação de patologias e alterações gonadais, que podem influenciar diretamente na produção e qualidade seminal, com impactos sobre a fertilidade do indivíduo (HELLGREN et al. 1989; KAHWAGE, 2010).

Figura 5: Ultrassonografia testicular em cateto (*Pecari tajacu*) – Fonte: Arquivo pessoal.

De acordo com Kahwage et al., (2010) a biometria testicular em catetos adultos é semelhante à relatada na literatura para animais criados na natureza, sendo pouco influenciada pelo peso corpóreo. Adicionalmente, Peixoto et al., (2012) relataram que os catetos mostram uma grande variação individual em relação a esse parâmetro, tendo demonstrado um volume testicular total médio de 33,6 ± 6,2 cm^3. Hellgren et al., (1989) observaram que o volume testicular e a circunferência escrotal mensal em catetos variaram significativamente (P <005) pela data de coleta. Os maiores valores ocorreram entre o final de outubro e final de fevereiro para ambas as características, enquanto os valores menores foram registrados nos meses de verão. A circunferência escrotal mensal média variou de 182 ± 12 mm (setembro de 1983) para 228 ± 8 mm (janeiro de 1983). As dimensões testiculares gerais foram de 28 ± 2 mm de largura e 49 ± 3 mm de comprimento.

2.6 Estacionalidade Reprodutiva

Estacionalidade reprodutiva é o fenômeno pelo qual algumas espécies apresentam atividade sexual reduzida durante determinado período do ano, regulado principalmente pelos diversas variáveis meteorológicas, como temperatura, umidade do ar e luminosidade, afetando a capacidade reprodutiva dos animais (AGUILAR et al., 2014). Em regiões de clima tropical, a alta temperatura ambiental, observada no período seco, é o principal fator limitante à eficiência reprodutiva, pois pode interferir na termorregulação testicular, repercutindo negativamente na espermatogênese e, consequentemente, na qualidade do sêmen (CHEMINEAU et al., 1991). A intensidade da radiação solar afeta diretamente o comportamento e a fisiologia dos animais domésticos e determina a sua adaptabilidade ao ambiente físico (ZHENGKANG et al., 1994).

A existência de um possível efeito estacional sobre a reprodução já vem sendo estudada em inúmeras espécies domésticas e silvestres. Nunes et al., (1997) relataram que o macho caprino criado sob clima semiárido apresenta uma determinada estacionalidade produtiva que depende de uma série de variáveis, intrínsecas, como raça, peso e idade, ou extrínsecas como o fotoperíodo, a latitude, a temperatura e a alimentação. Em ovinos, a influência do fotoperíodo é marcante tanto nos machos quanto nas fêmeas de raças oriundas do hemisfério norte, que iniciam seu ciclo reprodutivo anual em função da diminuição da duração das horas de luz/dia, sendo considerados animais poliéstricos estacionais ou "fotoperíodo de dias curtos" ou decrescente (TRALDI et al, 2007). Já em equinos, que são animais estacionais de dias longos, ou fotoperíodo crescente, o aumento da exposição à melatonina tem efeito oposto, inibindo a secreção de GnRH pelo hipotálamo e consequentemente pela redução da produção e liberação de LH pela hipófise anterior, no período de menor luminosidade (WILLIAMS et al., 2012).

No Brasil devido a sua extensa área territorial, em algumas regiões como o Sul e o Norte, os animais apresentam grande influência do fotoperíodo. Ao passo que no Nordeste, região onde não tem diferença significativa na duração dos dias e noites, geralmente os animais sofrem menos o efeito desse fenômeno, estando a estacionalidade reprodutiva mais associadas a carências nutricionais, sanitárias e eventos climáticos (GRANADOS et al., 2006). Cavalcante et al., (2005), acrescentaram que alterações nas características do sêmen e na libido de carneiros e bodes de diferentes raças são devido a variações estacionais. Sendo as causas dessas alterações, em grande parte, os fatores climáticos, como evidenciam trabalhos efetuados com respeito à intensidade de luz, à temperatura e ao regime de chuvas (NUNES et al., 1982; MAIA et al., 2011)

Na Caatinga, um dos habitats naturais para os catetos e onde predomina o clima semiárido, a temperatura é fator determinante do ritmo de vida, limitando a eficiência reprodutiva dos animais que ali habitam (TAKAHASHI, 2012). O semiárido é caracterizado por temperatura média anual de 27,4°C, média, máxima de 34,4°C e a mínima de 21,3°C, tendo janeiro como mês mais quente e julho o mais frio. O período chuvoso normalmente ocorre entre fevereiro e abril, e a precipitação média anual é de 765,8 mm. A umidade relativa do ar é de 70%, tempo de insolação chega a 2.700 horas anuais (KÖPPEN, 2006, SILVA et al., 2011). Tais características climáticas fazem com que os animais estejam em diversos momentos do dia fora da sua zona de conforto térmico, o que requer uma energia extra para termorregular em busca da homeotermia. Isto faz com que haja uma menor quantidade de energia disponível para os processos reprodutivos, devido a ajustes endócrinos e fisiológicos para reduzir a produção de calor endógeno (ZHENGKANG et al., 1994, NUNES et al., 2011).

A termorregulação testicular é um processo oriundo da evolução dos mamíferos terrestres através do posicionamento destes órgãos na área externa do corpo do macho com a finalidade de manutenção da temperatura dos testículos em patamar distinto do resto do corpo (KLEISNER, 2010). A natureza do saco escrotal, com pele fina, pouca gordura e pelos, altamente vascularizado, aliada a mecanismos físicos como troca de calor contracorrente, regulação do fluxo sanguíneo, posição dos testículos e sudorese, desempenham papel importante na manutenção da temperatura testicular 2 a 6 °C abaixo da temperatura corporal, em bovinos (KASTELIC et al., 1997).

Assim, a exposição dos animais a elevadas temperaturas ambientais altera os mecanismos de termorregulação testicular, podendo ocasionar, dependendo do gradiente térmico, uma degeneração do parênquima testicular que é a principal causa de subfertilidade e infertilidade em machos (MIEUSSET et al., 1992). Estudos conduzidos em várias espécies têm mostrado que a degeneração do epitélio germinativo gonadal causa redução na qualidade seminal, que é refletida pelo aumento das patologias espermáticas, reduções da motilidade e vigor (MOREIRA et al., 2001; GABALDI e WOLF, 2002). Isto ocorre devido ao comprometimento da espermatogênese, principalmente na fase intermediária (espermatócitos e espermátides) e por alterações no epitélio epididimário (ROSS e ENTWISTLE, 1979; BRITO et al., 2002, 2003; RAHMAN et al., 2011).

Ainda, Aguiar et al. (2013) avaliando a influência das estações seca e chuvosa no resfriamento do sêmen de caprinos, por meio da aferição das características espermáticas e bioquímicas do plasma seminal de animais criados no semiárido Nordestino, verificaram que os mesmos apresentam variações nos componentes bioquímicos do plasma seminal durante as estações.

Nunes et al., (1982), relataram que o aumento da umidade durante a estação chuvosa interferiu na composição do plasma seminal caprino, nessa região, promovendo variações sazonais na sobrevivência de espermatozoides *in vitro*. Os autores verificaram que o plasma seminal obtido na época chuvosa, com grande parte do volume seminal constituído pelas secreções das glândulas vesiculares, levou ao aumento da motilidade dos espermatozoides refrigerados/descongelados nesse período.

Kahwage et al., (2010) estudando catetos criados na Amazônia Oriental observaram flutuações nos parâmetros seminais dos ejaculados ao longo de um ano. Os autores demonstrarem uma redução nos valores para os parâmetros seminais, principalmente entre março e maio, com redução da motilidade e integridade de membrana plasmática e concomitante aumento no total de defeitos espermáticos. Nesse período, a motilidade espermática média foi de 40,1 ± 4,0%, o vigor médio, 2,0 ± 0,0, a MPI média, 41,9 ± 3,3%, a média de defeitos maiores foi de 27,4 ± 7,7%, a média de defeitos menores foi de 11,2 ± 1,7% e de defeitos totais foi de 38,5 ± 9%.

Hellgren et al (1989) verificaram que as concentrações máximas de testosterona no soro e medidas testiculares de catetos no sul do Texas, ocorreram entre outubro e março, coincidindo com o pico da época de reprodução desta espécie. Esses autores sugeriram que alterarações na temperatura ambiente também poderia modificar a fisiologia reprodutiva masculina, uma vez que, foi observada forte correlação negativa entre valores séricos de testosterona e temperatura ambiente. Demonstrando que apesar de ser fértil durante todo o ano, o macho de cateto pode apresentar uma atividade reprodutiva reduzida no final do verão, onde predomina as altas temperaturas.

De modo semelhante, com os catetos criados sob clima semiárido têm-se observado, empiricamente, durante a realização de alguns trabalhos (CASTELO et al, 2010; PEIXOTO et al., 2012; SILVA et al., 2013a; SANTOS et al., 2013) que em determinadas épocas do ano se obtêm ejaculados de melhor qualidade. Neste sentido, seria de sobremaneira importante avaliar a existência de um possível efeito estacional sobre a atividade reprodutiva dessa espécie, levando em consideração parâmetros climáticos próprios do clima semiárido, nas estações seca e chuvosa, tais como, pluviosidade, temperatura, entre outros.

2.7 Clima e variáveis meteorológicas

O Brasil é um país continental, que em virtude da sua localização e grande extensão, apresenta diferentes tipos de climas. No qual, dentro de um mesmo estado são observadas condições climáticas diferentes, tais como, no estado do Rio Grande do Norte, onde ocorre o clima tropical úmido, na costa potiguar e em pontos de maior altitude, e o semiárido, que predomina em todo o resto do território, especialmente no centro e no sul (SILVA et al., 2011; SILVA et al 2013b). Esta diferença climática pode influenciar de forma efetiva o equilíbrio térmico de uma mesma espécie animal (SILVA et al., 2011).

Regiões geográficas de baixa latitude, ou seja, próximas ao Equador, são caracterizadas por intensa radiação solar ao longo do ano (entre 2400 e 2700 horas), fator muito importante a se considerar, já que exerce influência direta sobre o clima local (OLIVEIRA et al 2014).

As regiões de clima quente caracterizam-se por um ciclo diurno de temperatura atmosférica que muda dependendo da localização geográfica da região e estação do ano, entre outros fatores (YALÇIN et al., 1997). A temperatura do ar (TA) em um determinado local é bastante variável, podendo ser influenciada por diversos fatores como, a latitude, época do ano, hora do dia, altitude, concentração de gases e aerossóis, características físicas da superfície do solo e presença de estruturas. A umidade relativa (UR), por sua vez, se caracteriza pela proporção da umidade observada, em relação àquela que existiria se a atmosfera tivesse saturada, à mesma temperatura (UR = pressão parcial de vapor à temperatura Ta /pressão de saturação à temperatura Ta). Adicionalmente, deslocamentos do ar constituem um processo importante na variação atmosférica, sendo o vento definido como um deslocamento do ar relativo a uma dada superfície, sendo importante para determinar alguns índices de conforto térmico (QUEIROZ et al., 2014). Essas variáveis podem ser obtidas com auxilio, entre outros equipamentos, de um termo-higro-anemômetro (CAMPBELL, NORMAN, 1998; THOMPSON 1998, SILVA, 2008, SILVA et al., 2010, 2014).

O psicrômetro, outro equipamento bastante útil no estudo das variáveis ambientais, é um conjunto de dois termômetros de mercúrio ou álcool, um dos quais tem seu bulbo envolvido em tecido de algodão, que é mantido úmido (termômetro de bulbo úmido), o outro termômetro (bulbo seco) é destinado a medição usual da temperatura do ar. Se a atmosfera não tiver saturada, a evaporação que ocorre causará uma queda na temperatura de bulbo úmido (Tu), do que resultará numa temperatura mais baixa que a Ta. Assim, quanto maior a diferença entre essas temperaturas, mais seca se acha a atmosfera (SILVA, 2008).

É inquestionável que as trocas térmicas por radiação entre os animais e seu meio ambiente assumam importância fundamental em climas tropicais. Nesse sentido, para pesquisa de conforto térmico, o ambiente radiante é geralmente avaliado pela temperatura radiante média (TRM) (SILVA et al., 2014). A TRM é a temperatura média do conjunto de todas as superfícies reais e virtuais ao redor do animal em um dado local. Esse animal troca com essas superfícies uma quantidade de energia determinada pela Carga térmica

Radiante (CTR), que pode ser definida como a quantidade de energia térmica trocada por um indivíduo através da reação com o ambiente (SILVA et al., 2002).

Para tais medidas termômetros de globo negro têm sido amplamente utilizados, se constituindo em dispositivo simples e apropriado para fluxos de radiação de onda longa, a temperatura indicada pelo globo negro provê uma estimativa dos efeitos combinados da energia térmica radiante procedente do meio ambiente em todas as direções possíveis, da temperatura do ar e da velocidade do vento, dando assim uma medida de conforto térmico proporcionado pelo ambiente nessas determinadas condições (SILVA et al., 2002, SILVA et al., 2014).

As temperaturas de bulbo seco (Ta), de globo negro e velocidade do vento são utilizadas para calcular a temperatura radiante média (T_{RM}, K) e a carga térmica radiante (CTR, W/m^2) a partir das equações adaptadas por Silva (2008) e Buffington et al. (1981).

$$\mathrm{T_{RM}} = \left(\frac{CTR}{\sigma}\right)^{\frac{1}{4}} \quad K \qquad [1]$$

$$\mathrm{CTR} = 1{,}053 \cdot h_G(\mathrm{T_G} - \mathrm{T_A}) + \sigma \cdot \mathrm{T}_G^4 \qquad W \cdot m^{-2} \qquad [2]$$

Em que T_G (K) é a temperatura do globo negro, $\sigma = 5{,}67051 \cdot 10^{-8}$ $W \cdot m^{-2} \cdot K^{-4}$ é a constante de Stefan-Boltzman e h_G ($W \cdot m^{-2} \cdot K^{-1}$) é o coeficiente de convecção do globo negro:

$$\mathrm{h_G} = \frac{k \cdot Nu}{d_G} \qquad [3]$$

Onde d_G (0,15 m) é o diâmetro do globo. No caso de esferas, o número de Nusselt para convecção forçada é dado pela seguinte equação:

$$Nu = 2 + (0{,}4 \cdot R_e^{1/2} + 0{,}06 \cdot R_e^{2/3})P_r^{0,4} \qquad [4]$$

e a convecção natural:

$$Nu = 2 + \frac{0{,}589(G_r \cdot P_r)^{1/4}}{[1 + (0{,}469 / P_r)^{9/16}]^{4/9}} \qquad [5]$$

Números adimensionais de Reynolds (Re), Prandtl (Pr) e o de Grashof (Gr) são dados por:

$$G_r = \frac{g \cdot d^3 (T_G - T_A)}{\nu^2 \cdot T_A} \quad [6]$$

$$P_r = \frac{\rho \cdot C_P \cdot \nu}{\kappa} \quad [7]$$

$$R_e = \frac{U \cdot d}{\nu} \quad [8]$$

Onde: U ($m \cdot s^{-1}$) é a velocidade do vento e ρ, CP, κ e ν são as propriedades físicas da atmosfera: densidade, condutividade térmica, calor específico e a viscosidade cinemática, respectivamente dadas pelas seguintes equações:

$$c_p = 1{,}00522 + 0{,}0004577 e^{T_a / 32{,}07733} \ \mathrm{J \cdot g^{-1} \cdot {}^{o}C^{-1}} \quad [9]$$

$$k = \rho \cdot c_p \left(1{,}888 \cdot 10^{-5} + 1{,}324 \cdot 10^{-7} \cdot t_A \right) \ \mathrm{W \cdot m^{-1} \cdot K^{-1}} \quad [10]$$

$$\nu = 1{,}3291 \cdot 10^{-5} + 9{,}0 \cdot 10^{-8} \cdot t_A \quad \mathrm{m \cdot s^{-1}} \quad [11]$$

$$\rho = 3484{,}358 \cdot P_a \cdot T_A^{-1} \quad \mathrm{g \cdot m^{-3}} \quad [12]$$

Onde, P_a é a pressão atmosférica dada por:

$$P_a = 101{,}325 \cdot e^{\left\{ - \frac{z \cdot g}{287{,}04 T_A} \right\}} \quad KPa \quad [13]$$

Em que g é a gravidade, dada por:

$$g = 9{,}78013 + 8{,}18 \cdot 10^{-5} \cdot L_t + 1{,}168 \cdot 10^{-5} \cdot L_t^2 - 3{,}1 \cdot 10^{-6} \cdot z \quad \mathrm{m \cdot s^{-2}} \quad [14]$$

A relação Gr/Re^2 será usada para especificar se a convecção será natural ($Gr/Re^2 > 3$), forçada ($Gr/Re^2 < 0{,}08$) ou mista ($0{,}08 < Gr/Re^2 > 3$).

Além da CTR e T_{RM} os dados poderão ser utilizados para o cálculo do Índice de temperatura de globo negro e umidade (ITGU), segundo modelo definido por Buffington et al (1981):

$$\mathrm{ITGU} = T_G + 0{,}36 T_{PO} + 41{,}5 \quad [15]$$

Onde: T_G é a temperatura do globo e T_{PO} é a temperatura de ponto de orvalho.

$$T_{PO} = \frac{240{,}97 \ln(p_v / 0{,}61078)}{17{,}502 - \ln(p_v / 0{,}61078)} \quad {}^{o}C \qquad [16]$$

Onde: p_v é a pressão parcial de vapor dada através do analisador de H_2O/CO_2 LI7000.

Nesse contexto, os índices de conforto são calculados a partir destas variáveis obtidas e, dentre eles, se destaca o Índice de Carga Térmica (ICT – Gaughan et al., 2002, 2008) este último foi classificado por Silva et al., (2007) como melhor índice, em trabalho realizado com vacas das raças Holandesa e Jersey exposta a radiação solar direta na região central do Ceará e na costa do Rio Grande do Norte, correlacionando os índices mais utilizados, tais como, Índice de Temperatura e Umidade (ITU), Índice de Globo Negro e Umidade (IGNU), entre outros, com a temperatura retal e frequência respiratória dos animais.

Adicionalmente, Silva et al., (2010) verificaram que o índice de Carga térmica radiante - CTR para radiação de ondas longas (342 w/m^2) representou 53,4% da radiação total absorvida por vacas leiteiras das raças Holstein e Guzerá, mantidas a pasto no Nordeste brasileiro. A partir do qual pôde se concluir que o ambiente térmico para vacas em uma região tropical pode ser descrita em termos de carga térmica radiante efetiva. Que leva em consideração o efeito da radiação de onda curta. Este valor pode ser utilizado para calcular também a temperatura média radiante do ambiente.

Com relação à catetos (*Pecari tajacu*), poucos estudos foram realizados no sentido de verificar a influência das variáveis meteorológicas sobre os parâmetros reprodutivos. Os estudos mais relevantes foram realizados por Hellgren et al., (1989), no estado do Texas – EUA, no qual foi avaliado a variação estacional na testosterona sérica, mensurações testiculares e características do sêmen. E por Kahwage et al., (2010) na Amazônia Brasileira, avaliando a biometria testicular e as características seminais ao longo do ano. No entanto, não foram calculados os índices de conforto térmico, muito importantes para avaliar as condições ambientais as quais os animais estão expostos, nem tampouco, o efeito de climas como o semiárido, com apenas duas estações bem definidas, um longo período seco e um curso período chuvoso, com chuvas irregulares e espaciais.

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4. JUSTIFICATIVA

A população mundial de catetos (*Pecari tajacu*) é classificada pela IUCN como menos preocupante em relação à extinção, no entanto, foi obeservado que a população está em declínio em seus habitats naturais (IUCN, 2017). No Brasil, em virtude dos efeitos da caça predatória, da perda de qualidade de habitat e da fragmentação tem-se observado uma redução populacional significativa, o que pode levar a espécie a se tornar vulnerável a extinção, em algumas regiões (Desbiez et al., 2012).

Uma alternativa diante o declínio dessa população seria sua criação em cativeiro, tanto para desenvolvimento de estratégias de conservação da espécie, como para fins comerciais. Embora seja uma espécie de interesse comercial, haja vista a qualidade de sua carne e pele, sua manutenção em cativeiro depende do conhecimento de sua biologia reprodutiva. Assim, os estudos necessários para tal deve passar obrigatoriamente sobre a compreensão dos mecanismos reprodutivos face as diferentes condições climáticas a que a espécie está submetida

Dessa forma, com o estudo de características reprodutivas, correlacionando-as às variáveis meteorológicas da região semiárida, poderá potencializar a conservação da espécie e prover técnicas adequadas de manejo para a exploração econômica da mesma em cativeiro.

Neste sentido, justifica-se a realização de estudos em catetos, referentes à avaliação do efeito das variáveis meteorológicas próprias da região semiárida, sobretudo dos períodos seco e chuvoso, sobre os parâmetros reprodutivos desta espécie ao longo do ano.

5. HIPÓTESES CIENTÍFICAS

1. As variáveis meteorológicas de uma região semiárida influenciam os aspectos reprodutivos de catetos, incluindo as características seminais, o perfil sérico de testosterona e a biometria testicular ao longo do ano;

2. Há correlação entre as variáveis meteorológicas do semiárido e os parâmetros reprodutivos dos catetos;

3. As variáveis meteorológicas do semiárido influenciam a congelabilidade do sêmen de catetos radicados no bioma Caatinga.

6 OBJETIVOS

6.1 Objetivo geral

Avaliar a influência das variáveis meteorológicas de uma região semiárida sobre a atividade reprodutiva e a congelabilidade de sêmen de catetos (*Pecari tajacu*) ao longo do ano.

6.2 Objetivos específicos

- Identificar em catetos, existência de influência das variáveis meteorológicas do semiárido nas seguintes caractetísticas reprodutivas:
    - Parâmetros seminais;
    - Perfil sérico de testosterona;
    - Biometria testicular;
    - Ecotextura testicular;
    - Congelabilidade espermática;

- Estimar qual o melhor período para realizar criopreservação de sêmen de catetos numa região semiárida;

- Correlacionar os parâmetros reprodutivos analisadas com as variáveis meteorológicas de temperatura do ar, umidade e velocidade do vento;

7 CAPÍTULO 1

Influence of Seasonality on Mammals Reproduction

(Influência da Estacionalidade sobre a Reprodução de Mamíferos)

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Influence of Seasonality on Mammals Reproduction

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Review Article

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INTRODUCTION

Reproduction is the most important biological activity for any animal population, since it is responsible for the origin of the individuals of the next generation, and it ensures the survival of the species. It multiplies the herds' activities for commercial breeding and generates an interface with selection and genetic enhancement work in research [1,2]. The reproductive management covers several aspects, such as feeding, mating system, the use of biotechnology for genetic improvement, establishment of criteria for the selection of breeders and matrices, and reproductive disease control[3].

The reproductive activity can be affected by several factors such as the influence of climate and its changes, which are decisive for the semen quality in several species[2,4-7]. Similarly, in females, exposure to stressors such as high temperatures, food, and water deprivation can act on the hypothalamic-pituitary-gonadal axis, jeopardizing their operation[8]. These factors reduce the release of gonadotropin-releasing hormone (GnRH) from the hypothalamus and determine a decrease in the frequency and amplitude of luteinizing hormone (LH) released from the pituitary gland, resulting in abnormal ovarian function due to the nonoccurrence of LH surge and thus inhibiting follicular development and ovulation[8,9].

In this context, the reproductive seasonality consists of the phenomenon by which some species have reduced sexual activity during a certain period of the year, mainly regulated by climatic factors such as temperature, air humidity, or photoperiod[4,5,10]. In tropical climates, high ambient temperature observed in the dry season is one of the main limiting factors for reproductive efficiency, and may affect testicular thermoregulation, interfering in the process of spermatogenesis and semen quality[11-14]. These characteristics, combined with the production of forage of poor nutritive value, require that the animals exhibit heat tolerance, skill conversion of roughage, and resistance to parasites, once the animal productivity depends of its degree of adaptation to the environment and their interactions[15]. Thus, reproduction is the most positive evidence of animals being able to adapt to a particular environment[16]. The reproductive performance is the main phenomenon responsible for the success of production, being fundamentally dependent on the genotype and environment[17].

This study aimed at conducting a review on the influence of climatic aspects, such as photoperiod, temperature, rainfall regimes, and food availability that may interfere with a mammal's reproduction.

Physiological mechanisms

Many mammalian species have various physiological mechanisms for maintaining their reproductive activity in the face of environmental adversity, among which testicular thermoregulation stands out. Among the different thermoregulation processes, the positioning of the testicles within the scrotum consists of a process derived from the evolution of most terrestrial mammals, which is based on the placement of this organ in the area outside of the male body, with the purpose of maintenance of a distinct temperature level in the rest of the body[18]. In addition, the nature of the scrotum, with thin skin, low fat, and low pelage, highly vascularized, coupled with physical mechanisms such as countercurrent heat exchange, blood flow regulation, the position of the testicles, and sweating, plays an important role in the maintenance of testicular temperature 2°C to 6°C below body temperature[19]. However, exposure of the animals to high environmental temperatures changes the thermoregulatory mechanisms, which may cause, depending on the thermal gradient, a degeneration of the testicular parenchyma, which is the main cause of subfertility and infertility in males[20].

Studies conducted in several species have shown that the degeneration of gonadal germinal epithelium leads to a reduced semen quality, which is reflected in the increase in sperm pathology, and reduced motility and sperm vigor[21,22]. This is due to impaired spermatogenesis, especially in the intermediate phase (spermatocytes and spermatids), and changes in the epididymal epithelium[23-25].

In females, the control of gonadotropin secretion during the estrous cycle requires a delicate balance between the complex hormonal interactions. Hypothalamic nuclei secretes GnRH, which through the hypothalamic-pituitary portal system stimulates the anterior pituitary to secrete LH and follicle-stimulating hormone (FSH), thus promoting the synthesis of estrogen and progesterone by the ovaries[26]. These latter two exert influences by positive or negative feedback mechanisms, directly on the pituitary or hypothalamus, making possible the continuity of the cyclic events that characterize the estrous cycle[26,27]. That cycle is determined by the pace of functional female reproductive organs that are established from puberty, comprising cyclic changes in the physiology and morphology of the genital organs and also in the related hormone profile[28,29].

In goats bred in tropical regions, the period of seasonal anestrus varies in intensity and duration depending on the latitude, race, lineage within the same race, the climate, and genetic and social factors. In addition, it can be influenced by the stage of lactation and management practices, such as the animal feeding. The nutritional deficiency is among the main factors that can interfere with reproduction of females created in this tropical regions, since the energetic support is usually not enough to maintain the production processes in the dry period[30,31].

Once the acclimatization to a high temperature environment involves processes that lead to the reduction of the thermal load, the immediate response is the reduction in food intake, increase in respiration rate and water intake, and changes in hormone levels that affect the responsiveness of the target tissue to environmental stimuli[32]. If the exposure to high temperatures is extended, the lower feed intake is followed by a decline in the secretion of hormones, such as growth, catecholamines, and glucocorticoids, as well as by thermogenic processes of digestion and metabolism, thereby reducing the production metabolic energy to be used in maintenance processes, production, and animal breeding[33].

Additionally, harsh environmental conditions and risk of predation and malnutrition are the most relevant stressors for free-living vertebrates[34]. The activation of the hypothalamic-pituitary-adrenal axis by stress generates a cascade of hormonal messages that culminate in increased concentrations of plasmatic cortisol, which in turn inhibits the activity of hypothalamus and pituitary gland, producing negative effects on animal reproduction[35,36].

Influence of photoperiod

The photoperiod is the duration period of light of a certain place, depending on the latitude and season, and is represented by the length of a day. The ability of animals to react to the duration of daily light that they are subjected to is called "photoperiodism." In seasonal animals, variations in the annual reproductive cycle occur due to changes in day length and melatonin secretion profile, thus being classified as the negative or positive photoperiod for animals[5,37].

In horses, animals that are long-day seasonal or positive photoperiod, the increased exposure of melatonin has the opposite effect, inhibiting the secretion of GnRH by the hypothalamus and, consequently, reducing the production and release of LH by the anterior pituitary. Thus, in the Southern Hemisphere, the period of greatest brightness is the most favorable season for breeding[38,39]. In addition, the domestic cat also behaves similar to a long-day animal, once the sperm production provides evidence of being influenced by the photoperiod, thus becoming greater with an increasing exposure to sunlight[40].

In sheep and goats, the influence of this phenomenon is striking in both male and female breeds coming from the northern hemisphere, which begins their annual reproductive cycle due to the decrease in the intensity of light daily, thus being considered animals presenting a negative photoperiod[41]. Thus, in the dark, the pineal gland synthesizes and secretes melatonin, which stimulates the hypothalamus, the pituitary gland, and the ovaries or testes to the return of the reproductive activity[31,42]. Buffalo (Bubalus bubalis) also behaves similar to a short-day seasonal poliestral animal (negative photoperiod) or as a continuous poliestral animal depending on the proximity to the Equator[2,43].

Most wild ruminants as the Alpine ibex (Capra ibex), the Iberian ibex (Capra pyrenaica) and the mountain goat (Oreamus americanus) present a short breeding season, usually from November to February, and the photoperiod is the main environmental cue regulating seasonal breeding activity in these species[5,13]. Santiago-Moreno et al.[14] also reports that testicular activity and secondary sexual characteristics development can be changed by manipulating the photoperiod. The male goral (Naemorhedus griseus) of the Thailand showed seasonal variation in testicular and adrenal steroidogenic function, with greater activity in the rainy season and winter. Given that resources for this animal are consistent throughout the year, reproduction may be primarily regulated by photoperiod in this species[44]. Camels are also considered seasonal breeders, under the influence of the photoperiod; however, when these animals are raised near the equator, factors such as rainfall, nutrition, and management may override the effects of photoperiod and allow breeding to occur throughout the year[45].

Therefore, in regions where there is not much differentiation in the length of days and nights, the animals suffer less due to the effect of this phenomenon, with the reproductive seasonality being more related to thermal stress, nutritional deficiencies, health, and the husbandry system[28,46].

Influence of thermal stress

The intensity of solar radiation directly affects the behavior and physiology of domestic animals and determines their adaptability to the environment. Mammals adjust their physical, biochemical, and behavioral processes in an attempt to contain the negative effects of thermal stress, which involves heat dissipation to the environment and reduces the production of endogenous heat, reflecting the restriction of reproductive activity[47-50].

Mathevon et al.[51] reported that the concentration of semen, under temperate conditions, and the number of sperm and mobile cells ejaculated by bulls are lower in summer than in winter and spring. In regions where the predominant climate type is hot and humid, the increase of the temperature and humidity index (THI) ranging from 63.9 to 82.6 enables the respiratory rate and skin temperature to rise in the female buffalo, whereas there is a decrease in sweat rate and consequently a decrease in the loss of body heat, causing thermal stress[52]. In addition, Garcia[2] states that this phenomenon can adversely affect the reproductive physiology of buffaloes, generating a change in reproductive behavior, reducing the manifestation of estrus and conception rates, and causing a decline in pregnancy maintainability, which reduces fertility. The male buffaloes subjected to the same weather conditions have reduced sperm quality and changes in semen composition[2]. In horses, normal intratesticular spermatogenesis occurs at a temperature of 35°C on average, and most of the testicular problems of stallions are related to changes in their ability to control testicular temperature[53].

Under semiarid climate, Nunes et al.[54] reported that the male goat may present a certain seasonal production that depends on a series of intrinsic variables such as race, weight, and age; extrinsic variables such as latitude, temperature, and power. In sheep, the seminal characteristics most affected by high temperatures are motility, vigor, concentration, and morphology[16]. Maia et al.[4] attributed the high percentage of sperm morphology damage observed in Dorper and Santa Inês sheep to the negative effect of ambient temperature on spermatogenesis and sperm maturation, since the samples were collected in the summer, hightemperature environment period. As a result, rectal temperature was elevated to approximate values of 39°C to 39.5°C, resulting in the appearance of abnormal spermatozoa in the ejaculate, especially with tail pathologies, isolated heads, and cytoplasmic droplets, with these types of defects directly related to thermal stress[4].

Pigs are very sensitive to hot conditions, mainly due to the low capacity of the species to heat loss. In feral pigs, Macchi et al.[55] evaluated the reproductive seasonality in two northeastern areas of Italy: one in alpine climate, characterized by coniferous forest, and the other in a plain, grazing area, experiencing a warm climate. The authors identified the existence of different patterns of reproductive rates between the two geographically distinct populations created in these regions because of, among other factors, the various environmental conditions. Studying peccaries (Pecari tajacu) in captivity, Hellgren[56] determined that the highest testosterone concentrations occurred between the months of October and March, coinciding with the time of the highest number of species mating in southern Texas, USA. In addition, the authors described the relationship between the circulating levels of the hormone with the season and ambient temperature, suggesting a reduction in the reproductive performance of males during late summer, due to the occurrence of high temperatures[56].

Influence of rainfall regime and food availability

In the semiarid region, as well as due to direct climate action, animals suffer from the poor quality of food that is almost exclusive pastures produced in low fertility soils, which is the result of intense leaching that causes forage production irregularity[15,4]. In addition, there is the effect of seasonality of rainfall and other weather conditions that do not allow uniform production throughout the year, with excess in the rainy season and shortages in the dry season and the low digestibility of forage due to the low protein content and high fiber[33,57]. Low[58] proposed that the high ambient temperature or nutritional stress induced by drought or protein forage decreases during the late summer, can reduce male reproductive activity reflecting acyclicity females, since nutritional factors are important for both the establishment of puberty and the maintenance of ovarian cyclicity, resumption of cyclicity postpartum, and maintenance of pregnancy in buffaloes[2].

According to Cavalcante et al.[59] changes in semen characteristics and libido of sheep and goats of different breeds are due to variations in climate, as evidenced by studies that described the influence of light, temperature, and rainfall in breeding animals. So, in some tropical or subtropical regions, the goats can behave similar to continuous polyestrous, with the nutrition and health status being among the main factors that can interfere with reproduction[60,61]. Thus, the effect of season on the quality of the different breeds of sheep semen has been the subject of several studies[62] in which wooless races have generally well adapted to the region, suffering due to less influence of climatic factors on semen quality[4]. However, in Santa Inês and Somalis sheep, the time of year affected the volume and sperm concentration, with a larger volume and a lower concentration in the rainy season[63]. In addition, in the rainy season, we observed a significant increase in mass motility and sperm concentration in semen of the same kind in the Santa Ines breed, otherwise observed in the dry season, when there was impaired spermatogenesis, whose disability substrates for energy production resulted in nutritional deficiency of animals[64].

Similar studies were carried out by Coimbra[65] under humid tropic weather while evaluating seminal characteristics of zebu semen donors, taurine, and buffaloes that were kept in an artificial insemination center in the state of Pará. The author analyzed ejaculations collected during the most rainy season (January to June) and during the less rainy season (July to December); the results showed that the buffalo bulls showed differences in semen quality, which was always favorable to ejaculate produced in the rainy season of the year. Sperm motility was the attribute that showed greater differences in both the semen in natura as post freezing. In addition, Garcia[2] reported that nutrition in the same species is an important element and where deficit, it reduces the body condition of the animals, with negative impacts on the age at puberty, the age at first calving, and calving interval. In cattle, the reduction in energy consumption due to poor intake of food results in a negative energy balance, and it partly explains why the cows lose significant amounts of weight and body condition when subjected to thermal stress and nutritional deficiency[66].

Regarding wildlife, Dubost et al.[67] investigated the reproductive seasonality of three species of rodents in the rainforest of French Guiana: the Myoprocta exilis, the Dasyprocta leporina, and Agouti paca. The authors concluded that Myoprocta exilis showed the most evident features of seasonality, with 56% of births concentrated between the months of November and January; the Dasyprocta leporina also performed seasonally, but to a lesser extent; and the Agouti paca apparently produced during most of the year. The authors attributed this to the seasonality in the production of some fruits of the forest, which were important in the diet of these animals, and also reported that less seasonal species had a more diverse diet for the poorest season of these fruits. However, under the conditions of the humid tropical Amazon, the agouti presented a continuous reproduction being classified as poliestric, both in captivity and in the wild[68]. However, it is noteworthy that Weir[69] reported that one reproductive seasonality period occurs in captivity for this species in temperate conditions.

Trillmich et al.[70] showed that in guinea pigs (Cavia aperea) the photoperiod is an important factor in determining the reproductive seasonality of the species, whereas Bauer et al.[71] stated that the seasonal level varies with nutritional components such as proteins, which are essential for perinatal development of this kind.

In desert, where unpredictable rainfall arises and a large variation in temperature occurs, severe energetic and water related constraints influence the native small mammals reproduction. The harsh conditions associated with arid environments can constrain reproduction in rodents to the favorable period which maximizes the growth and survival of offspring[72]. Sarli et al.[6] reported that the Acomys imidiatus placed in arid region of Saudi Arabia reproduces seasonally with the cessation of reproduction during winter. This appears to be tightly linked to the rainfall and indirectly to other factors as the salinity in the desert vegetation that serves as food for such animals.

The hairy nose wombat (Lasiorhinus latifrons) breeding occurs between July and December (winter and spring) and is highly dependent of annual rainfall and the nutritional content of vegetation with cause changes in semen quality, plasma testosterone secretion and bulbourethral size[7].

In collared peccary, Lochmiller et al.[73] reported decreased serum levels of testosterone and scrotal circumference after nine weeks followed by nutritional deficiency. Hellgren[56], in turn, determined that the concentration of this hormone in peccaries did not vary significantly by season, but seasonal values were higher in winter (February–March). In addition, testicular volume and scrotal circumference in captive animals varied significantly, with higher measures between late October and late February for both traits. However, smaller amounts were recorded in the summer months, also noting that the average monthly scrotal perimeter ranged from 182 ± 12 mm in September to 228 ± 8 mm in January[56].

While studying peccaries created in eastern Amazonia, Kahwage et al.[74] observed fluctuations in seminal parameters of ejaculates over a year. In this study, the authors demonstrated a reduction in the values for semen parameters, especially between the months of March and May, with reduced motility and plasma membrane integrity and a concomitant increase in total sperm defects. As for animals of the same species maintained in semiarid climate, conditions have not yet been developed for long-term studies and under the environmental conditions monitored.

With regard to the reproduction of marine mammals, environmental, nutritional or social influences may affect the oestrus cycle, thus affecting the reproductive season[75]. For example, the values for serum testosterone in mature male killer whale (Orcinus orca) (>13 years) demonstrated periods of elevation from spring to fall. Despite these apparent seasonal rhythms in testosterone secretion (particularly within each animal), sperm production did not exhibit an obvious seasonal change with conceptions occurring throughout the year[76].

Regarding social influences on reproduction, some researchers say non-human primates differed from all other mammals since its sexual activity occur without interruption throughout the year. On the other hand, many other primates mate seasonally and in the rest of the year display a complete cessation of true copulation. In fact, a discrete reproductive period may be thought to allow gestation, births and early development to occur when the environmental conditions are most favorable. A growing body of evidence confirming that many primate species, if not completely seasonal, do at least tend to indulge in sexual activity more often at one time of the year than another[77]. Although ecological factors are undoubtedly important in timing seasonal transitions, there is considerable evidence in squirrel monkeys and other seasonally breeding animals that the coordination of reproductive changes between individuals is mediated by social stimulation[78,79]. Schiml et al. [78] evaluating seasonality in Squirrel Monkeys (Saimiri sciureus) found that the distribution of births was highly seasonal. The initial birth peak (November-December, 1989) followed group formation by 6-7 months. Estimates of the timing of conceptions indicated that the first breeding season included the months February through June, 1990, and the second breeding season commenced in February and ended in May, 1991, when behavioral and endocrine data collection ended. In wild Cebus apella the birth periods occur during the early to mid-wet season, both at this site, and elsewhere throughout their geographic range[80,81]. In direct contrast, plasma testosterone levels in captive Cebus apella show no circannual rhythm, and births in captivity may occur throughout the years[82].

CONCLUSION

From what has been mentioned earlier, it can be inferred that animals subjected to semiarid climate suffer little or no interference from the photoperiod. However, the factors related to thermal stress and poor food availability caused by erratic rainfall can reduce the reproductive capacity of these animals, thus creating a period of reproductive seasonality in some species during the months of higher and lower temperature availability of fodder in the dry season (July to December). Some steps that can be taken to minimize the negative effects of climatic aspects on animal reproduction are deploying shading areas in pastures, or heat dissipation mechanisms in plants and even nutritional supplementation, which can be realized through conservation techniques, forage such as silage or hay, and grazing areas when conditions permit water availability, according to each breeding system.

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Semen production in collared peccaries (*Pecari tajacu* Linneaus, 1758) is associated to natural meteorological variations in the Caatinga biome

(Caracterização de parâmetros reprodutivos de catetos (*Pecari tajacu* Linneaus, 1758) radicados no bioma Caatinga sob influência de diferentes índices pluviométricos)

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Abstract

The objective was to characterize the influence of natural variations in a semiarid region on semen production in collared peccaries (*Pecari tajacu*). Reproductive metrics (semen quality, testicular morphometry and testosterone serum profiles) of 10 mature males were measured monthly during 18 months. Meteorological data (air temperature, relative humidity, wind speed and rainfall) also were recorded during the same period. Rainfall index was used to define different classes (Class 1: months with no rain; Class 2: months with up to 50 mm of rain; and Class 3: months with >50 mm of rain). It was confirmed that a significant difference ($P < 0.05$) in the average air temperature (TA, °C), wind speed (WS, m/s) and relative humidity (RH, %), as well as values of radiant heat load to the sun (RHL, w/m²) among rainfall classes. These differences did not lead to significant changes in the reproductive metrics that were evaluated. However, based on the meteorological data collected five days prior to semen collection, positive correlations ($P < 0.05$)

were found between rainfall and semen volume (R = 0.26) and sperm concentration (R=0.22). Air temperature and relative humidity also were positively correlated ($P < 0.05$) with semen motility parameters determined by computerized analysis as straightness – STR (R=0.39) and beat cross frequency – BCF (R=0.28). In addition, negative correlations ($P < 0.05$) were identified between air temperature and BCF (R = -0.31) and STR (R = -0.35). Based on meteorological data collected 51 to 55 days before the semen collection (which corresponds to the interval related to spermatogenesis in the species) positive correlations ($P < 0.05$) were found between rainfall and semen volume (R = 0.22) and amplitude lateral head – ALH (R = 0.25), as well as between relative humidity and velocity straight line – VSL (R = 0.20) and STR (R = 0.25). Lastly, negative correlations ($P < 0.05$) were found between air temperature and semen volume (R = 0.21) and BCF (R = -0.31). This study demonstrates for the first time that meteorological conditions in a semiarid region have a short to medium term impact on the semen production in a model species like the peccary. While no seasonal changes could be detected overt the 18 month period, it is expected that long term environmental changes in a biome like the Caatinga will influence the reproductive physiology of species leaving in that habitat.

Keywords: *Tayassu tajacu*; wild pig; temperature; rainfall; semiarid weather; wildlife.

Introduction

Climate changes, especially the increase in ambient temperature, has had a major impact on ecosystems (Thompson 2010; Qu et al. 2016), influencing the survival of several species. The climate predictions for the Caatinga biome, constituted by a dry and seasonal Neotropical forest, over the next three decades have shown a rise in temperature in the order of 0.5 to 1.0°C and a reduction in rainfall index around 10 to 20%. At the end of the century, a temperature increase of up to 4.5°C is expected, accompanied by a rainfall reduction of between 40 and 50% (PBMC 2013). Such changes will certainly affect the vegetation of the region (Carvalho et al. 2014), thus reducing the food resources for the native animals. Under the influence of **climatic** conditions, native animals, such as collared peccary (*Pecari tajacu* Linnaeus, 1758), need to regulate their physiological functions to adapt to certain periods of stress.

Currently, the world population of collared peccaries, a kind of wild pig native of Americas, has been classified as stable (IUCN 2017). However, their status in all biomes requires monitoring (IUCN 2017) because various factors as the continuous rates of habitat destruction and excessive poaching of this species have been considerably reducing its population (Gongora et al. 2011), making it vulnerable to extinction in some biomes (Desbiez et al. 2012). Collared peccaries present ecological importance as seed dispersers and as a prey for large carnivores (Gongora et al. 2011; Bodmer et al. 2014). Moreover, they serve as a model for the establishment of strategies for the conservation of vulnerable closely related species as the white-lipped peccary (*Tayassu pecari*) and the Chacoan peccary (*Catagonus wagneri*).

It is a consensus among researches that males can have a substantial effect on population dynamics (Ranking and Koko, 2007). In this context, various studies has been focusing at elucidating physiological mechanisms of peccaries' male reproduction in order to establish rational strategies for its conservation. The duration of spermatogenesis in this species was determined as 55.1 ± 0.7 days (Costa et al., 2004). The peccaries' semen presents three fractions: the first being clear and secreted by the accessory glands, the second rich in spermatozoa, and a jelly fraction as the third one. However, fractions are currently not collected isolate through electroejaculation, the method of choice for semen obtaining in the species (Hellgren et al., 1989; Costa and Paula, 2005). Regarding semen characteristics and testicular biometry, Peixoto et al. (2012) identified a marked individual variation for the species. All this information, although, were derived from punctual studies, and the long-term variation in reproductive performance of peccaries, especially in Caatinga biome, is unknown.

Following these statements, different studies have recently evidence the existence of interactions between **meteorological** characteristics of the semiarid weather typical of the Caatinga biome and the reproductive parameters of different species that live there as ovine (Maia et al. 2011) and caprine (Van Tilburg et al. 2015). For the peccaries, however, existence of seasonality derived from natural variations on semiarid weather of Caatinga along the year is unknown. Additionally, there is a lack on the information how the **meteorological** characteristics in the moment immediately before or in the day of semen collection could influence peccaries' reproductive parameters as the sperm characteristics, testicular biometry and testosterone production. Moreover, the effect of natural **meteorological** variations of Caatinga on the sperm production of the species is unknown. This knowledge of their reproductive biology would be relevant to the adequacy of their management in captivity, as well as support for conservation strategies of the free-ranging individuals. Therefore, the objective was to characterize the influence of natural **meteorological** variations in a semiarid **region** on reproductive parameters of collared peccaries.

Methodology

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

Evaluation of meteorological **parameters**

The rainfall index (mm) was obtained daily from the automatic station of the National Institute of Meteorology - INMET, located in Mossoró, RN, Brazil. During the experiment, a total rainfall of 650 mm was observed, presenting irregular and below expected rains for the rainy period (March, April and May) in the region. Rain peaks were observed in the months of January 2015 and February 2017 (Figure 1). The 18 months of the experiment were grouped according to the rainfall index as follows: Class 1 (rainless) with 7 months, Class 2 (up to 50) with 8 months, and Class 3 (>50) with 3 months.

On the days of collection of semen, on each hour, from 6 am to 6 pm, the air temperature, relative humidity and wind speed were monitored by means of a thermo-hygrometer-anemometer (Instruterm-HT300, São Paulo, Brazil) (Silva 2008). The humid bulb (Tu, °C) and dry (Ta, °C) temperatures were measured using a psychrometer (Bacharach, New Kensington, Pennsylvania, USA). Additionally, temperatures of the black globes (Tg, ºC), one exposed to the sun and another to shade, measured by means of a mercury thermometer, were registered in the center of a black copper globe with 15 cm of diameter, connected to a digital thermometer (MINIPA, MT-600, São Paulo, Brazil). The equipment was positioned in the picket where the animals were located, at an approximate height of the animals' torso, according to the methodology described by Silva (2008). The dry bulb, black globe and wind speed temperatures were used to determine the radiant heat load (RHL, W/m^2), which is a thermal comfort index, calculated from the equations proposed by Silva et al. (2010). On the days of semen collection, a significant difference ($P < 0.05$) was observed for the average air temperature (°C), wind speed (m/s) and relative humidity (%), as well as the values of radiant heat load to the Sun (w/m2) among the evaluated rainfall classes (Figure 2).

To verify the influence of **meteorological** conditions during the management and preparation of the animals on semen parameters, we monitored the **meteorological** variables at the five days immediately before the semen collection. Moreover, in order to verify the effect of **meteorological** variables on semen production of peccaries, we conducted a retrospective analysis and monitored these variables at 50 to 55 days before semen collection. Thus, data related to air temperature (Ta, °C) and relative humidity (RH, %) were obtained from an automatic weather station (Campbell Scientific Brasil, São Paulo, Brazil) located 50 m from the experiment site (latitude: 5 ° 12'48 " S, longitude: 37 ° 18'44"W and altitude: 37 m above sea level), Mossoró, RN, Brazil. Based on these data, it was possible to trace the monthly profile of these meteorological parameters during the 18 months of the experimental period (Figure 3). Note that the air temperature remained high throughout the year, regardless of the rainfall index.

Animal management and restraint

Ten male adult collared peccaries aging 3.1 ± 1.5 years and weighting 21.9 ± 1.3 kg at the beginning of the experiment. They reached an average age of 4.6 ± 1.5 years and weight of 23.2 ± 1.2 kg at the end of the study. The peccaries were separated into groups of 2 to 3 animals, kept in pickets (20 m x 3 m), with a covered area (3 m x 3 m) under natural 12h photoperiod. They were fed with commercial ration for pigs, fruits and water at will.

On the days of collection, the animals were submitted to food fasting of 12h before the beginning of the procedures. The animals were initially restrained with a hand net. Subsequently, they were anesthetized with propofol (Propovan®, Cristália, Fortaleza, Brazil) at an intravenous dose of 5 mg/kg in their bolus. Throughout the experimental procedure, the animals were submitted to fluid therapy (sterile saline solution 0.9%) and their heart and respiratory rate were monitored (Souza et al. 2009).

Immediately after restraint, a digital thermometer (Mesure Technology, Park Dongting Town, China) determined the rectal temperature of the individuals. (Lima et al., 2014). Moreover, surface temperature of each individual was obtained through a surface infrared thermometer (Fluke, Everett, Washington, USA.) at each hour from 6 a.m. to 6 p.m. This infrared thermometer was positioned at 2 m from the individual and the laser sensor was pointed to its dorsum (Lima et al., 2014).

Semen collection and evaluation

During 18 months, the animals were submitted to a semen collection per month. On these occasions, anesthetized animals were placed in lateral decubitus position, and submitted to an electroejaculation protocol previously described for the species (Castelo et al. 2010). A portable device (Autojac®, Neovet, Campinas, SP, Brazil) connected to a 12V source was used, to which a rectal probe 15cm long and 1.3cm in diameter was attached. The probe was inserted approximately 12cm into the rectum of the animal. The semen of the animals was collected in plastic tubes and immediately evaluated.

Semen volume was measured with the aid of micropipettes. Sperm concentration (in millions of sperm / mL) was determined in a Neubauer chamber. From this concentration and volume, the total number of sperm in the ejaculate was calculated (Peixoto et al. 2012). For analysis of sperm morphology (%), smears stained in Rose Bengal were evaluated by light microscopy (1000x), counting 200 cells per slide (Sousa et al. 2013). Sperm membrane functionality was determined by evaluating the osmotic response of the sperm to the hyposmotic test using distilled water (0 mOsm / L). A total of 200 cells were examined and those with swollen and coiled tail were considered as having a functional membrane (%) (Santos et al. 2013).

For the analysis of sperm plasma membrane integrity (%), a fluorescent solution of carboxyfluorescein diacetate (CFDA) and propidium iodide (PI) was used. The samples were incubated for 10 min at 27 ° C in the CFDA + PI solution and then evaluated by epifluorescence microscopy (Episcopic Fluorescent attachment "EFA" Halogen Lamp Set; Leica, Kista, Sweden). For each sample, 200 sperm were counted and those marked totally green (CFDA) were classified intact, while those marked totally or partially red (PI) were considered with the non-intact membrane (Souza et al. 2015).

The kinetic patterns of sperm motility were determined by computerized analysis (IVOS 7.4G, Hamilton-Thorne ResearchTM, Beverly, MA, USA), according to previously determined configurations for the species (Souza et al. 2016). The following parameters were evaluated: total motility (%), VAP velocity average pathway (μm / s), velocity straight line (VSL; μm / s), velocity curvilinear (VCL; μm / s), amplitude lateral head (ALH, μm), beat cross frequency (BCF, Hz), straightness (STR; %), and linearity (LIN; %) as well as the sperm subpopulations: rapid, medium, slow and static.

Blood collection and measurement of serum testosterone

For the occasion of semen collection, in order to determine serum concentrations of total testosterone, two blood samples from each animal were collected by puncture of the saphenous vein, at intervals of 30 minutes between each one. The first sample was obtained immediately after the animal was restrained, and the second sample was taken at the end of the semen collection process. Blood was passed to 3 mL vials (Vacutainer®, BD Diagnostics, Franklin Lakes, NJ, USA), and immediately placed in an icebox. After collection, the tubes were taken to the laboratory and centrifuged (5000 g, 60 min, 4 ° C) for serum separation, which was aliquoted into microtubes (1.5 mL) and stored (-20 ° C) until the hormonal analysis (Maia et al. 2014).

For analysis of serum testosterone, it was used the liquid phase radioimmunoassay (RIA) and a commercial kit (ImmuChem™ Double Antibody Testosterone RIA-MP Biomedicals) in a Wizard device detector (PerkinElmer of Brazil Ltda). The intra-assay coefficient was 8.54% and 7.31% inter-assay. All concentrations determined were between the minimum and maximum points of the curve (Aquino-Cortez et al. 2017).

Testicular morphometry

When animals were restrained in order to collect semen, their scrotal circumference was measured in the largest portion of the testicular sac, using a metric tape measure (Peixoto et al. 2012). Then, testicular ecotexture and echogenicity were evaluated by ultrasonography, using a 7.5 MHz sector transducer coupled to a portable ultrasound equipment (Aquila vet, Pie Medical®, Nutricell, Campinas, Brazil) (Peixoto et al. 2012). The testicular biometry was evaluated by taking the measurements of length (L), width (W) and height (H) of each testicle. These measures were used to calculate the testicular volume (V) by the Lambert formula (V = L x H x W x 0.71, Lambert 1951). This analysis was performed on each animal, monthly, on semen collection days, shortly after animal restraint, for 12 months (September 2015 to August 2016).

Experimental design and statistical analysis

The rainfall index was used to group the months in different classes, and their effect on the meteorological and reproductive parameters of collared peccaries located in the Caatinga biome was verified. In this way, class 1 comprised the months without rain (rainless); class 2, the months in which it rained to 50 mm (up to 50) and class 3, with rainfall above 50 mm (above 50).

All variables were expressed as average and standard error and were transformed by log (x + 1) or arc-sine (√ (x / 100)), when necessary. An analysis of variance (ANOVA) was performed using the least squares method (Silva, 1993), considering class (1, 2 or 3) as the only source of variation on the reproductive parameters studied. Tukey-Kramer test (P <0.05) was used to compare the averages. In these statistical procedures the general linear model procedure (PROC GLM) of the Statistical Analysis System (SAS 1999) was used.

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

Results

Semen parameters

The rainfall class did not influence the efficiency of semen ($P > 0.05$). In class 1 (rainless), 70 collection attempts were performed, and 47 semen samples were obtained (67.1%). In class 2 (up to 50), 80 attempts were performed, which resulted in 59 semen samples (73.8%). Finally, in class 3 (above 50), 19 samples were obtained from 30 attempts of semen collection (63.3%). For the evaluation of the sperm parameters, only those samples with sufficient volume for the analyses were counted.

The rainfall classes did not lead to significant differences in semen volume, sperm concentration, total sperm count, sperm morphology, functionality and membrane integrity (Table 1). In addition, the computerized analysis did not identify significant differences between the rainfall classes regarding the analysis of the kinetic parameters of sperm motility in collared peccaries (Table 2).

Serum testosterone profile and testicular morphometry

The values for serum testosterone concentration of the animals (Figure 4) remained constant throughout the three rainfall classes evaluated ($P > 0.05$). In addition, there was maintenance of the scrotal circumference and testicular morphometry (right, left and total testicular volume) during the experiment (Figure 5), without influences of the rainfall classes ($P > 0.05$). Based on the values obtained on the days of semen collections, a significant correlation was found between serum testosterone concentrations and scrotal circumference ($R = 0.21$, $P = 0.02$).

Peccaries' temperature evaluation

The average values for the rectal and surface temperature of the animals are shown in Figure 6. The rainfall classes did not influence these physiological parameters (P> 0.05).

The surface and rectal temperatures obtained on the day of semen collection did not influence serum testosterone profile, scrotal circumference or testicular morphometry (P> 0.05). The surface temperature also did not influence the sperm parameters. However, the rectal temperature showed a negative correlation with total motility (R = -0.20, P = 0.02), VAP (R = -0.23, P = 0.01) 20, P = 0.02), and rapid sperm subpopulations (R = -0.23, P = 0.01), as well as positive correlation with static sperm (R = 0.20, P = 0.02).

Correlations between meteorological data and reproductive parameters

The meteorological variables determined on the day of semen collection did not influenced serum testosterone levels, scrotal circumference or testicular morphometry of the peccaries (P> 0.05). However, there was a positive correlation between air temperature and BCF (R = 0.20, P = 0.03), average relative humidity and STR (R = 0.19, P = 0.04) and negative correlation between RHL in the sun and STR (R = -0.21, P = 0.01).

When evaluating the meteorological variables in the five days prior to semen collection, significant positive correlations were identified between rainfall and semen volume (R = 0.26), sperm concentration (R = 0.22) and number of sperm per ejaculate (R = 0.24), as well as between relative humidity and STR (R = 0.39) and BCF (R = 0.28). Besides, negative correlations (P < 0.05) were identified between air temperature and BCF (R = -0.31) and STR (R = -0.35).

When evaluating the influence of the meteorological variables determined between 51 and 55 days before the semen collection with the reproductive parameters, significant positive correlations were identified between rainfall and semen volume (R = 0.22) and ALH (R = 0.25), as well as between relative humidity and VSL (R = 0.20) and STR (R = 0.25). Again, negative correlations (P < 0.05) were found between air temperature and semen volume (R = 0.21) and BCF (R = -0.31).

Discussion

The Caatinga is a dry and seasonal Neotropical forest constituted by numerous areas suffering from anthropic disturbances or in desertification processes, which should be considered as high priority for its preservation and restoration (Sánchez-Azofeifa et al. 2005). In a 2012 study, Desbiez et al. have pointed out that although the population of collared peccaries in this biome is usually considered of least concern, in fact, there are no estimates of density and abundance of the species in there. In addition, these authors report that in this biome, the species is threatened by different factors such as alteration, deforestation and fragmentation of its habitat, excessive hunting, burning and mining. In this sense, the importance of the present research is reflected in the first long-term study evaluating the effects of a semiarid region on the reproductive parameters of the collared peccaries, contributing with fundamental information for the development of strategies for its *in situ* and *ex situ* conservation. In addition, this study serves as a basis for the reproductive follow-up of males of the species over the coming decades, in view of the estimated climatic changes for that biome (PBMC 2013).

Through an approach of multiple reproductive parameters such as the monitoring of semen quality, serum testosterone levels and testicular morphometry of the species, a profile of these characteristics and their pattern could be traced during the long dry period and the short rainy period, characteristic of the Caatinga biome. In this sense, we demonstrated that it is possible to obtain semen from the species by means of electroejaculation, independently of the rainfall index. Thus, we observed that the reproductive performance of the male collared peccary remains constant throughout the year, without being negatively influenced by the irregular rainfall of the biome. At first glance, this leads us to believe that these peccaries seem to be a specie well suited to the semiarid climate of the Caatinga. In fact, according to Zervanos (1975), the collared peccaries have the ability to maintain their surface temperature above ambient temperature, which would facilitate their loss of heat to the environment when at high temperatures. This information contradicts the statement of Albuquerque et al. (2012), according to which the wild mammals living in this biome do not have pronounced physiological adaptations to this region but exhibit behavioral adaptations that allow them to survive in this environment. In fact, Bissonete (1978) demonstrated that under high temperatures, the collared peccaries decrease their diurnal activities under the sun and usually present more activities, including feeding, from the end of the afternoon.

Currently, it seems that the meteorological characteristics of the Caatinga are not significant enough to promote alterations in the reproductive performance of male collared peccaries when different rainfall indexes are compared. In fact, under all rainfall conditions, the semen quality of the peccaries was constant and with values similar to those previously described for individuals raised in this same biome (Peixoto et al. 2012; Silva et al. 2013b, Souza et al. 2015). However, under meteorological conditions of a different biome, in the case of the Eastern Amazon with its semi-humid Equatorial climate, Kahwage et al. (2010) identified fluctuations in the semen parameters of the species throughout the year, evidencing a significant improvement of these parameters during periods of higher rainfall. It is necessary to point out that in the eastern Amazonia there is a total annual rainfall of 2,900 mm, ranging from 2,000 to 3,800 mm (Pachêco et al. 2011), well above the annual average of 765.8 mm observed in the Caatinga (Koppen 2006). It is emphasized that although the influence of the semi-humid climate of the Eastern Amazon on the reproduction of the male collared peccaries was identified, this influence was not of sufficient magnitude to indicate reproductive seasonality in these animals (Kahwage et al. 2010), which remain apt to perform activities through all the years as that observed in the Caatinga.

With regard to domestic species raised in semiarid regions, fluctuations in the reproductive parameters of small ruminants, particularly of exotic breeds introduced in there, have been reported. In Saanen goats, Van Tilburg et al. (2015) reported an increase in sperm production during the rainy period. The volume of semen remained unchanged between different periods, however, there was a reduction in sperm concentration, percentage of motile spermatozoa ($p < 0.05$) and increase in sperm morphological defects during the dry period (Van Tilburg et al. 2015). On the other hand, when evaluating goats natives of Caatinga, Aguiar et al. (2010) did not find differences regarding the sperm motility, vigor, volume and concentration between the dry and rainy periods of the region. It seems that in the native animals, changes in semen quality remain within the limits of the physiological measures to overcome the thermal stress, allowing the maintenance of their reproductive performance throughout the year (Marai et al. 2004), similar to verified for the collared peccaries of the present study. In fact, peccaries are rustic species apparently already acclimated to the semiarid region. This is mainly due to their already mentioned adaptive flexibility in their physiological parameters, which allows them to adjust to the seasonal changes in their environment, through adaptation in metabolic heat production, adjustments in evaporative and heat flow capacities, and changes in tolerance to extreme temperatures of the dry period (Zervanos 1975).

In parallel to the semen parameters, serum levels of testosterone from the collared peccaries remained constant against the different rainfall classes of the semiarid region in the Caatinga biome. As already mentioned, it is probable that the meteorological metrics of this biome do not vary sufficiently during the year to the point of affecting the reproduction of these males. These findings, however, differ from those reported for the same species when settled in Texas, USA, under arid climate conditions (Hellgren et al. 1989). In that region, the animals showed a seasonal trend in serum testosterone concentrations ($P < 0.05$), with an increase in values during the winter and a decrease in these values during the summer. It is worth noting, however, that there are discrepancies among researchers regarding the variations in testosterone concentrations in males of mammalian species, since some animals with seminal quality affected by environmental thermal stress may not present any hormonal alteration (Coelho et al. 2008). In addition, the constancy of the testosterone levels in the Caatinga biomes confirms their adaptability, since even under stressful conditions (high temperatures and very low relative humidity, typical of the dry season of the semiarid region), the hormonal production of these animals remained stable.

Similar to the sperm parameters and serum testosterone levels, the scrotal circumference and testicular morphometry of the collared peccaries located in the Caatinga biome were not influenced by the different rainfall indexes of the semiarid climate. These findings also differ from those reported for the collared peccaries settled in Texas, USA, under arid climate conditions (Hellgren et al. 1989). In these individuals, the estimated testicular volume and scrotal circumference presented significantly higher values during the rainier periods when compared to the records performed in the driest period, correlating directly with serum testosterone values. The authors suggested that this male remains reproductively fertile throughout the year, but may suffer an optional quiescence in the dry period, which may be influenced by the ambient temperature and social factors (Hellgren et al. 1989). In the Caatinga, a positive correlation of testosterone with scrotal circumference was observed in peccaries, as described by Hellgren et al. (1989), however, there were no fluctuations of these parameters along the different rainfall indexes, and no type of reproductive quiescence was identified, denoting its possibility of reproduction throughout the year in this biome.

Among the different classes of rainfall indexes evaluated in the present study, differences regarding the meteorological variables stand out, but above all the radiant heat load in the sun, are an important indicator of thermal comfort (Silva et al. 2010). In this sense, a lower RHL to the sun was verified in the rainiest months, resulting in a better thermal comfort for the animals, directly influencing the temperature of the animals. In the collared peccaries, the negative correlations observed between rectal temperature and important kinetic parameters of sperm motility show that high body temperatures could affect sperm quality. In fact, in different mammals such as goats (Nunes et al. 1997), cattle (De Rensis et al. 2003), sheep (Maia et al. 2011), and domestic pigs (Macchi et al. 2010), it has already been observed that thermal stress might decrease reproductive efficiency. Despite the meteorological influence, the collared peccaries located in the Caatinga biome seem to maintain their reproductive parameters within acceptable limits throughout the year.

This study demonstrate for the first time that conditions in meteorological a semiarid region have a short to medium term impact on the semen production in a model species like the peccary. While no seasonal changes could be detected overt the 18 month period, it is expected that long term environmental changes in a biome like the Caatinga will influence the reproductive physiology of species leaving in that habitat.

We demonstrated that not only the meteorological variables of the day of semen collection, but also those of the immediately previous days can influence the reproductive parameters of the collared peccaries. Over all, it has been shown that the meteorological variables seems to exert a significant influence on the sperm production of the individuals, since the relationships between the reproductive parameters and the meteorological conditions obtained between 51 and 55 days before collection of the semen were found. In collared peccaries, spermatogenesis lasts approximately 55.1 ± 0.7 days (Costa et al. 2004), a period very similar to spermatogenesis in pigs, which are extremely sensitive to high temperatures (Cameron and Blackshaw 1980). In mammals, in general, there is limited studies that relate different meteorological factors to spermatogenesis, and these studies mainly focus on the effect of temperature alone (Cameron and Blackshaw 1980; Yaeram et al. 2006; Shadmehr et al. 2018). In amphibians, however, it has recently been shown that the association of air temperature and rainfall may influence the success of the spermatogenesis process (Chaves et al. 2017), as observed in the collared peccary. In this sense, the unpublished information presented here will allow monitoring of the sperm production of the species during the future climate changes estimated for the Caatinga biome (PBMC 2013).

This study demonstrates for the first time that meteorological conditions in a semi-arid region have a short to medium term impact on the semen production in a model species like the peccary. While no seasonal changes could be detected overt the 18-month period, it is expected that long term environmental changes in a biome like the Caatinga will influence the reproductive physiology of species leaving in that habitat. Thus, the information generated here can be applied to the management of collared peccaries within the said biome, either in captivity or in free life, in order to develop strategies for its reproduction and conservation.

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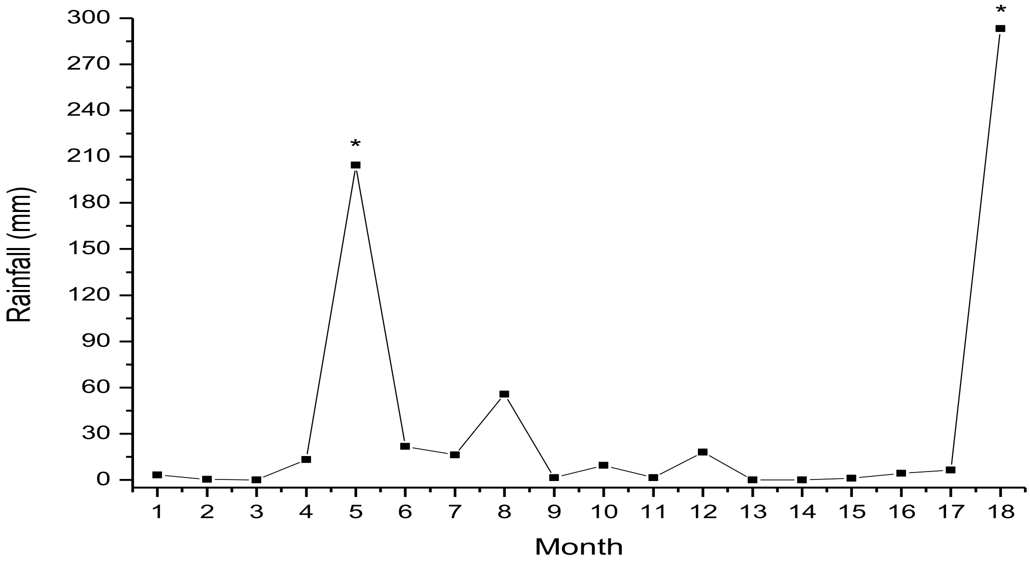


Figure 1. Cumulative rainfall index per month, from September 2015 (month 1) to February 2017 (month 18) in the Caatinga biome. Asterisks (*) evidence rainy peaks (*) in January 2016 (month 5) and February 2017.

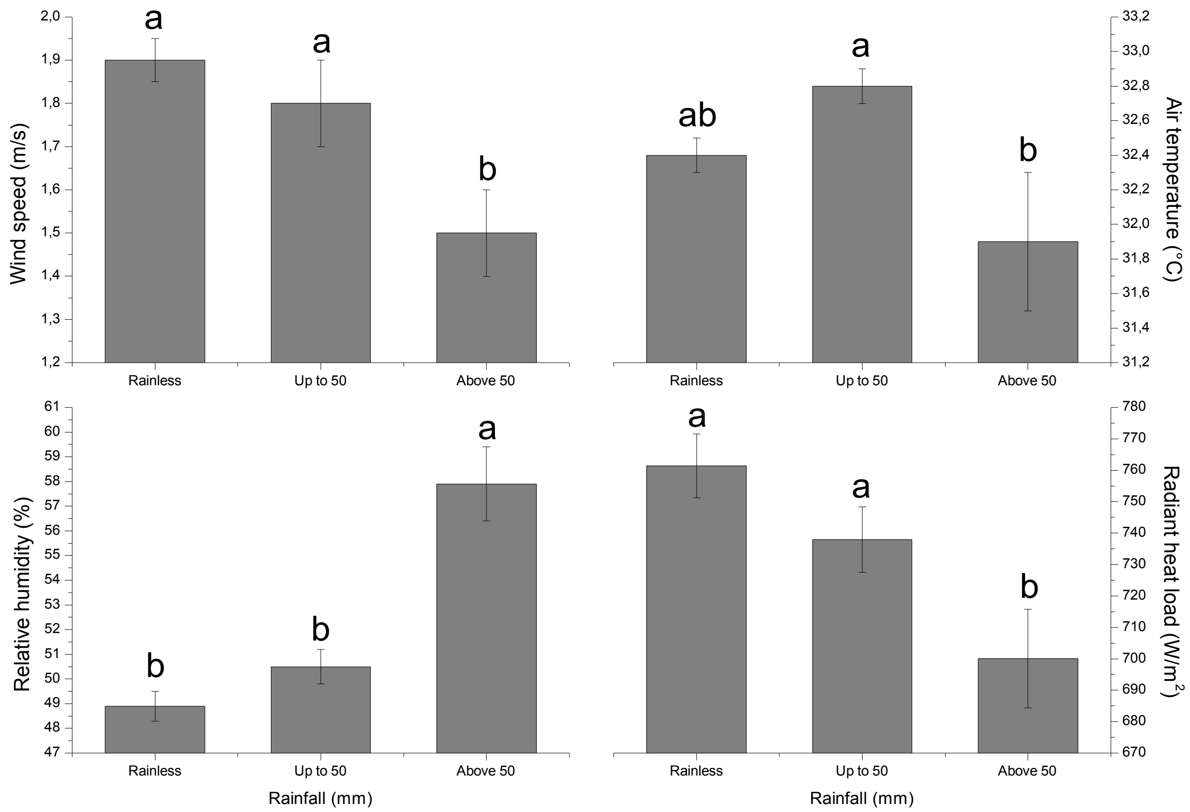
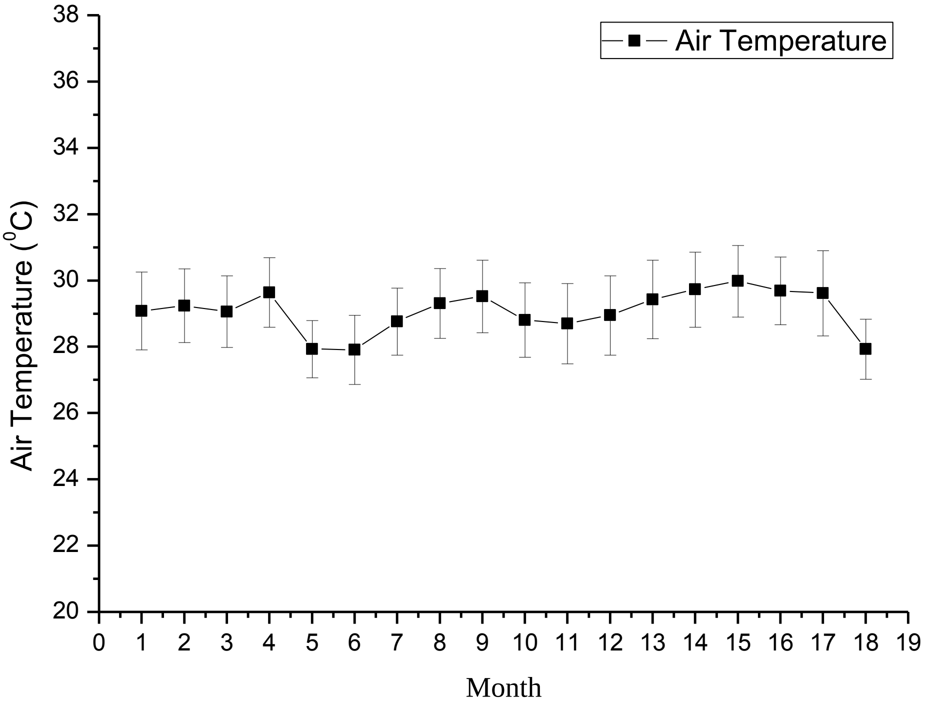


Figure 2. Mean values (± SEM) for the meteorological variables (air temperature, ° C, relative humidity, %, wind speed, m/s) and radiant heat load sun, (W / m^2) obtained in the day of collared peccaries' semen collection, from September 2015 to February 2017 under different rainy classes (class 1 – rainless; class 2 – up to 50; class 3 – above 50) in the Caatinga biome.

[a,b] Means with different letters differ statistically (p<0,05)



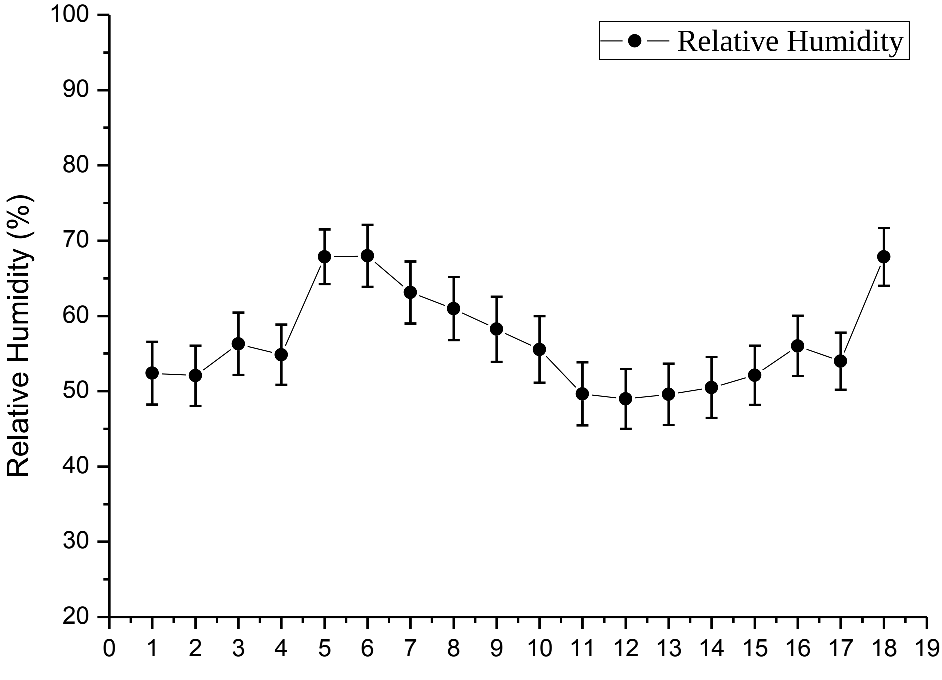


Figure 3. Mean values (± SEM) for the air temperature (° C) and relative humidity (%) obtained per month, from September 2015 (1) to February 2017 (18), in the Caatinga biome.

Table 1. Mean values (± SEM) for sperm parameters derived from collared peccaries (*Pecari tajacu*) raised in the Caatinga biome. Data obtained from September 2015 to February 2017 under different rainy classes (rainless, up to 50, and above 50).

Sperm parameters	**Classes***		
	Rainless	**Up to 50**	**Above 50**
Number of samples evaluated	39	46	16
Volume (mL)	3.1 ± 0.4	3.72 ± 0.6	3.5 ± 0.8
Concentration (x10^6 sperm/mL)	339.8 ± 53.3	280.9 ± 28.2	226.3 ± 55.0
Sperm/ejaculate (x10^6)	817.6 ± 149.8	912.1 ± 140.0	742.96 ± 212.1
Normal morphology (%)	86.6 ± 1.4	86.3 ± 1.1	91.2 ± 1.3
Membrane funtionality (%)	78.3 ± 1.6	76.9 ± 2.2	80.2 ± 3.1
Membrane integrity (%)	78.5 ± 2.0	79.9 ± 2.0	76.7 ± 2.6

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

Table 2. Mean values (±SEM) for kinematic parameters of sperm motility evaluated by computerized analysis of semen derived from collared peccaries (*Pecari tajacu*) raised in the Caatinga biome. Data obtained from September 2015 to February 2017 under different rainy classes (rainless, up to 50, and above 50).

Sperm parameters	**Classes***		
	Rainless	**Up to 50**	**Above 50**
Number of samples evaluated	46	56	18
Total motility (%)	80.9 ± 2.8	81.4 ±2.3	74.1 ± 5.9
Velocity average pathway (µm/s)	47.5 ± 1.7	50.7 ± 1.9	49.8 ± 2.7
Velocity straight line (µm/s)	26.8 ± 1.09	30.4 ± 1.5	29.2 ± 1.8
Velocity curvilinear (µm/s)	105.7 ± 3.1	110.1 ± 3.1	105.1 ± 4.6
Amplitude lateral head (µm)	6.5 ± 0.1	6.6 ± 0.1	6.7 ± 0.2
Beat cross frequency (Hz)	35.5 ± 0.7	36.1 ± 0.7	33.9 ± 1.1
Straightness (%)	55.3 ± 1.3	57.2 ± 1.0	55.7 ± 1.4
Linearity (%)	27.1 ± 1.0	30.0 ± 0.7	27.6 ± 0.7
Subpopulations			
Rapid (%)	59.0 ± 3.4	61.4 ± 2.9	54.9 ± 5.8
Medium (%)	16 ± 1.7	14.6 ± 1.1	13.7 ± 1.8
Slow (%)	5.9 ± 0.5	5.6 ± 0.4	5.3 ± 0.6
Static (%)	19 ± 2.5	18.5 ± 2.1	25.9 ± 5.6

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

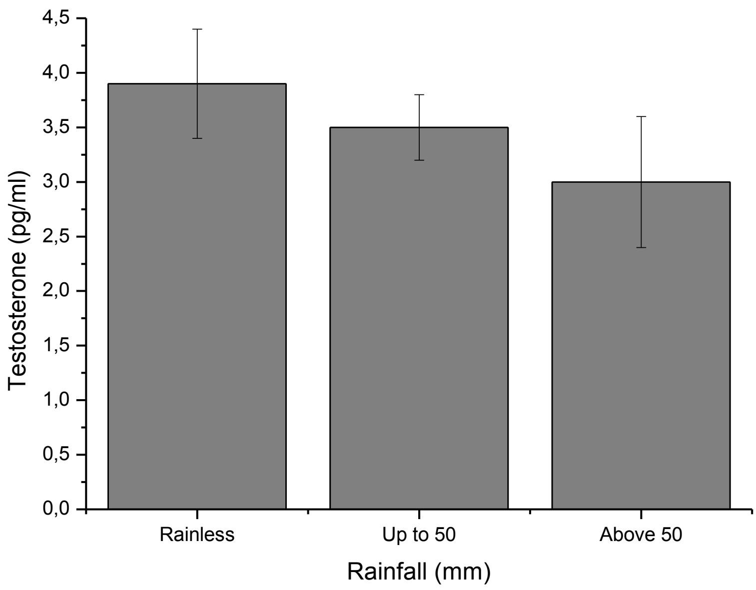


Figure 4. Mean values (± SEM) for serum testosterone in collared peccaries (*Pecari tajacu*) raised in the Caatinga biome. Data obtained from September 2015 to February 2017 under different rainy classes (rainless – n=39 samples; up to 50 – n=46 samples; above 50 – n=16 samples), P > 0.05.

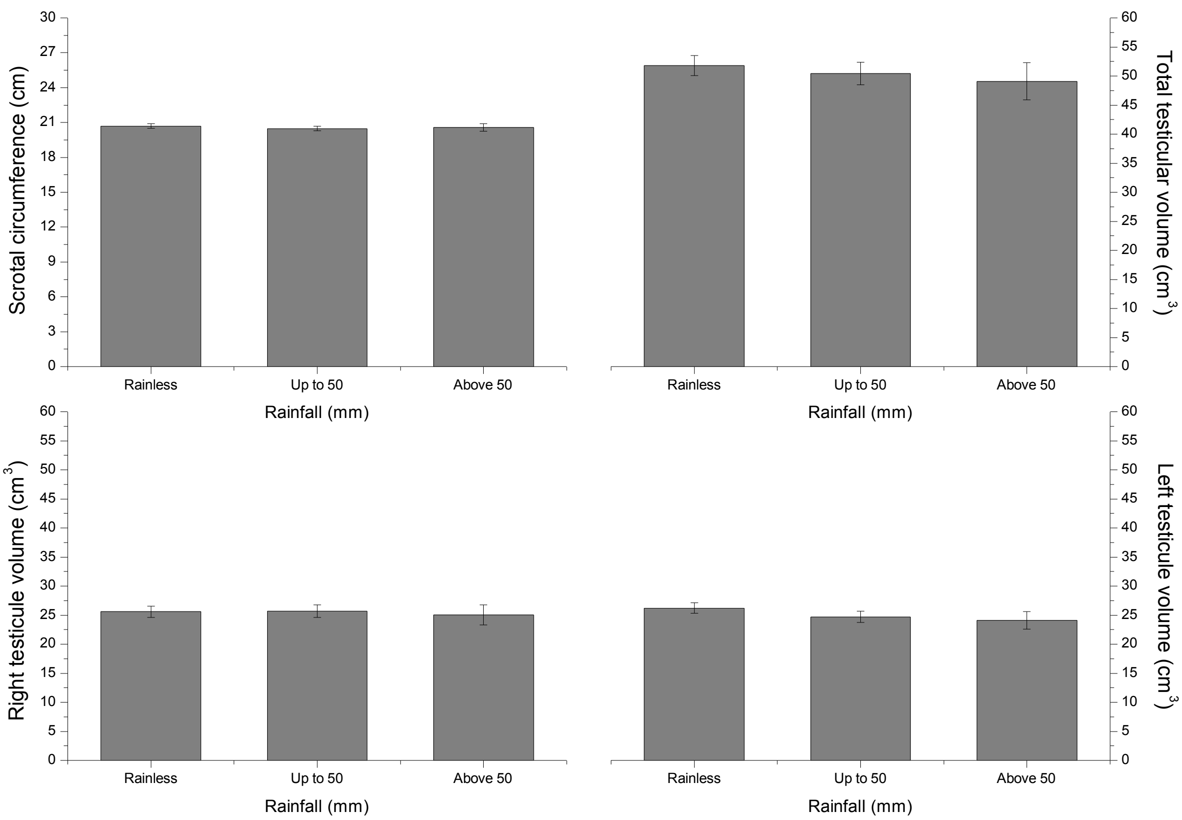


Figure 5. Mean values (± SEM) for scrotal circumference (cm) and testicular morphometry (cm^3) in collared peccaries (*Pecari tajacu*) raised in the Caatinga biome. Data obtained from September 2015 to February 2017 under different rainy classes (rainless – n=39 samples; up to 50 – n=52 samples; above 50 – n=19 samples).

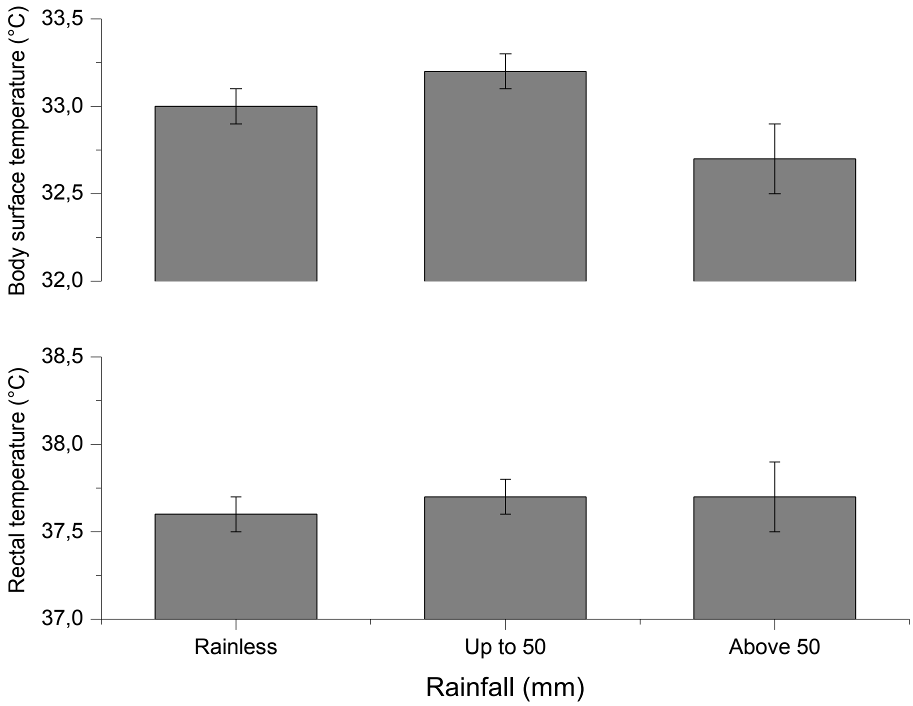


Figure 6. Mean values (± SEM) for the rectal and surface temperatures (°C) from collared peccaries (*Pecari tajacu*) raised in the Caatinga biome. Data obtained from September 2015 to February 2017 under different rainy classes (class 1 – rainless; class 2 – up to 50; class 3 – above 50), $P > 0.05$.

Environmental factors related to a semiarid climate influence the freezability of sperm from collared peccaries (*Pecari tajacu* Linnaeus, 1758)

(Fatores ambientais relacionados a um clima semiárido influenciam a congelabilidade de espermatozoides de cateto (*Pecari tajacu* Linnaeus, 1758))

Keilla M. Maia, Ana L. P. Souza, Erica C. G. Praxedes, Luana G. P. Bezerra, Andreia M. Silva, Livia B. Campos, Samara S. J. Moreira, Carlos A.C. Apolinário, Alexandre R. Silva

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Qualis Capes B1

Environmental factors related to a semiarid climate influence the freezability of sperm from collared peccaries (*Pecari tajacu* Linnaeus, 1758)

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Abstract

The influence of environmental factors in a semiarid climate on characteristics of fresh and frozen-thawed sperm collected from collared peccaries (*Pecari tajacu*) was assessed. Semen from 11 male collared peccaries was collected by electroejaculation during the peaks of the dry and rainy periods while rainfall indices, air temperatures, relative humidity levels, and wind speeds were measured. The number, motility, morphology, osmotic response, and membrane integrity of sperm in the collected ejaculates were assessed. Samples were then frozen in liquid nitrogen, thawed, and reassessed. The rainfall index of the rainy period (73.2 mm) was significantly higher than that of the dry period (13.6 mm) and the relative humidity was significantly higher during the rainy period (74.6%) than it was during the dry period (66.8%). Air temperature and wind speed did not differ between the two periods. Characteristics of sperm in the fresh samples were not affected by environmental parameters. In contrast, computerized analysis revealed that sperm in samples frozen during the rainy period exhibited better post-thaw membrane integrity (28.6 ± 6%), motility (29.5 ± 7.7%) and rapid sperm population (13.7 ± 6.2%) than did sperm in samples frozen during the dry period (23.4 ± 3% membrane integrity, 14.6 ± 4.1% motility and 4.1 ± 1.2 % rapid sperm; P <0.05). Other characteristics of the frozen-thawed sperm did not differ depending on the period in which they were collected. We demonstrated that environmental parameters did not affect the quality of fresh sperm, but could influence the freezability of sperm collected from collared peccaries raised under a semi-arid climate.

Keywords: *Tayassu tajacu*; Wildlife; Caatinga; Biobank; Semen.

Introduction

The collared peccary (*Pecari tajacu)* is a species of wild pig native to the Americas.[1] Although the International Union for the Conservation of Nature (IUCN) classifies the collared peccary as a least concern (LC) species,[2] poaching and deforestation are reducing populations in some biomes.[3] One such biome is the Caatinga, which is characterized by a semiarid climate, irregular rainfall, and high average annual temperatures.[4]

Seasonal changes in temperature can influence the metabolisms[5] and activities of peccaries.[6] Thus, temperature and other environmental factors might also influence the reproductive traits of peccaries, such as sperm quality, as has been shown to occur for other mammals living in the Caatinga.[7,8] Environmental factors have also been shown to affect the freezability of the sperm of some wild species, such as the Iberian ibex (*Capra pyrenaica*).[9] However, how the environment influences the freezability of sperm from collared peccaries remains unknown.

To gather information that will contribute to the establishment of an efficient biobank, the current study aimed to evaluate the influence of certain environmental parameters of Caatinga's semiarid climate on the characteristics of fresh and frozen-thawed sperm collected from collared peccaries.

Material and Methods

Animals

The Ethics Committee of Animal Use of the Federal Rural University of Semi-Arid (CEUA/UFERSA; no. 23091.008820/2016-03) approved this study. Sperm samples were taken from 11 male collared peccaries that were 40.7 ± 1.6 months old and weighed 22.5 ± 2.8 kg. The animals belonged to the Centre of Multiplication of Wild Animals (CEMAS/UFERSA), located in Mossoró, RN, Brazil (5º10 = S, 37º10 = W). The climate there is typically semiarid, with an average annual temperature of 27 °C. The animals were maintained under a 12-h natural photoperiod in groups of six in paddocks (20 × 3 m) with a covered area of 3 × 3 m. Animals were fed pig food and fruits and were provided with water *ad libitum*.

Evaluation of environmental parameters

The experiment presented here was conducted during the peaks of the dry period (October – December, 2015) and rainy period (March – May, 2016). The rainfall index (mm^3) for each period was measured automatically by a station of the National Meteorological Institute (INMET).[10] Air temperature, relative humidity, and wind speed were measured with a thermo-hygrometer-anemometer (INSTRUTERM-HT300, São Paulo, Brazil).[11] These parameters were measured every hour, from 6 AM to 6 PM, on days when sperm was collected.

Sperm collection and evaluation

During the peaks of the two climatic periods, semen was collected from each male once per month for three months. For sperm collection, animals were anesthetized with propofol (5 mg/kg IV; Propovan, Cristalia, Fortaleza, Brazil) after fasting for 12 h. Semen was then collected into plastic graduated tubes by electroejaculation as previously described for the species.[12] Sperm concentration was measured with a Neubauer chamber and the total number of sperm was calculated.[13] Sperm morphology was assessed by analyzing a smear of 100 cells stained with bromophenol blue under light microscopy (×1000).[13] Sperm membrane functionality was determined by measuring response to hypoosmotic shock induced with distilled water (0 mOsm/L).[14] Membrane integrity was assessed via fluorescence microscopy using carboxyfluorescein diacetate (CFDA) and propidium iodide (PI) as probes.[15] Sperm kinetic motility patterns were analyzed by a computer-aided semen analysis (CASA) system (IVOS 7.4G, Hamilton-Thorne Research, Beverly, MA, USA) using configurations determined for the species.[15]

Sperm freezing and thawing

Semen samples were diluted at 27 °C in a Tris extender supplemented with 20% Aloe vera, incubated for 40 min to reach 15 °C, and then incubated again for 30 mins at 5 °C. Glycerol was then added to the samples to a final concentration of 3% (resulting in samples containing 100 x 10^6 sperm/mL) and the samples were packed in 0.25-mL straws. The samples were frozen via exposure to nitrogen (N_2) vapors for 5 min and then plunged into liquid N_2 for storage. After two weeks, the samples were thawed by bathing in water at 37 °C for 1 min[15] and their sperm were analyzed in the same manner as were the fresh sperm.[15]

Statistical analysis

Data (Mean ± SE) were tested for normality with the Shapiro-Wilk test and asymmetries and kurtosis were assessed with the univariate procedure provided by the Statistical Analysis System application (SAS 8.0, SAS Institute Inc., Cary, NC, USA). When necessary, data were transformed by log (x + 1) or arc sine (√ (x / 100)). Effects of individuals and periods (dry and rainy) on semen parameters were evaluated by an analysis of variance at using a general linear model (Proc GLM; SAS). Comparison of means was conducted by Student's t tests ($P \leq 0.05$). Efficiency of semen collections during dry and rainy periods was evaluated by Chi-square test ($P \leq 0.05$).

Results

The total rainfall index measured during the peak of the dry period (13.6 mm) was significantly lower than that measured during the rainy period (73.2 mm) ($P <0.05$). Values for environmental parameters that were measured during the experiment are reported in Table 1. The relative humidity during the rainy period was higher than that during the dry period ($P < 0.05$).

There was no effect of climatic period on the efficiency for semen collection ($P > 0.05$). All the males provided at least one ejaculate per period. During dry period, 14 semen samples were obtained from 33 attempts (14/33; 42.4%); three males provided two ejaculates while the other eight males ejaculate only once. During the rainy period, from the 33 attempts conducted, 11 ejaculates were collected (11/33; 33.3%), being one ejaculate provided per male.

Fresh sperm collected during the dry period were not found to differ from fresh sperm collected during the rainy period (Tables 2 and 3). In contrast, semen cryopreserved during rainy period presented more intact membrane than that frozen during dry period (Table 2). Moreover, CASA (Table 3) revealed that cryopreserved sperm collected during the rainy period were more motile and rapid than were cryopreserved sperm collected during the dry period ($P < 0.05$).

Discussion

There is growing concern that genetic materials from valuable wild species should be safeguarded in biobanks. Doing so will require more knowledge about basic aspects of reproductive biology and cryobiology.[16] Because of the ecological and economic importance of collared peccaries, recent studies have focused on developing protocols for preserving their reproductive[15] and somatic cells.[17] In addition to helping to preserve collared peccaries, advances in these protocols could be extrapolated to help preserve other related species, like the vulnerable white-lipped peccary (*Tayassu pecari* Link, 1795) and the endangered Chacoan peccary (*Catagonus wagneri* Rusconi, 1930).[2] The results of the present study contribute to the development of a protocol for efficient biobanking by demonstrating that sperm from peccaries raised under semiarid conditions would be cryopreserved better if collected during the rainy period, even though the quality of their fresh sperm is not affected by environmental factors.

We demonstrated that it is possible to obtain semen from peccaries raised in a semiarid region independently from the climatic period. However, not all individuals provided samples in all the attempts for semen collection. This is probably a consequence of an individual variation related to the electroejaculatory procedure observed in the species, as previously reported by Peixoto et al.[13]

Differences in the rainfall indexes and relative humidity levels of the rainy and dry periods did not affect the number of sperm produced by peccaries. This finding contrasts with reports that goats raised in the same semiarid region produced more sperm during rainy periods than they did during dry periods.[8] Seminal plasma from those animals showed higher concentrations of lysine-specific demethylase 5D (KDM5D), a protein that is a key determinant of spermatogenesis,[18] during the rainy period than it did during the dry period.[8] However, this protein was not identified in the proteome of seminal plasma from peccaries.[19]

The characteristics of the fresh sperm collected from collared peccaries were not affected by semiarid weather parameters. This finding contrasts with how climate-related environmental parameters have been observed to affect the reproductive characteristics of peccaries living in tropical[20] and semi-humid equatorial[21] climates. In those climates, changes in rainfall index that occur at the peaks of summer and winter correlate with significant changes in ambient temperature that influence the reproductive characteristics of peccaries, including the quality of their sperm. Under a semiarid climate, however, the temperature remains constant across dry and rainy periods and differences in rainfall index and humidity seem to be insufficient to affect the quality of peccaries' fresh sperm. The absence of such effect reflects that collared peccaries are robust and well-adapted to a semiarid climate of the Caatinga such that the species maintains good reproductive performance throughout the entire year.[22]

Interestingly, sperm collected from collared peccaries during the rainy period exhibited better freezability than did sperm collected during the dry period, as reflected by the higher membrane integrity, motility and rapid subpopulation. Many authors have pointed out that sperm motility is commonly measured to gauge "sperm quality" because it indirectly indicates metabolic activity and sperm membrane integrity.[23,24] In this sense, Campos *et al.*[25] recently demonstrated that sperm motility (r = 63.6%, P <0.0) and membrane integrity (r = 65.1%; P = 0.03) are highly related to the ability of a peccary's sperm to bind to the zona pellucida of heterologous substrates.

The present findings suggest that a protective factor might exist that is conferred to sperm collected from collared peccaries during the rainy period. In sheep, it is hypothesized that the composition of the seminal plasma may exert a protective effect on sperm during cryopreservation via the presence of specific proteins.[26, 27] In domestic swine, the presence of proteins with molecular masses of 18, 19, 44, 65, 80, and 100 kDa in seminal plasma was found to be a potential marker for the tolerance of their sperm for cryopreservation.[28]

Echoing the findings reported here for sperm from collared peccaries, Aguiar *et al.*[7] recently demonstrated that the freezability of sperm from goats bred under a semiarid climate is negatively affected by the dry period. According to the authors, the biochemical components of the goats' seminal plasmas varied across climatic periods, suggesting that the presence of a greater concentration of the enzyme phospholipase A2 in their seminal fluids during the dry period compared to during the rainy period might be responsible for reducing the viability of their sperm. A study of peccaries' proteome revealed that phospholipase A2 was not identified,[19] but proteins involved in spermatic protection, such as clusterin and T-complex protein 1, and proteins with antioxidant activities, like glutathione peroxidase (GPx-5), ceruloplasmin, albumin, and transferrin, were identified in their seminal plasmas. Whether the concentrations or activities of these or other proteins vary across different climatic periods and whether such variation could influence the efficiency of cryopreservation of sperm from peccaries is not known.

In conclusion, the environmental parameters measured in this study did not affect the quality of fresh sperm collected from collared peccaries, but did influence the freezability of sperm collected from collared peccaries raised in a semi-arid climate. Therefore, the results of this study suggest that collecting semen from collared peccaries living in a semiarid climate during its rainy periods will benefit efforts to biobank their sperm.

Author Disclosure Statement

No conflicting financial interests exist.

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Table 1. Mean values (minimun and maximun) for the environmental parameters verified during dry (october, november and december 2015) and rainy (march, april and may 2016) periods in Caatinga's semiarid climate

Period	Wind velocity (m/s)		Air temperature (°C)		Relative humidity (%)	
	Minimum	Maximum	Minimum	Maximum	Minimum	Maximum
Dry	0.4	4	27.1	36	34	66.8 [b]
Rain	0.06	2.9	26.7	37.2	42.2	74.6 [a]

[a, b] Means without common superscript letters, differ within a row ($p < 0.05$).

Table 2. Values (mean± S.E.M.) for semen characteristics of fresh and frozen-thawed samples derived from collared peccaries (*Pecari tajacu*) bred under semiarid conditions, and collected by electroejaculation during the dry (October, November and December 2015; n = 14) and rainy (March, April and May 2016; n = 11) periods of the Caatinga's semiarid climate

Sperm Variables	**Fresh semen**		**Thawed semen**	
	Dry period	**Rainy period**	**Dry period**	**Rainy period**
Sperm/ejaculate	1336.5 ± 296.8	1888.6 ± 590.5	-	-
Membrane integrity (%)	84.4 ± 2.4	74.4 ± 7.6	23.4 ± 3 [b]	28.6 ± 6 [a]
Osmotic response (%)	79 ± 4.5	78.6 ± 6.3	23.6 ± 3	24.3 ± 4.3
Normal morphology (%)	86.9 ± 2.5	86.8 ± 3.2	76.8 ± 1.2	79 ± 2.1

[a,b] superscripts mean differences between semen collected in different seasons.

Table 3. Values (means _ standard error of the mean) for kinetic parameters of sperm motility of fresh and thawed semen derived from collared peccaries (Pecari tajacu) bred under Caatinga's semiarid climate during dry (October, November and December 2015; n = 14) and rainy (March, April and May 2016; n = 11) periods.

Sperm parameters	**Fresh semen**		**Thawed semen**	
	Dry period	**Rainy period**	**Dry season**	**Rainy period**
Total motility (%)	78.6 ± 4.4	85.7 ± 3.3	14.6 ± 4.1[b]	29.5 ± 7.7[a]
Velocity average pathway (mm/s)	42.6 ± 2.6	49.5 ± 3.5	22.5 ± 1.6	24.3 ± 1.4
Velocity straight line (µm/s)	27.1 ± 2.2	32.6 ± 2.8	13.5 ± 1.1	16.2 ± 1.4
Velocity curvilinear (µm/s)	100 ± 4.4	106.7 ± 5.9	58.9 ± 4	59.2± 3
Amplitude lateral head (µm)	5.9 ± 0.2	6.2 ± 0.2	5.2 ± 0.5	5.5 ± 0.7
Beat cross frequency (Hz)	40.1 ± 1.2	36.8 ± 1.2	38.2 ± 0.8	35 .8± 1.7
Straightness (%)	62.7 ± 2	64.4 ± 1.3	64.8 ± 1.9	65.7 ± 3.3
Linearity (%)	30 ± 1.6	31.7 ± 1.3	31.5 ± 1.4	33.2 ± 3.7
Subpopulations				
Rapid (%)	54.7 ± 5.4	68.1 ± 6	4.1 ± 1.2 [b]	13.7 ± 6.2 [a]
Medium (%)	21.1 ± 2.5	17.8 ± 3.2	14.2 ± 4.2	15.8 ± 3
Slow (%)	5.7 ± 0.7	3.8 ± 0.6	6.6 ± 1.4	6.4 ± 1.2
Static (%)	18.5 ± 4.2	10.3 ± 2.9	75.1 ± 6.3	64.1 ± 8

[a,b] superscripts mean differences between semen collected in different seasons.

10 CONCLUSÕES GERAIS

1. Os catetos não apresentam estacionalidade reprodutiva sob influência das variáveis meteorológicas da região semiárida do bioma Caatinga.

2. As correlações entre as variáveis meteorológicas e parâmetros reprodutivos indicam a existência de influência dos primeiros sobre os últimos, no entanto, ao momento, estas não são marcantes o suficiente para causar alterações no desempenho reprodutivo dos catetos.

3. As variáveis meteorológicas influenciaram o processo de criopreservação de sêmen de cateto, com melhor resultado pós descongelação no sêmen obtido durante o período chuvoso. Portanto, o sêmen de catetos coletado no período chuvoso na região semiárida é o mais indicado para ser submetido a criopreservação;

11 CONSIDERAÇÕES E PERSPECTIVAS

O trabalho realizado, através de uma abordagem de múltiplos parâmetros reprodutivos, como o monitoramento da qualidade seminal, dos níveis de testosterona sérica e da morfometria testicular da espécie, foi importante para traçar um perfil destas características e de seu padrão no decorrer do longo período seco e do curto período chuvoso, característicos do bioma Caatinga.

Além disso, contribuiu com informações importantes a respeito das variáveis meteorológicas as quais os catetos estão submetidos, não só no dia da coleta de sêmen, mas também aquelas dos dias imediatamente prévios, que podem vir a influenciar os parâmetros reprodutivos dos catetos, uma vez que, muitas correlações entre as variáveis meteorológicas e os parâmetros reprodutivos foram verificadas, salientando que futuras modificações nos primeiros podem vir a afetar o desempenho reprodutivo da espécie.

Ainda, este estudo serve como base para o acompanhamento reprodutivo dos machos da espécie ao longo das próximas décadas, em vista das modificações climáticas estimadas para o referido bioma. Desse modo, as informações aqui geradas podem ser aplicadas ao manejo de catetos, seja em cativeiro ou em vida livre, no intuito de desenvolver estratégias para sua reprodução e conservação.

12 ANEXOS

I. Resumo: Seasonal variation of electroejaculatory response and semen parameters of collared peccaries (*Pecari tajacu*) bred under semiarid conditions

II. Resumo: Influência dos parâmetros ambientais sobre a cinética espermática de catetos (*Pecari tajacu*) criados no semiárido nordestino brasileiro

III. Carta de submissão: Biodiversity and Conservation

IV. Carta de submissão: Biopreservation and Biobanking

Seasonal variation of electroejaculatory response and semen parameters of collared peccaries (*Pecari tajacu*) bred under semiarid conditions

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It is known that the reproductive activity of various mammals can be affected by several factors such as the climate and its changes, which are decisive for the semen quality in several species. In semiarid regions, animals suffer the effect of high temperatures, especially during dry season. This research was conducted in order to obtain information regarding a possible influence of seasonality on semen collection efficiency and sperm kinetic parameters in collared peccaries (*Pecari tajacu*) bred under semiarid conditions. These are preliminary data obtained from September 2015 to June 2016. The experiment was conducted in the Centre for Multiplication of Wild Animals/UFERSA, located in Mossoró, RN, in the Northeast Brazil, under the following geographical coordinates 5º10'S, 37º10'W. Eleven sexually matured male were monthly monitored. The animals were isolated from females 3 months before the commencement of the study and kept under a 12 h natural photoperiod. They were maintained outdoors in groups of five and six animals in paddocks (20m×30m) with a covered area of (3m×3m). The animals were fed with sow food and seasonal fruits. Water was freely available. For occasion o semen collection, they were anesthetized with propofol (5 mg/kg; Propovan®, Cristália, Fortaleza, Brazil) and the semen was collected with the aid of an electroejaculator (Autojac®, Neovet, Campinas, SP, Brasil) connected to a source of 12 V using a rectal probe. Semen was collected in graduated plastic tubes and immediately evaluated for the kinetic parameters of sperm motility by a computer assisted semen analysis (CASA IVOS 12.0, Hamilton-Thorne, Beverly, USA). Environmental parameters such as ambient temperature (°C), relative humidity (%) and rainfall were obtained by an automated meteorological station from the National Weather Institute – INMET. The efficiency of semen collection per month was expressed as percentage and analyzed by the Fisher's exact test. Effect of monthly seasonal variation on sperm parameters was checked by two way-ANOVA for repeated followed by the Tukey test ($P < 0.05$). The efficiency of semen collection per month was 81.8% in September, 63.6% in October, 72.3% in November, 63.4 %, in December, 36.4%, in January, 90.9% in February, 72,3% in March, 54.5% in April, 72.3 %, in May and 54.5 %, in June. In February we verified an increase on such efficiency, when compared to January ($P<0.05$), highlighting that it is positively influenced by beginning of the rainy season. Moreover, January presented the most unsatisfactory values for efficiency of semen collection, probably because of the previous long dry season. In fact, from September to November 2015, no rainfall was detected in the region. In December, rainfall values of 13.2 mm were determined, reaching 204.6 mm in January, and 21.8 mm in February. Moreover, significant variations were detected concerning temperature and relative humidity from December (28.2 °C, 65.9 %) to January (27.3 °C, 79.1%) and February (27.9°C, 74.9 %). We believe that the positive effect of rainfall was only verified on the semen collection in February due to the spermatic cycle that lasts 55.1 ± 0.7 days in peccaries. In addition, few variations were verified regarding sperm kinetic parameters, and only a difference on values for velocity straight line (VSL) was found between February (32.8 ± 2.6 µm/s) and June (32.8 ± 3.0 µm/s) when compared to November (19.7 ± 2.6 µm/s). In conclusion, these preliminary results suggest that seasonality can influence the efficiency for semen collection, but not the quality of sperm kinetic parameters in collared peccaries raised under semiarid conditions. These are valuable information to be used for the management of peccaries destined to reproduction, which are not indicated to be used for reproductive assisted techniques during and immediately after the dry season peak.

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Influência dos parâmetros ambientais sobre a cinética espermática de catetos (*Pecari tajacu*) criados no semiárido nordestino brasileiro

Influence of environmental parameters on the sperm kinetics of collared peccaries (Pecari tajacu) bred in Brazilian Northeast semiarid

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Na Caatinga, um dos principais habitats naturais para os catetos (*Pecari tajacu*), predomina o clima semiárido caracterizado por uma longa estação seca e um curto período chuvoso, onde a temperatura é fator determinante do ritmo de vida, limitando a eficiência reprodutiva dos animais que ali habitam. Nesse contexto, objetivou-se avaliar a existência de um possível efeito dos parâmetros ambientais sobre a cinética espermática do sêmen fresco e descongelado de catetos criados no bioma Caatinga. Foram utilizados os ejaculados de cinco catetos coletados por eletroejaculação em dois períodos distintos: o pico do período seco (outubro – dezembro/ 2015) e o pico do período chuvoso (Março – Maio/ 2016). As amostras foram avaliadas a fresco quanto aos parâmetros cinéticos da motilidade por meio de análise computadorizada (Hamilton Thorne IVOS 5.0, Beverly, MA, EUA). Em seguida, foram diluídas em Tris com *Aloe vera* 20% e glicerol 3%, congeladas a -196 $_{\circ}$C, descongeladas após uma semana em banho maria (37$_{\circ}$C/1min) e reavaliadas. As variáveis ambientais observadas foram: temperatura do ar, umidade relativa e índice pluviométrico, obtidas por meio de um termo-higrômetroanemômetro (INSTRUTEMP-HT300, São Paulo, Brasil). Os dados foram expressos em média ± erro padrão, sendo comparados pela ANOVA seguida do teste t de Student ($P < 0,05$), e por suas assimetrias e curtoses, por meio do procedimento UNIVARIATE (SAS 8.0, SAS Institute Inc., Cary, NC, EUA), ($P < 0,05$). Não foi verificada influência do período chuvoso ou seco no que se refere ao sêmen fresco quanto a nenhum dos parâmetros cinéticos, tendo sido verificada motilidade total de 86,2±4.7% e 79±7,3%, respectivamente. Por outro lado, após a descongelação, verificou-se uma melhor qualidade do ejaculado criopreservado quando coletado no período chuvoso com motilidade total de 34.1±9.7%, do que no período seco, 20.1±5.4% ($P > 0,05$). Além disso, os dados relativos a amplitude lateral da cabeça – ALH (µm), frequência de batimento flagelar cruzado – BCF (Hz) e índice de linearidade – LIN (%) foram significativamente melhores quando a coleta foi realizada no período chuvoso ($P < 0,05$). Necessário salientar que não se observou diferença quanto a temperatura do ar, cuja máxima foi de 36 ºC no período seco e 37,2 °C no período chuvoso. Ainda, a precipitação pluviométrica foi abaixo da média tanto para o período seco (13,6 mm^3) como para o chuvoso (73,2 mm^3) ($P > 0,05$), tendo sido observada diferença significativa apenas entre a umidade máxima observada no período seco, 66.8% e período chuvoso de 74.6% ($P < 0,05$). É possível que a influência da estação seca ou chuvosa apenas tenha se manifestado após a descongelação devido a alterações específicas nas proteínas do plasma seminal ou da membrana espermática, as quais são suscetíveis à influência estacional, conforme já demonstrado em suínos domésticos, e as quais poderiam ter efeito direto sobre a congelabilidade do sêmen desses animais. Com base nos resultados, sugere-se que, para um melhor manejo dos catetos em cativeiro, o sêmen destes animais seja coletado com fins de criopreservação, preferencialmente, durante o período chuvoso no bioma Caatinga.

Palavras-chave: criopreservação, temperatura do ar, umidade relativa, índice pluviométrico.
Keywords*: cryopreservation, air temperature, relative humidity, rainfall index.*
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