

Morphologic analysis of sperm from two neotropical primate species: comparisons between the squirrel monkeys *Saimiri collinsi* and *Saimiri vanzolinii*

Wlaysia V. Sampaio^{2,5}, Karol G. Oliveira^{2,3}, Danuza L. Leão³, Maria C. Caldas-Bussiere⁴, Helder L. Queiroz⁵, Fernanda P. Paim⁵, Regiane R. Santos¹ and Sheyla F.S. Domingues²

Faculty of Veterinary Medicine, Federal University of Pará, Castanhal, Pará; Institute of Sustainable Development Mamirauá, Tefé, Amazonas; National Primate Center, Ananindeua, Pará; and State University of Norte Fluminense 'Darcy Ribeiro', Campos dos Goytacazes, Rio de Janeiro, Brazil

Date submitted: 13.08.2016. Date accepted: 11.10.2016

Summary

Sperm morphometry can be applied to identify different animal groups and species and to evaluate sperm quality. Furthermore, knowledge on species-specific differences will help to enhance biological information, as well as to develop efficient reproductive technologies. The aims in the present study were to describe sperm morphometry from the recently characterized species *S. collinsi* and *S. vanzolinii*, to verify if the morphometric sperm patterns are similar or different between both species, and to determine if the sperm morphometry is affected by the levels of sperm defects using the *S. collinsi* as a model. Semen was collected from *S. collinsi* ($n = 10$) and *S. vanzolinii* ($n = 2$) monkeys, and sperm was submitted to morphological analysis. From the 10 samples from *S. collinsi*, five presented sperm of poor quality and two subgroups were formed for this species, i.e. high and poor quality sperm. Data on sperm motility and vigour were analysed, as well morphometric parameters on sperm head and tail. It was observed the normal morphometry was correlated with high quality sperm. Poor quality sperm presented smaller and 7% more ellipticity in their head, when compared with high quality sperm. Sperm from *S. vanzolinii* presented larger head than those from *S. collinsi*, but tail lengths were similar. Sperm morphometry can be used as a complementary tool to predict sperm motility and vigour for the *S. collinsi* species, and *S. collinsi* appear as a suitable model for *S. vanzolinii*.

Keywords: Morphological defects, Non-human primates, Sperm morphometry

Introduction

Morphometric analysis is a practical tool to measure male gametes and to identify changes within a

determined animal group (Micklethorn & Sanderson, 2001). This procedure can be used to rapidly determine sperm quality based on gold standard measurements (Valle *et al.*, 2012) and to compare sperm morphology from different species (Phetudomsinsuk *et al.*, 2008; Steinberg *et al.*, 2009).

Several studies have reported species-specific variation in the form and size of sperm (Gage & Freckleton, 2003). These findings are supportive for taxonomic and phylogenetic studies, as well as for understanding reproductive evolution (García *et al.*, 2003). Furthermore, such knowledge will support the development of assisted reproductive techniques to preserve genetic material from endangered species (Andrabi & Maxwell, 2007). Studies in primates suggest that increase in sperm size is positively correlated with sperm competition, as observed in males from the genus *Macaca*

¹All correspondence to: Regiane R Santos. Faculty of Veterinary Medicine, Federal University of Pará, Laboratory of Wild Animal Biology and Medicine, BR 316 Km 61, CEP 68740-970, Castanhal, Pará, Brazil. Tel.: +55 91 33114707. E-mail: R.Rodriguesdossantos@pq.cnpq.br

²Laboratory of Wild Animal Biology and Medicine, Faculty of Veterinary Medicine, Federal University of Pará, Castanhal, Pará, Brazil.

³National Primate Center, Ananindeua, Pará, Brazil.

⁴Laboratory of Animal Reproduction and Breeding, State University of Norte Fluminense 'Darcy Ribeiro,' Campos dos Goytacazes, Rio de Janeiro, Brazil.

⁵Institute of Sustainable Development Mamirauá, Tefé, Amazonas, Brazil.

and *Papio*, in which evolution selected the sperm with the longest tail facilitating a more rapid swimming to reach the oocyte (Gomendio & Roldan, 1993). This fact was also recently demonstrated by the sperm morphometry comparison of the monogamic (low male-male competition) night monkeys (*Aotus lemurinus*) with the polygamic squirrel monkey species (*Saimiri boliviensis*) (high male-male competition). It was clear that the sperm from the night monkeys were much smaller than those from squirrel monkeys (Nakazato *et al.*, 2015). Other studies indicated a relationship between sperm morphometry and male body mass, female reproductive tract dimensions, oestrous length (Gage, 1998), and sperm quality (Valle *et al.*, 2012, 2013a,b; Nakazato *et al.*, 2015). Besides the sexual evolution, sperm morphometry indicates also evidences when searching for common ancestors for primate groups (Gould & Martin, 1978).

In recently published revisions on phylogeny and gender systematics of neotropical primates from genus *Saimiri* (Lynch Alfaro *et al.*, 2015; Mercês *et al.*, 2015), some subspecies were recognized as new species, for example the *S. collinsi* previously considered a subspecies of *S. sciureus* (Paglia *et al.*, 2012; Rylands *et al.*, 2012). Consequently, all the reproductive aspects from the new species are now under investigation.

The population of the *S. collinsi* is usually located at Marajó island and in the south of the Amazonas river (Brazil). In Brazil there is a colony of captive *S. collinsi* at the National Primate Center (1°22'57"S 48°22'52"W; Ananindeua). Some of these animals are maintained in captivity for scientific research purposes. However, there is still a lack of information on the reproductive biology of these species (Oliveira *et al.*, 2016a,b). Another species, *S. vanzolinii* presents a much more restrict geographic location, being considered the smallest geographic distribution and one of the smallest population among all the other neotropical primates (Paim *et al.*, 2013). Almost the entire population of this species is located in the Mamirauá Sustainable Development Reserve, in Brazil (03°08'–02°36'S, 65°45'–67°13'W) (Paim *et al.*, 2013). A combination of the restricted habitat and small population resulted in the inclusion of the *S. vanzolinii* in the IUCN list as a threatened species (IUCN, 2016). Both squirrel monkey species, together with the *S. ustus*, form a monophyletic group within the genus *Saimiri* (Lynch Alfaro *et al.*, 2015). Hence, reproductive aspects studied and determined in captive *S. collinsi* most probably will be valid for extrapolations to *S. ustus* and *S. vanzolinii*.

The aims in the present study were: (i) to describe sperm morphometry from the species *S. collinsi* and *S. vanzolinii*; (ii) to verify if the morphometric sperm patterns are similar or different between both species; and (iii) to determine if the sperm morphometry is

affected by the levels of sperm defects using the *S. collinsi* as a model. For all these analysis, only morphologically normal sperm were measured.

Materials and methods

Ethical aspects

S. collinsi

All experimental protocols were approved by environmental authorities (Ministry of the Environment – System of Authorization and Information on Biodiversity – SISBIO/ICMBio/MMA no. 31,542-2, for captive animals/no. 29,906-3, for wild animals), by the Ethical Committee in Animal Research (no. 0010/2011/CEPAN/IEC/SVS/MS, for captive animals).

S. vanzolinii

All experimental protocols were approved by the Ethical Committee in Animal Research of the Mamirauá Institute for Sustainable Development (no. 002/2012) and by the System of Authorization and Information in Biodiversity (SISBIO/ICMBIO/MMA no. 299006-1). All procedures were supervised and controlled by a veterinarian.

Study site

We conducted our study at two different locations. The captive males (*S. collinsi*) were examined and maintained at the National Primate Center, Ananindeua, Brazil (1°22'57"S, 48°22'52"W), where the climate is tropical humid, with an average annual temperature of 28°C. The free-living males (*S. vanzolinii*) were captured at Mamirauá Sustainable Development Reserve. The reserve is a protected area located at the confluence of the Solimões and Japurá rivers (03°02'22"S to 64°51'41"W). Monthly average precipitation is 131.1 mm, and the climate is also tropical humid, with an annual average temperature of 27.5°C.

Animals

Sexually mature and healthy *S. collinsi* males ($n = 10$; >5-year-old) were collectively housed in cages of 4.74 m × 1.45 m × 2.26 m (length, width, and height, respectively), under natural photoperiod (i.e., 12 h of light and 12 h of dark). The diet consisted of fresh fruit, milk and commercial pellet chow (MEGAZOO® P18, Protein 18%, Fiber Maxi. 6.5%, Betim, MG, Brazil), and cricket larvae (*Zophobas morio*). Vitamins, minerals, and eggs were supplied once a week, and water was available *ad libitum*.

Sexually mature and healthy *S. vanzolinii* males ($n = 2$; >5-year-old) were captured using a Tomahawk Live Trap (0.7 m \times 0.4 m \times 0.4 m; length, width, and height, respectively) according to Paim & Rabelo (2015). Traps were set up in the early morning and checked after 4 h and at mid-afternoon. The animals caught were handled by a trained animal caretaker wearing leather gloves. Semen collection was performed at the site of capture and, after anaesthetic recovery, animals were left free at same local. For these animals, semen collection was performed at capture points to avoid the removal of the caught animals from their places of origin.

The age of all animals was estimated on the basis of dentition considering tooth eruption, intraosseous tooth formation, and tooth wear (Smith, 1989).

Semen collection

Physical restraint was done with netting and leather glove, by a trained animal caretaker. Semen was collected at the same period of the day, i.e. in the morning before feeding and throughout 5 months for *S. collinsi* (July to December, 2011) and during two expeditions at Mamirauá Reserve for *S. vanzolinii* (November 2012 and October 2013). After physical restraint, all studied animals were anesthetized with ketamine hydrochloride (Vetanarcol 15 mg/kg IM; König S.A., Avellaneda, Argentina) and xylazine hydrochloride (1 mg/kg IM; König S.A.) by a veterinarian (Oliveira *et al.*, 2015).

After total anaesthetic effect, the males were placed in dorsal recumbence, and the genital region was then sanitized with a mild soap and distilled water (1:10) and gauze. The prepuce was retracted with the thumb and index fingers for a more efficient cleansing of the penis with saline solution.

Animals were stimulated by electro-ejaculation (EEJ; Autojac-Neovet, Uberaba, Brazil) with a rectal probe as indicated by Oliveira *et al.* (2015): 0.6 cm diameter and 12.5 cm length with a rounded end, bearing two metal plates (2 cm in length and 0.8 cm wide) on opposite sides. The probe was smeared with a sterile lubricant jelly (KY Jelly, Johnson and Johnson Co., Arlington, TX, USA), introduced in the rectum (~5 cm deep), and electrical stimuli were delivered. The stimulation session consisted of three series (8 min), composed of 35 electrical stimuli (12.5–100 mA) within an interval of 30 s between series (Oliveira *et al.*, 2015). Ejaculate (liquid and coagulated fractions) was collected into microtubes (1.5 ml). If a male did not ejaculate after the session, no further attempts were made to collect semen in the case of wild animals. In the case of captive animals, another EEJ was attempted after intervals of at least 30 days. A veterinarian monitored the animals during EEJ and after recovering from anaesthesia.

Seminal analysis

Immediately after ejaculation in a graduated micro-tube, semen was placed in a water bath at 37°C. The seminal liquid fraction was measured with the aid of a pipette. Coagulum volume was calculated as the total volume minus the liquid volume. Only the liquid fraction *in natura* was used, once coagulated fraction must be liquefied mechanically or enzymatically, which could affect morphometric parameters (Maree *et al.*, 2010). Therefore, coagulated fraction was discarded in the present study. Vigour was subjectively evaluated on a scale of 0 to 5 as previously described (Oliveira *et al.*, 2016a). In brief, no sperm motility was considered 0, slight movement with >75% of sperm showing vibration only was represented by 1, moderate forward movement in about >50% of sperm was represented by 2, forward movement in about 70% of sperm was represented by 3, and when ~90% or >95% of sperm presented very active forward movement, scales 4 and 5 were used, respectively. Sperm motility was expressed as the percentage of cells actively moving in a forward direction. Sperm vibrating in place were not considered to be motile (Dong *et al.*, 2008). To measure percentage of progressive forward sperm motility, 10 μ l of semen was placed in a pre-warmed (37°C) glass slide with cover slip and 200 sperm were counted. Normal sperm morphology and plasma membrane integrity were evaluated by a smear prepared adding 5 μ l of eosin nigrosin stain (Vetec, Rio de Janeiro, Brazil) to 5 μ l of semen on a pre-warmed (37°C) glass slide.

Morphologic defects detected in sperm were classified according to Bloom (1973), as major or minor. Major defects are those that affect fertility, and minor defects are of less importance. All evaluations were performed under a light microscope (Nikon, Tokyo, Japan), at a magnification \times 100, by the same measurer.

Selection of samples

Collected samples ($n = 10$ from *S. collinsi*; $n = 2$ from *S. vanzolinii*) from each species were classified for their morphological quality according to Bloom (1973). During the analysis, it was found that half of the *S. collinsi* males presented less than 70% of morphologically normal sperm. Therefore, we divided the animals of this group into two subgroups as follow: the group denominated as high quality ($n = 5$ males) was characterized by *S. collinsi* males presenting $\geq 70\%$ normal sperm, with not more than 10% of major defects and not more than 20% of minor defects, while the group denominated as poor quality ($n = 5$ males) was characterized by *S. collinsi* males presenting <70% normal sperm. Both *S. vanzolinii* males presented $\geq 70\%$ normal sperm, with not more

Table 1 Description of the selected morphometric parameters for sperm head and tail

Parameters	Unit	Description
<i>Spermhead</i>		
Area	μm^2	[A] The sum of all pixel area contained within the boundary
Perimeter	μm	[P] The sum of external pixels that make the boundary
Length	μm	[L] The sum of all pixels that divide the sperm head in two equal parts (longest distance)
Width	μm	[W] The sum of all pixels that divide the sperm head in two equal parts (shortest distance)
<i>Head shape-derived</i>		
Ellipticity	μm	[EP] Represented by (L/W)
Elongation	μm	[EL] Represented by (L-W)/(L+W)
<i>Spermtail</i>		
Tail length	μm	[TL] The sum of all pixels that divide the sperm tail in two equal parts (longest distance)
Total sperm length	μm	[TSL] Represented by (L+ TL)

Adapted from Martí *et al.* (2011).

than 10% of major defects and not more than 20% of minor defects. Therefore, no subgroups were used for this species. Both *S. vanzolinii* studied males presented more than 70% of morphologically normal sperm.

Sperm morphometry

Sperm analysis was performed with the help of a light microscope (Leica DM LS 2, Leica Microsystems, Aargau, Switzerland) with a $\times 1000$ magnification. All the images were recorded with a Canon® Power Shot S50 and processed with the software Canon® Utilities Zoom Browser (EX 690 1996–2004, 5.0, Australia). Only sperm without morphological defects were evaluated, and when it was possible to achieve an optimal resolution for head and tail. Measurements were performed with the software Image J® 1.46r (Wayne Rasband National Institutes of Health, IH, Bethesda, MD, USA) using the tools Threshold, Wand (tracing) tool and Straight (segmented line). Five parameters related to linear dimensions from spermatid head and tail were measured as described in Table 1. Area (μm^2), perimeter (μm), length (μm), and width (μm) of sperm head, as well as tail length (μm) and sperm length (μm) were firstly expressed as pixels and transformed to micrometres by ImageJ®. Besides these linear parameters, derived parameters such as head shape (ellipticity end elongation), were measured (Yániz *et al.*, 2015).

Table 2 Data on semen collection by electro-ejaculation and the distribution (number of animals, number of attempts per animal, ejaculates obtained per animal and samples used in the study)

Animals	Number of attempts	Ejaculates obtained	Used seminal sample ^a
<i>S. collinsi</i>			
1 (AT-AAA)	4	3	1
2 (AT-AIM)	4	3	1
3 (AT-AHR)	4	3	1
4 (AT-BAA)	2	1	1
5 (AT-BAD)	5	3	1
6 (AT-BAH)	5	4	1
7 (AT-BAJ)	5	5	1
8 (AT-BAO)	5	3	1
9 (AT-BBC)	10	8	1
10 (AT-BEX)	4	2	1
Total	38	34	10
<i>S. vanzolinii</i>			
1 (SV 14)	1	1	1
2 (SV 15)	1	1	1
Total	2	2	2

^aOnly those with semen *in natura* for analysis, and when it was possible to achieve an optimal resolution for sperm head and tail.

Statistical analysis

Due to the number of *S. vanzolinii* animals used in this study ($n = 2$), data from these species are included in the results tables as means of visual comparison. All data are expressed as mean \pm standard deviation (SD) and analysed by the Minitab software (Minitab Inc., Copyright© 2005, version 14.20). Plasma membrane integrity, normal sperm morphology, motility, vigour and morphometry were compared within the high quality and poor quality *S. collinsi* groups. Data were evaluated using one-way analysis of variance (ANOVA) followed by Fisher's protected least significant difference (LSD) as a *post hoc* test, and a P -value < 0.05 was considered as statistically significant.

Results

From the 10 *S. collinsi*, 32 ejaculates were obtained, but only 10 were suitable for the study, while from the two *S. vanzolinii*, one ejaculate from each male was used for evaluation (see details in Table 2).

Table 3 shows the percentages of sperm with normal morphology and the percentages of major and minor defects within the *S. collinsi* subgroups (high and poor quality), as well as for *S. vanzolinii*. The *S. collinsi* group with the lowest percentages of morphologically

Table 3 Mean (\pm SD) percentages of normal sperm and sperm morphology (with major and minor pathologic defects) of *in natura* semen of *S. collinsi* (high and poor quality group) and *S. vanzolinii*. Data from distinct males ($n = 12$)

Morphological sperm classification	Species		
	<i>S. collinsi</i>		<i>S. vanzolinii</i> ($n = 2$)
	High quality group ($n = 5$)	Poor quality group ($n = 5$)	
Normal sperm	75 \pm 3.7 ^a	42 \pm 6.2 ^b	91 \pm 7
Major defects	(5.7)	(19)	(4)
Retro Axial Tail	0.4 \pm 0.2	0.2 \pm 0.2	0.0 \pm 0.0
Proximal cytoplasmic droplet	1.4 \pm 1.2	0.0 \pm 0.0	0.0 \pm 0.0
Mid-pieces lender	0.2 \pm 0.2	0.0 \pm 0.0	0.0 \pm 0.0
Strongly coiled tail	3.1 \pm 1.8 ^a	19 \pm 6.8 ^b	3.5 \pm 2.5
Pseudo droplet	0.6 \pm 0.4	0.0 \pm 0.0	0.0 \pm 0.0
Minor defects	(19)	(39)	(5)
Bent tail	11 \pm 1.8	19 \pm 5.1	0.0 \pm 0.0
Coiled tail	7.9 \pm 1.2 ^a	20 \pm 4.0 ^b	4.5 \pm 4.5
Distal cytoplasmic droplet	0.2 \pm 0.2	0.4 \pm 0.4	0.0 \pm 0.0
Distal cytoplasmic pseudo droplet	0.0 \pm 0.0	0.2 \pm 0.2	0.0 \pm 0.0

^{a,b}Different letters in a row indicate significant statistical differences among high and poor quality groups in *S. collinsi* ($P < 0.05$).

normal sperm also presented more than 10% of major defects and almost 40% of minor defects, being significantly different from the group with high quality sperm. Based on these data, collected sperm from *S. vanzolinii* could be considered of high quality. When considering the morphologic defects in *S. collinsi*, the poor quality semen contained a significantly higher percentage of sperm with coiled tail, when compared with high quality semen.

The seminal volume was highly variable and did not differ among species or *S. collinsi* samples. Although plasma membrane integrity tended to be lower in sperm from poor quality semen, this difference was not significant. Importantly, sperm motility and vigour were significantly lower in poor quality sperm *S. collinsi* than in those presenting high quality semen. *S. vanzolinii* presented sperm with motility and vigour similar to those *S. collinsi* of high quality semen (Table 4).

When morphometry of morphologically normal sperm was evaluated, it was observed that head parameters were significantly larger in *S. collinsi* from the high quality sperm group when compared with the one with poor quality sperm *S. collinsi* group. The inverse was observed when comparing sperm head ellipticity. Usually, sperm from *S. vanzolinii* presented a larger head than those from *S. collinsi* (independently on the group), but tail lengths were similar (Table 5).

Discussion

Sperm morphometric studies were performed in many primate families (*Cercopithecidae*; *Hylobatidae*;

Pongidae; *Hominidae*; *Callithricidae* and *Cebidae*) (Gage & Freckleton, 2003), including *S. sciureus* (Bennett, 1967; Martin *et al.*, 1975; Laverde *et al.*, 2001) and *S. boliviensis* (Steinberg *et al.*, 2009; Nakazoto *et al.*, 2015). For the best of our knowledge, up to now no studies reported sperm morphometric characteristics from the neotropical primates *S. collinsi* and *S. vanzolinii*, and its relationship with sperm quality, e.g. motility and vigour. In the present study we used the captive *S. collinsi* as a model to determine the importance of morphometric analysis to access sperm quality. Samples from two *S. vanzolinii* specimens were collected to compare with the *S. collinsi* data.

We showed that sperm from all studied *S. collinsi* presented similar tail length, corroborating with Humphries *et al.* (2008) who demonstrated that the swimming speed is not dependent on the size of the tail, but from the head size, which may retard the propulsion given by the sperm tail. We also observed a variable morphometry for sperm head when high and poor quality sperm groups were compared, where sperm of poor quality presented a more elongated head, which was 100% more elongated than *S. collinsi* high quality sperm and similar to *S. vanzolinii* sperm and not higher. *S. collinsi* sperm from the poor quality group presented a smaller head when compared with the high quality group. It has been shown that the most common defect in morphologically normal sperm occurs in the chromatin (Saacke, 2008). Beletti *et al.* (2002) reported that, in bovine, such chromatin defect is accompanied by a decrease in the sperm head area. Although we did not evaluate sperm chromatin, our data on *S. collinsi* substantiates these previous findings in bovine. Sailer *et al.* (1996)

Table 4 Mean (\pm SD) of the volume of liquid and coagulated fractions of ejaculate (μ l), sperm plasma membrane integrity (%), sperm motility (%) and vigour (grade) of fresh semen of *S. collinsi* (high and poor quality group) and *S. vanzolinii*

Parameters	Species		
	<i>S. collinsi</i>		<i>S. vanzolinii</i> (n = 2)
	High quality group (n = 5)	Poor quality group (n = 5)	
Volume of liquid fraction	203 \pm 88	110 \pm 73	83 \pm 68
Volume of coagulated fraction	177 \pm 89	24 \pm 19	93 \pm 58
Sperm plasma membrane integrity	77 \pm 9	48 \pm 17	71 \pm 11
Sperm motility	79 \pm 13 ^a	16 \pm 8 ^b	95 \pm 5
Vigour	4 \pm 0.5 ^a	1 \pm 0.5 ^b	5 \pm 0

^{a,b}Different letters in a row indicate significant statistical differences among high and poor quality groups in *S. collinsi* ($P < 0.05$).

Table 5 Mean (\pm SD) of area (μm^2), perimeter (μm), length (μm), width (μm), ellipticity, elongation, tail length and total sperm length of *in natura* semen of *S. collinsi* (high and poor quality group) and *S. vanzolinii*. Data from distinct males (n = 12)

Parameters	Sperm morphometry of <i>Saimiri</i> spp.		
	Species		<i>S. vanzolinii</i> (n = 2)
	<i>S. collinsi</i>		
	High quality group (n = 5)	Poor quality group (n = 5)	
<i>Head sperm</i>			
Area	21.0 \pm 0.10 ^a	20.2 \pm 0.11 ^b	25.4 \pm 0.09
Perimeter	17.4 \pm 0.04 ^a	17.2 \pm 0.04 ^b	19.1 \pm 0.03
Length	6.2 \pm 0.01	6.2 \pm 0.02	6.8 \pm 0.01
Width	4.3 \pm 0.01 ^a	4.1 \pm 0.01 ^b	4.8 \pm 0.01
<i>Head shape-derived</i>			
Ellipticity	1.4 \pm 0.005 ^a	1.5 \pm 0.005 ^b	1.4 \pm 0.004
Elongation	0.1 \pm 0.002 ^b	0.2 \pm 0.002 ^b	0.2 \pm 0.001
<i>Tail sperm</i>			
Tail length	70.5 \pm 0.19	70.6 \pm 0.19	69.2 \pm 0.09
Total sperm length	76.7 \pm 0.19	76.9 \pm 0.19	76.1 \pm 0.09

^{a,b}Different letters in a row indicate significant statistical differences ($P < 0.05$).

pointed that a variation in sperm morphometry can be considered a potential marker for male sub-(in)fertility and abnormal chromatin structure.

Regarding other neotropical primates, sperm morphometry was described for *Callithrix jacchus* (Moore et al., 1984; Cummins & Woodall, 1985; Valle et al., 2012; Swanson et al., 2016), *Lagothrix* sp. (Cummins & Woodall, 1985), *Cebus apella* (Gage & Freckleton, 2003), *Callimico goeldii* (Valle et al., 2013b; Swanson et al., 2016), *Alouatta caraya* (Valle et al., 2013a; Swanson et al., 2016), *Aotus lemurinus* (Nakazoto et al., 2015) and *Atelles geoffroyi* (Swanson et al., 2016). Comparing these reported data with our study, we can show that linear parameters from sperm head and tail from *S. collinsi* and *S. vanzolinii* are larger than in other species cited above, except for *Cebus apella* that presents larger heads (Gage & Freckleton, 2003) and *S. boliviensis* (Nakazoto et al., 2015) that presents longer sperm. This brings in evidence that sperm

morphometry can vary within neotropical primates. However, comparisons among different studies are not completely accurate once fixation and staining methods may affect sperm morphometry if reagents are not isosmotic (Maree et al., 2010). Therefore, it is important to check if different studies adopted the same or similar protocols. The observed sperm morphometric differences between *S. collinsi* and *S. vanzolinii* can be an indicative that this parameter may be used to characterize species (Martin et al., 1975), being a tool to support taxonomic and phylogenetic studies (Steinberg et al., 2009; Sousa et al., 2013).

It was stated that there is a relationship between sperm size and male–male competition. Gomendio & Roldan (1991) described that within primates and rodents, sperm length is larger in accordance to the degree of male–male competition in the species under analysis. Comparative studies indicate that, in mammals, larger sperm are faster swimmers (Gomendio

& Roldan, 1991, 2008; Tourmente *et al.*, 2011), as an evolutionary adaptation for post copulatory male-male competition (Simmons & Fitzpatrick, 2012).

Although no complete information is available on the mating system from *S. collinsi* and *S. vanzolinii*, it is known that the *Saimiri* genus presents a multimale-multifemale system, probably with high sperm competition (Izar *et al.*, 2009). This may explain the larger sperm from these species when compared with monogamic neotropical species.

Sperm morphometry can be used as a complementary tool to predict sperm motility and vigour for the *S. collinsi* species. This can be used as an extra parameter when selecting males for reproductive programs and biotechniques. Furthermore, *S. collinsi* appear as a good model for *S. vanzolinii*.

Competing interests

The authors declare that there is no conflict of interest that can be perceived as prejudicing the impartiality of the research reported.

Acknowledgements

The authors thank National Primate Center (CENP), Brazil and Mamiraua Institute for Sustainable Development (IDSMD), Brazil for the logistical support. W.V. Sampaio was supported by CNPq, Brazil.

References

- Andrabi, S.M.H. & Maxwell, W.M.C. (2007). A review on reproductive biotechnologies for conservation of endangered mammalian species. *Anim. Reprod. Sci.* **99**, 223–43.
- Beletti, M.E., Costa, L.F. & Guardieiro, M.M. (2002). Morphometric features and chromatin condensation abnormalities evaluated by toluidine blue staining in bull spermatozoa. *Braz. J. Morphol. Sci.* **22**, 85–90.
- Bennett, J. P. (1967). Semen collection in the squirrel monkey. *J. Reprod. Fertil.* **13**, 353–5.
- Bloom, E. (1973). The ultrastructure of some characteristic sperm defects and a proposal for a new classification of the bull spermogram. *Nord. Vet.* **25**, 83–91.
- Cummins, J. M. & Woodall, P. F. (1985). On mammalian sperm dimensions. *J. Reprod. Fertil.* **75**, 153–75.
- Dong, Q., Rodenburg, S.E., Huang, C. & VandeVoort, C.A. (2008). Cryopreservation of rhesus monkey (*Macaca mulatta*) epididymal spermatozoa before and after refrigerated storage. *J. Androl.* **29**, 283–92.
- Gage, M.J. (1998). Mammalian sperm morphometry. *Proc. Roy. Soc. Lond. B: J. Bio. Sci.* **265**, 97–103.
- Gage, M.J.G. & Freckleton, R.P. (2003). Relative testis size and sperm morphometry across mammals: no evidence for an association between sperm competition and sperm length. *Proc. R. Soc. Lond. B.* **270**, 625–32.
- García, D.A., Montiel, J.L.C., Pérez, O.H., Pichardo, E.M., Reza, F.A.C. & García, R.A. (2003). Estudio comparativo de los testículos, epidídimos, glándulas sexuales accesorias y espermatozoides em tres especies de lagomorfos (*Romerolagos diazi*, *Lepus californicus* y *Oryctolagus cuniculus*). *Acta. Zool. Mex.* **88**, 257–69.
- Gomendio, M. & Roldan, E.R.S. (1991). Sperm competition influences sperm size in mammals. *Proc. R. Soc. Lond. B: Biol. Sci.* **243**, 181–185.
- Gomendio, M. & Roldan, E.R.S. (1993). Coevolution between male ejaculates and female reproductive biology in eutherian mammals. *Proc. R. Soc. Lond. B: Biol. Sci.* **252**, 7–12.
- Gomendio, M. & Roldan, E.R.S. (2008). Implications of diversity in sperm size and function for sperm competition and fertility. *Int. J. Dev. Biol.* **52**, 439.
- Gould, K.G. & Martin, D.E. (1978). Comparative morphology of primate spermatozoa using scanning electron microscopy. II. Families Cercopithecidae, Lorisidae, Lemuridae. *J. Hum. Evol.* **7**, 637–42.
- Humphries, S., Evans, J.P. & Simmons, L.W. (2008). Sperm competition: linking form to function. *BMC Evol. Biol.* **8**, 319.
- IUCN, 2016. IUCN Red List of Threatened Species, Version 2011.2. Disponible in: <https://doi.org/10.2305/IUCN.UK.2008.RLTS.T19839A9023022.en> Downloaded on 03 May 2016.
- Izar, P., Stone, A., Carnegie, S. & Nakai, E.S. (2009). Sexual selection, female choice and mating systems. In *South American Primates: Comparative Perspectives in the Study of Behavior, Ecology and Conservation* (Garber, P.A., Estrada, A., Bicca-Marques, J.C., Heymann, E.W. & Strier, K.B. eds). Series Developments in Primatology: Progress and Prospects. University of Chicago. Springer. New York, pp. 175–89.
- Laverde, H.J., Medina, V.M. & Casallas, C.P.E. (2001). Contribución al conocimiento de las características semifinales del mono ardilla *Saimiri sciureus*. Universidad de los Llanos-Escuela de Medicina Veterinaria y Zootecnia Villavivencio- Colombia, 2001.
- Lynch Alfaro, J.W.L., Boubli, J.P., Paim, F.P., Ribas, C.C., Silva, M.N.F., Messias, M.R., Rohe, F., Mercedes, M.P., Silva Junior, J.S., Silva, C.R., Pinho, G.M., Koshkarian, G., Nguyen, M.T., Harada, M.L., Rabelo, R.M., Queiroz, H.L., Alfaro, M.E. & Farias, I.P. (2015). Biogeography of squirrel monkeys (genus *Saimiri*): South-central Amazon origin and rapid pan-Amazonian diversification of a lowland primate. *Mol. Phylogenet. Evol.* **82**, 436–54.
- Maree, L., Du Plessis, S.S., Menkveld, R. & Van der Horst, G. (2010). Morphometric dimensions of the human sperm head depend on the staining method used. *Hum. Reprod.* **25**, 1369–82.
- Martí, J.I., Aparicio, I.M. & García-Herreros, M. (2011). Sperm morphometric subpopulations are differentially distributed in rams with different maturity age in cryopreserved ejaculates. *Theriogenology* **76**, 97–109.
- Martin, D.E., Gould, K.G. & Warner, H. (1975). Comparative morphology of primate spermatozoa using scanning

- electron microscopy. I. Families *Hominidae*, *Pongidae*, *Cercopithecidae* and *Cebidae*. *J. Hum. Evol.* **4**, 287–92.
- Mercês, M.P., Alfaro, J.W.L., Ferreira, W.A., Harada, M.L. & Júnior, J.S.S. (2015). Morphology and mitochondrial phylogenetics reveal that the Amazon River separates two eastern squirrel monkey species: *Saimiri sciureus* and *S. collinsi*. *Mol. Phylogenet. Evol.* **82**, 426–35.
- Micklem, K. & Sanderson, J. (2001). Digital imaging in pathology. *Curr. Diagn. Pathol.* **7**, 131–40.
- Moore, H.D.M., Hartman, T.D. & Holt, W.V. (1984). The structure and epididymal maturation of the spermatozoon of the common marmoset (*Callithrix jacchus*). *J. Anat.* **138**, 227–35.
- Nakazato, C., Yoshizawa, M., Isobe, K., Kusakabe, K.T., Kuraishi, T., Hattori, S., Matsumoto, H., Fukui, E., Kuwahata, A., Ochi, M., Kiso, Y. & Kai, C. (2015). Morphological characterization of spermatozoa of the night monkey. *J. Mamm. Ova. Res.* **32**, 37–40.
- Oliveira, K.G., Leão, D.L., Almeida, D.V.C., Santos, R.R. & Domingues, S.F.S. (2015). Seminal characteristics and cryopreservation of sperm from the squirrel monkey, *Saimiri collinsi*. *Theriogenology* **84**, 743–9.
- Oliveira, K.G., Santos, R.R., Leão, D.L., Brito, A.B., Lima, J.S., Sampaio, W.V. & Domingues, S.F.S. (2016a). Cooling and freezing of sperm from captive, free-living and endangered squirrel monkey species. *Cryobiology* **72**, 283–9.
- Oliveira, K.G., Santos, R.R., Leão, D.L., Queiroz, H.L., Paim, F.P., Vianez-Júnior, J.L. S.G. & Domingues, S.F.S. (2016b). Testicular biometry and semen characteristics in captive and wild squirrel monkey species (*Saimiri* sp.). *Theriogenology* **86**, 879–87.
- Paglia, A.P., da Fonseca, G.A., Rylands, A.B., Herrmann, G., Aguiar, L.M.S., Chiarello, A.G., Leite, Y.L.R., Costa, L.P., Siciliano, S., Kierulff, M.C.M., Mendes, S.L., Tavares, V. da C., Mittermeier, R.A. & Patton, J.L. (2012). Annotated checklist of Brazilian mammals. Occasion. *Papers Conserv. Biol.* **6**, 1–76.
- Paim, F.P. & Rabelo, R. (2015). Methods for capturing wild squirrel monkeys in a floodplain forest: a comparison of two techniques and two species. *Neotrop. Primates.* **22**, 7–11.
- Paim, F.P., Valsecchi, J., Harada, M.L. & Queiroz, H.L. (2013). Diversity, geographic distribution and conservation of squirrel monkeys, *Saimiri* (Primates, *Cebidae*), in the flood plain forests of Central Amazon. *Int. J. Primatol.* **34**, 1055–76.
- Phetudomsinsuk, K., Sirinarumit, K., Laikul, A. & Pinyopummin, A. (2008). Morphology and head morphometric characters of sperm in Thai native crossbred stallions. *Acta Vet. Scand.* **50**, 41.
- Rylands, A.B., Mittermeier, R.A. & Silva, J.S. (2012). Neotropical primates: taxonomy and recently described species and subspecies. *Int. Zoo Yearbook.* **46**, 11–24.
- Saacke, R.G. (2008). Sperm morphology: Its relevance to compensable and uncompensable traits in semen. *Theriogenology.* **70**, 473–78.
- Sailer, B.L., Lorna, K.J. & Donald, P.E. (1996). Bull sperm head morphology related to abnormal chromatin structure and fertility. *Cytometry.* **24**, 167–73.
- Simmons, L.W. & Fitzpatrick, J.L. (2012). Sperm wars and the evolution of male fertility. *Reproduction.* **144**, 519–34.
- Smith, B.H. (1989). Dental development as a measure of life history in primates. *Evolution* **43**, 683–8.
- Sousa, P.C., Santos, E.A.A., Bezerra, J.A.B., Lima, G.L., Castelo, T.S., Fontenele-Neto, J.D. & Silva, A.R. (2013). Morphology, morphometry and ultrastructure of captive six-banded armadillo (*Euphractus sexcinctus*) sperm. *Anim. Reprod. Sci.* **140**, 279–85.
- Steinberg, E.R., Nieves, M., Ascunce, M.S., Palermo, A.M. & Mudry, M.D. (2009). Morphological and genetic characterization of *Saimiri boliviensis*. *Int. J. Primatol.* **30**, 29–41.
- Swanson, W.F., Valle, R.R., Carvalho, F.M., Arakaki, P.R., Rodas-Martínez, A.Z., Muniz, J.A.P.C. & García-Herreros, M. (2016). Sperm morphology assessment in captive neotropical primates. *Reprod. Dom. Anim.* **51**, 623–7.
- Tourmente, M., Gomendio, M. & Roldan, E.R. (2011). Sperm competition and the evolution of sperm design in mammals. *BMC Evol. Biol.* **11**, 12.
- Valle, R.D.R., Nayudu, P.L., Leal, C.L.V. & Garcia-Herreros, M. (2012). Sperm head morphometry in ejaculates of adult marmosets (*Callithrix jacchus*): a model for studying sperm subpopulations and among-donor variations. *Theriogenology*, **78**, 1152–65.
- Valle, R.R., Arakaki, P.R., Carvalho, F.M., Muniz, J.A.P.C., Leal, C.L.V. & García-Herreros, M. (2013a). Identification of sperm head subpopulations with defined pleiomorphic characteristics in ejaculates of captive Goeldi's monkeys (*Callimico goeldii*). *Anim. Reprod. Sci.* **137**, 93–102.
- Valle, R.R., Carvalho, F.M., Muniz, J.A.P.C., Leal, C.L.V. & García-Herreros, M. (2013b). Differential distribution of sperm subpopulations and incidence of pleiomorphisms in ejaculates of captive howling monkeys (*Alouatta caraya*). *Naturwissenschaften.* **100**, 923–33.
- Yániz, J.L., Soler, C. & Santolaria, P. (2015). Computer assisted sperm morphometry in mammals: a review. *Anim. Reprod. Sci.* **156**, 1–12.