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RESEARCH ARTICLE

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Male fattening is related to increased seminal quality of squirrel monkeys (*Saimiri collinsi*): Implications for sperm competition

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Abstract

Saimiri are neotropical primates with seasonal reproduction, males develop a seasonal fattening condition that has been suggested as a pre-copulatory sexual selection strategy. Furthermore, females mate with multiple males in the same season. This could also favor the evolution of a postcopulatory sexual strategy by sperm competition. In this study, we tested the hypothesis that there is a relationship between the fatted condition and advantageous seminal characteristics in *Saimiri collinsi* and evaluated its implications for sperm competition. Adult males ($N = 10$), aged 5–15 years, housed in mixed or only-male groups, were analyzed from June, 2015 to July, 2016. Measurements of weight, axilla, and arm skinfold, and testicular volume were taken monthly, and semen was collected by electroejaculation. A fattening index was developed to quantify and identify fatted males, biometrics, and seminal parameters were compared between the non-fatted and fatted conditions. The fatted males present a larger testicular volume. This is related to the increase in spermatogenic activity necessary to sustain a high ejaculation frequency during the mating season. An increase in seminal volume and in frequency of semen coagulation were detected in fatted males, advantages related to sperm protection in the female reproductive tract. Age and social context were not significant sources of variation for both morphological and seminal traits. A decrease in response from the fatted males in obtaining semen and an increase in the frequency of azoospermic ejaculates were observed. These unexpected results may be due to intense reproductive activities in a short period. The fattening phenomenon has many implications in the sexual selection of squirrel monkeys, and they are still not entirely unveiled. Our results corroborate the idea that, in *S. collinsi*, the fatted male condition is related to sexual selection, and we found evidence suggesting it may be also expressed by a post-copulatory component, sperm competition.

KEYWORDS

neotropical primate, reproduction, seasonality, sperm competition

1 | INTRODUCTION

Sexual selection is a process that affects the reproductive success of organisms, conferring certain advantages over others of the same sex and species (Brennan, 2010). In males, disputes over access to females have resulted in the development of pre-copulatory mechanisms that favor mating opportunities, by excluding competitors and by attracting or retaining partners (Brennan, 2010). The phenomenon of pre-copulatory sexual selection has been shown to include male and female selection based on morphological and behavioral traits in paleotropical primates such as *Mandrillus* spp. (Setchell & Dixson, 2001) and *Macaca* spp. (Petersdorf et al., 2017) and also in neotropical primates such as *Brachyteles* spp. (Tokuda et al., 2014), *Sapajus* spp. (Lynch Alfaro et al., 2014), and *Alouatta* spp.(Dunn et al., 2015). Furthermore, post-copulatory selection via cryptic female choice and sperm competition, wherein characteristics that increase the success of fertilization by an ejaculate/sperm under competitive conditions are positively selected (Pizzari & Parker, 2009), also play a role. Post-copulatory sexual selection is comparatively less well-studied but has already been documented in some primate genera such as *Gorilla* spp., *Hylobates* spp., *Pan* spp. (Jensen-Seaman & Li, 2003), *Pongo* spp. (Kinoshita et al., 2021), *Callithrix* spp. (French et al., 2003), *Ateles* spp. (Hernández-López et al., 2008), and *Sapajus* spp. (Lima et al., 2017). In sperm competition, selected traits are generally related to an improvement in fertilization probability (Martinez & Garcia, 2020). This includes an increase in the motility of the gamete, or enhancement in physiological traits, such as the fructose concentration of the seminal fluid, which is essential for sperm metabolism and motility (Dixson, 1998). Fertilization probability can also be increased by changes in morphological characteristics, such as an increased volume of the sperm midpiece in polyandrous mating systems (Dixson & Anderson, 2004).

Saimiri is a genus of neotropical primates known for its strictly seasonal reproduction (Stone, 2007), with a mating season lasting 2–3 months followed by a birth season 5 months later (Baldwin, 1968; Boinski, 1987; Coe & Rosemblum, 1978; DuMond, 1968; Stone, 2007; Trevino, 2007). The mating system is polygamous in multimale–multifemale groups (38–45 individuals: Boinski, 1987; 25–75 individuals: Boinski, 1999; 25–50 individuals: Izar et al., 2008; Mitchell, 1990; 40–50 individuals: Stone, 2007), and the males undergo marked morphophysiological changes shortly before and during the mating season. These changes result in the deposition of water and fat in the arms, shoulders, and thorax (Boinski, 1987; Mendoza et al., 1978a; Stone, 2014), which is known as the “fatted” condition (DuMond & Hutchinson, 1967). It has been suggested by researchers investigating different species of squirrel monkeys that a fatted male may be a product of pre-copulatory sexual selection. Fatter males are able to engage in courtship behavior for longer periods (Mitchell, 1990; Stone, 2014), and have more positive outcomes in male–male competition to access females (Mitchell, 1990). In fact, fatted males can obtain ~70% of all copulations during the mating season (Boinski, 1987, 1992).

However, other aspects of the reproductive biology of squirrel monkeys suggest that postcopulatory sexual selection, mainly sperm competition, may be also related to the fattening phenomenon, since females mate with multiple males in the same season (Dixson & Anderson, 2004; Dixson, 1995, 2018; Matinez & García, 2020). Studies have demonstrated that the fatted condition is androgen dependent with a seasonal increase in testosterone (Coe, Smith, et al., 1985; Nadler & Rosenblum, 1972). Furthermore, this condition coincides with seasonal spermatogenesis, an increased sperm concentration in the seminiferous tubules (DuMond & Hutchinson, 1967; It & Cavazos, 1971), and increased testes size (DuMond & Hutchinson, 1967; It & Cavazos, 1971; Nadler & Rosenblum, 1972). This suggests that the fattening phenomenon may also be related to sperm competition, a post-copulatory sexual selection strategy, which has implications for semen characteristics, as well as cryptic female choice. These ideas can be investigated since, in several species of the genus, semen has been studied in detail (Chen et al., 1981; Coe, Rosenblum, et al., 1985; Dukelow, 1983; Oliveira et al., 2015, 2016a, 2016b).

Previous studies have used various approaches to understand the fattening phenomenon, but its function remains unclear (Stone, 2014; Zimbler-Delorenzo & Stone, 2011). This is partly because the quantification of fattening has not been well explored (Chen et al., 1981; DuMond & Hutchinson, 1967; Mendoza et al., 1978a, 1978b), and the condition is poorly quantified. Studies have used different subjective methods to visually distinguish the fatted condition (Nadler & Rosenblum, 1972; Stone, 2014) or used only one measure, such as weight, shoulder circumference, or arm skinfold, to discriminate between the fatted and non-fatted states (Ausman et al., 1981; Baldwin, 1985; Boinski, 1999; Chen et al., 1981; DuMond & Hutchinson, 1967; Mendoza et al., 1978b; Nadler & Rosenblum, 1972). These limitations prevent the accurate assessment of individual variations in fattening, which is necessary to test hypotheses related to sexual selection (Dixson, 2018).

In the present study, we aimed to identify the possible advantages of the fatted condition in male squirrel monkeys (*Saimiri collinsi*) that live in eastern Amazonia. We tested the hypothesis that there is a relationship between the fatted condition and advantageous seminal characteristics in squirrel monkeys, and evaluated its implications for sperm competition. The objectives of our study were to: (i) develop an objective method for the quantification and identification of fatted males and to subsequently classify males into non-fatted and fatted categories using an index of fattening based on their biometric parameters; (ii) compare the variation in testicular volume and its relationship with biometric parameters involved in the discrimination of the two conditions; (iii) compare seminal characteristics of males in non-fatted and fatted conditions; and (iv) rule out the effect of age and social context as main source of variation in morphological and seminal characteristics.

2 | METHODS

2.1 | Ethical approval

All experimental protocols were approved by environmental authorities (Ministério do Meio Ambiente, Sistema de Autorização e

Informação em Biodiversidade-SISBIO/ICMBio/MMA no. 47051-3) and the Ethical Committee in Animal Research of the Instituto Evandro Chagas (no. 0026/2014/CEPAN/IEC/SVS/MS and no. 02/2015 CEPAN/IEC/SVS/MS), in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 8023, revised 1978) and the ASP Principles for the Ethical Treatment of Non-Human Primates.

2.2 | Locality

This study was conducted at the Centro Nacional de Primatas (CENP), Ananindeua, Pará, Brazil (1°22′58″S; 48°22′51″W), where the climate is humid and tropical, with an average annual temperature of 28°C.

2.3 | Animals

Sexually mature adult male *S. collinsi* ($n = 10$), approximately 5–15 years old, were selected by andrological examination (e.g., inspection and palpation of the testes to verify size, consistency, symmetry, mobility, and seminal parameters). The animals underwent an adaptation period of 6 months (January–May 2015) to form social groups. The four captive experimental groups consisted of 28 squirrel monkeys, three mixed social groups were composed of adult males ($n = 2$ or 3), adult females ($n = 3$), and juveniles and infants ($n = 3$), while one group consisted only of adult males ($n = 3$). The groups were held in 4.74 × 1.45 × 2.26 m (length × width × height) cages inside sheds with covered roof and open sides with screen protectors under natural conditions of photoperiod (12 h light; 12 h dark), temperature, humidity, and precipitation. There was also visual, olfactory, and auditory contact between the groups.

The animals were subjected to the same nutritional conditions throughout the experimental period with the same quantity of food offered at the same time twice a day (early morning and late afternoon). Their diet consisted of commercial pellet chow (MEGAZOO P18™, Protein 18%, Fiber Maxi. 6.5%). Eggs, fresh fruit, milk, and beetle larvae (*Zophobas morio*) were used as food supplements, and water was available ad libitum.

2.4 | Physical and chemical contention

Physical restraint was undertaken using netting and a leather glove by a trained animal caretaker. After physical restraint, the animals were subdued chemically with ketamine hydrochloride (20 mg/kg; IM; Vetanarcol, König S.A.) and xylazine hydrochloride (1 mg/kg; IM; Kensol, König S.A.), and monitored by a veterinarian.

2.5 | Body measurements

The weight and subcutaneous fat thickness of all experimental males were measured every month from June 2015 to July 2016 at the same time of day (in the morning before feeding). Animal body weight was measured using a digital weight scale (Balança Digital Toledo Prix 3 Plus 30 kg, Toledo do Brasil). Furthermore, two skinfold measurements were taken for the arm and axilla, since the accumulation of water and fat occurs specifically in these parts (Boinski, 1987; DuMond & Hutchinson, 1967; Mendoza et al., 1978a; Nadler & Rosenblum, 1972; Stone, 2014).

For each animal, the subcutaneous fat thickness was measured using a skinfold caliper (digital body fat caliper). Two measurements were taken, and the average was used as the skinfold measurement. The animals were placed in the left lateral decubitus position for the bicep site (arm skinfold) with the right arm hanging freely at their side. The caliper was then applied vertically (~1 cm) at a marked level. For the axilla site (axilla skinfold), a caliper was applied at the point where a vertical line from the mid-axilla (middle of the armpit) intersects with a horizontal line level with the bottom edge of the xiphoid process (the lowest point of the breast bone) (~1 cm), as described by the National Health and Nutrition Examination Survey (NHANES, 2007).

2.6 | Testicular volume and semen collection

Testicular biometry was determined using a universal caliper. Testicular length, width, height, circumference, and volume were measured. To obtain the volume of the testes, we used the equation described by Oliveira et al. (2016b).

Semen was collected monthly through electroejaculation (Autojac-Neovet®) as described by Oliveira et al. (2015). Before semen collection, the animal was placed in dorsal recumbency, the bladder was emptied, and the genital region was sanitized with a mild soap (Ypê, Química Amparo) and distilled water (Quimis®) (1:10). The prepuce was retracted for more efficient cleaning of the penis with saline solution. A rectal probe was used according to the method described by Bennett (1967). The probe was smeared with a sterile lubricant jelly (KY™ Jelly, Johnson & Johnson Co.) for introduction into the rectum (~2.5 cm deep) where electrical stimuli were delivered. The stimulation session consisted of three series (7–8 min) composed of 35 electrical stimuli (12.5–100 mA) with an interval of 30 s between series. Ejaculates (liquid and coagulated fractions) were collected in 1.5-ml microtubes. Semen was collected at the same time each day during the collection period from June 2015 to July 2016 (in the morning before feeding, with a 30-day interval between procedures).

2.7 | Semen analysis

Immediately after ejaculation, the microtubes containing semen were placed in a water bath at 37°C. The volume of liquid and coagulated fractions was evaluated in a graduated tube with the aid of a pipette. Appearance and consistency were assessed subjectively, that is, based on color (colorless, yellowish, or whitish) and opacity (opaque or transparent). The degree of seminal coagulation was evaluated according to the classification widely accepted in the literature (Dixson & Anderson, 2002), from Grade I (fluid) to Grade IV (firm). Seminal pH was estimated using a pH strip (Merck Pharmaceuticals). Sperm concentration was analyzed to verify the absence or presence of spermatozoa in the ejaculate. Briefly, after 1 h of dilution in ACP-118® (1:1) (powdered coconut water; ACP Biotecnologia) (Oliveira et al., 2015), 1 µl of semen was added to 99 µl of 10% formalin solution (Sigma-Aldrich®), and cells were counted in a Neubauer chamber (Optik-Labor). The analysis was performed under a light microscope (E400, Nikon) at a magnification of ×100.

2.8 | Statistical analysis

All statistical analyses were performed using Minitab 17 (Minitab Statistical Software, Inc., version 17).

2.8.1 | Fattening index

To determine the Fattening index (FI), the morphological characteristics of weight (g) and skinfolds of the axilla and arms (mm^2) were considered. To calculate the index from this multivariate database (Kubrusly, 2001; Silva & Silva, 2015), principal component analysis (PCA) for each animal was applied.

In brief, the Bartlett sphericity test was preliminarily performed on the variables to test normality and ensure that the use of PCA for each animal was possible. After PCA was performed, the eigenvalues and eigenvectors generated for each male were used to construct the index according to a theoretical and empirical model described elsewhere (Silva & Silva, 2015). For FI calculus, only eigenvalues >1 were used, since these corresponded to higher variances (>70%) of the main components, as well as their respective eigenvectors (Manly & Alberto, 2008). After obtaining FI values, the cutoff point 1.1 was adopted, which corresponded to the intersection of principal components 1 and 2 when both presented significant growth simultaneously, so all animals in the non-fatted condition showed FI scores <1.1, while all animals in the fatted condition had FI scores ≥1.1. For more details about the model adopted and eigenvalues and eigenvectors used, see Tables S1 and S2.

2.8.2 | Statistical analyses of body, testicular volume, and seminal quality

All morphometric and seminal data are expressed as mean ± standard deviation (*SD*) and standard error (*SE*), and data were checked for normality using the Kolmogorov–Smirnov test.

Based on the FI, the body biometric and seminal quality data were grouped according to non-fatted or fatted condition. To compare the parameters between the non-fatted and fatted conditions, analysis of variance (ANOVA) (Fisher) and paired *t* test were used when the distribution was normal, and the non-parametric Wilcoxon test was used when the distribution was not normal.

For comparison between ages, males were grouped into three age classes (5–6 yo $N = 5$ males, 10–12 yo $N = 2$ males, and 15 yo $N = 3$ males), and a general linear model (ANOVA for *k* samples) was used with the Tukey post hoc test (when the data showed a normal distribution), or Kruskal–Wallis test followed by the post hoc Dunn test (when the data showed a non-normal distribution). Finally, to compare males in different social contexts of the experimental groups (male-only group $N = 3$ males × mixed-sex groups $N = 7$ males), we used one-way ANOVA (for data with normal distribution) and paired *t* test, or the Mann–Whitney test (when the data showed a non-normal distribution). The critical *p* value was 0.05.

3 | RESULTS

3.1 | Fattening index

A total of 140 data points were collected (14 per animal) for each biometric parameter (weight, axilla, and arm skinfold). In the PCA analysis conducted for all animals, only principal component 1 (PC1) presented eigenvalues >1 (Table S2, supplementary material). The FI scores (Table 1) varied from −3.80 to 4.38, and when the FI was >1.1 (highlighted in Table 1), the animal was considered to be in the fatted condition (Figure 1). Although all males showed some degree of fattening, some of them presented a more pronounced transformation, which was reflected in the high increment of the FI in a short interval.

All males were in the fatted condition for an average of 3.4 months (range: 3–5 months). Except for male 1, all other animals had discrete phases in the fatted and non-fatted states, and the fatted phases were almost entirely synchronous. Scores >1.1 were concentrated from March to May 2016, with some individual variations. Again, male 1 was the exception and did not follow the general pattern.

3.2 | Biometric comparison between non-fatted and fatted conditions

The non-fatted and fatted condition datasets corresponded to 106 and 34 measurements for each of the three parameters (weight, axilla, and arm skinfold). A significant increase in the three biometric parameters can be observed in Figures 1 and 2, when animals expressing the fatted condition presented average monthly gains ranging from 37 to 194 g (4%–15%) in weight, 0.3 to 1.8 mm^2 (14%–60%) in the axilla skinfold, and 0.25 to 1.3 mm^2 (6%–52%) in the arm skinfold. During the period when the animals were in the non-fatted condition, we observed variations in the gains and losses in weight and skinfolds (see Table S3).

TABLE 1 Monthly distribution of the Fattening Index (FI) in *Saimiri collinsi* males over 14 months

Social group	Group 1			Group 2			Group 3		Group 4	
Animal	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)
Age (years)	10	15	15	15	5	5	5	5	10	5
June/15	−1.90	−0.87	0.35	−0.66	−1.66	−1.42	−0.41	0.15	0.14	−2.04
July/15	−3.80	−1.16	0.05	−1.15	−2.57	−1.41	0.82	0.14	−0.37	−1.75
Aug/15	−0.74	−1.03	−0.11	−1.27	−0.69	−1.62	−1.40	−0.07	−0.89	−0.44
Sept/15	0.37	−1.08	−0.52	0.11	0.10	−1.36	−1.87	−2.01	−1.24	−0.19
Oct/15	0.98	−0.08	−0.81	−2.07	−0.21	−0.56	−2.50	−1.85	−0.41	0.24
Nov/15	1.46	−1.44	−0.82	−0.52	−0.50	−0.81	−1.50	−1.60	−0.35	0.12
Dec/15	1.48	−1.23	−0.84	−0.43	0.78	−0.71	−1.00	−1.26	−0.01	0.33
Jan/16	1.76	−1.14	−1.84	1.39	1.10	−0.08	0.46	−0.64	−0.23	0.33
Feb/16	0.22	−0.67	−1.17	1.83	1.79	1.16	−0.08	−0.31	1.10	0.83
Mar/16	−1.17	3.93	1.23	2.93	1.65	1.94	0.74	3.00	2.45	1.70
Apr/16	−0.11	2.63	1.90	1.35	1.74	2.85	2.02	3.16	3.04	2.34
May/16	0.18	1.97	4.38	0.71	2.00	3.17	2.21	1.69	0.43	1.44
June/16	1.02	0.60	0.09	−0.76	−0.97	−0.48	1.55	0.47	−1.25	0.93
July/16	0.25	−0.42	−1.89	−1.45	−2.56	−0.66	0.95	−0.87	−2.41	−3.84

Note: The FI values of each individual and the months that the animals were fatted are highlighted (Group 1 all-males, Groups 2, 3, and 4 mixed-sex).

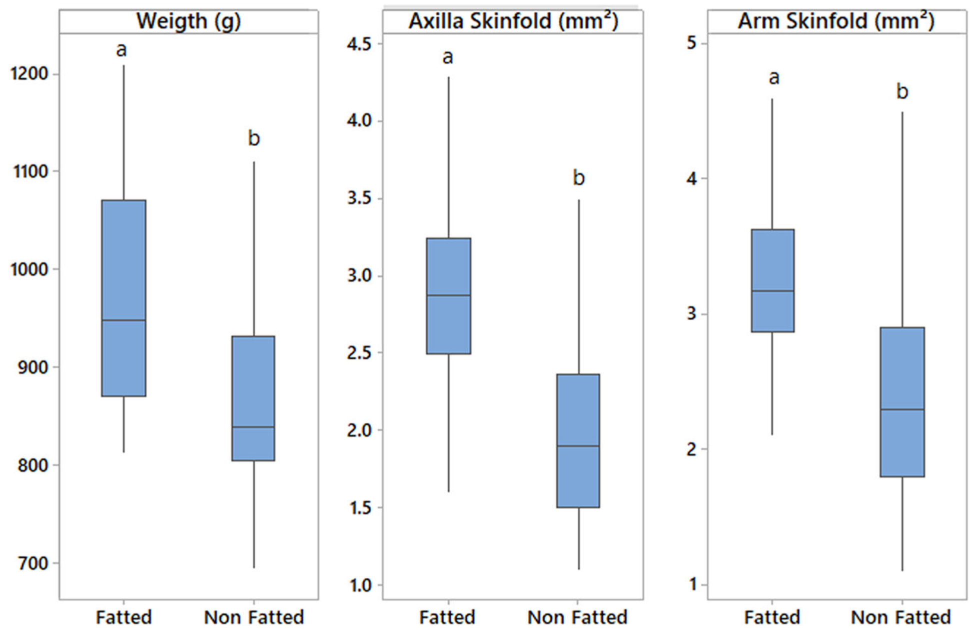

FIGURE 1 Box plot of the biometric body parameters (mean ± *SEM*) of 10 male squirrel monkeys (*Saimiri collinsi*) in fatted and non-fatted conditions. ab Different letters indicate significant statistical differences among fatted and non-fatted conditions ($p < 0.05$)

3.2.1 | Comparison of testicular volume and seminal quality between non-fatted and fatted conditions

A total of 140 data points were collected (14 per animal) for testicular volume, a dataset corresponding to 106 and 34 measurements for non-fatted and fatted conditions, respectively. We attempted to collect ejaculate samples in all 14 months of the experiment. From the 140 electroejaculation attempts, 114 (81.4%) semen samples were obtained, of which 88 (77.2%) were collected from males in the non-fatted condition, and 26 (22.8%) were from those in the fatted condition.

The success rate of obtaining ejaculates (Table 2) was higher in the non-fatted condition ($Z = 6.4403$, $p = 0.0001$). Cases of azoospermic semen differed significantly between conditions ($Z = 6.6255$, $p < 0.0001$), corresponding to 53.85% (14) of fatted samples and 18.2% (16) of non-fatted samples. Testicular volume ($Z = 5.7601$, $p < 0.0001$) and seminal volume ($Z = 4.5943$, $p < 0.0001$) both increased significantly during the fatted state, and seminal pH was alkaline for both conditions; however, during fattening, the pH tended to be approximately neutral ($Z = 7.3939$, $p < 0.0001$).

Interestingly, the presence of one or more semen coagulation degree in the same sample was sometimes observed. Coagulation

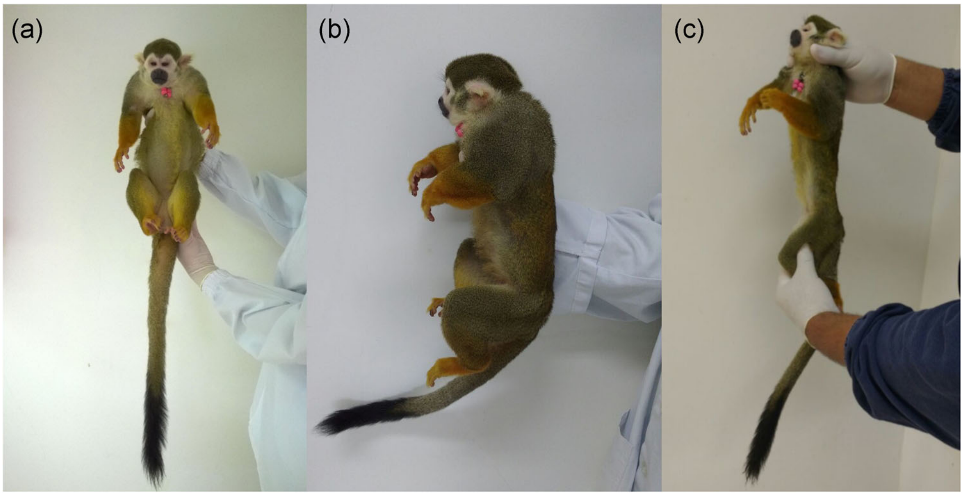

FIGURE 2 Fatted and non-fatted conditions in the same male squirrel monkey (*Saimiri collinsi*). (a) Frontal photo of fatted male, highlighting swollen or fat shoulders, arms and chest. (b) Profile of male expressing fatted, increased arm, and (c) the same animal in non-fatted condition

TABLE 2 Testicular volume, seminal parameters and degrees of seminal coagulation (mean, *SD* and *SE*), of squirrel monkeys (*Saimiri collinsi*) during 14 months: comparisons between non-fatted and fatted conditions

	Male condition		
Parameters	Non-fatted	Fatted	
Testicular volume (cm^3)	2.21 ± 0.96 (0.09)	2.92 ± 0.84 (0.15)	$Z = 5.7601$, $p < 0.0001$
Success in EJJ attempts (%)	83.02 ± 37.73 (3.66)	76.47 ± 44.06 (7.38)	$Z = 6.4403$, $p = 0.0001$
Presence of sperm in the semen (%)	81.82 ± 38.8 (4.14)	46.15 ± 50.84 (10)	$Z = 6.6255$, $p < 0.0001$
Seminal Volume (µl)	165.9 ± 148.8 (16)	227.3 ± 178.5 (35)	$Z = 4.5943$, $p < 0.0001$
pH	7.61 ± 0.55 (0.06)	7.29 ± 0.46 (0.09)	$Z = 7.3939$, $p < 0.0001$
Degree I (%)	88.64 ± 31.92 (3)	81 ± 40.2 (8)	$Z = 6.303$, $p < 0.0001$
Degree II (%)	37.5 ± 48.7 (5.2)	50 ± 51 (10)	$Z = 3.0957$, $p = 0.002$
Degree III (%)	31.82 ± 46.84 (5)	50 ± 51 (10)	$Z = 2.6234$, $p = 0.0087$
Degree IV (%)	10.23 ± 30.5 (3.25)	27 ± 45.23 (9)	$Z = 0.4395$, $p = 0.6603$

degree I was present in 86.8% (99) of the samples over 14 months. Significant differences were observed in coagulation degree I ($Z = 6.303$, $p < 0.0001$), which was more frequent in non-fatted samples, while coagulation degrees II ($Z = 3.0957$, $p = 0.002$) and III ($Z = 2.6234$, $p = 0.0087$) were more frequent in fatted samples. In contrast, no significant differences in frequency between non-fatted and fatted samples were found for coagulation degree IV ($Z = 0.4395$, $p = 0.6603$), as shown in Table 2.

3.2.2 | Comparison of age and social context on body biometry and semen

We also assessed whether different social contexts and age affected biometric parameters involved in the discrimination between non-fatted and fatted conditions and the related seminal characteristics. For the analysis of variation between the different age groups, we observed significant differences only for arm skinfold and testicular volume. The animals in the two first age classes (5–6 and 10–12 years old) presented a thicker skinfold of the arm than those in the 15-year-old group ($p < 0.0001$). The opposite was observed for testicular volume, wherein the animals of the first two age classes had lower testicular volume compared to those in the 15-year-old group ($p = 0.0003$). Considering the variation in all body and seminal parameters measured between the social contexts of the experimental groups, only weight and testicular volume showed a significant difference. The group composed of only males was heavier ($Z = 3.5218$, $p = 0.0004$) and had a higher testicular volume ($Z = 3.083$, $p = 0.0022$) (Tables 3 and 4).

4 | DISCUSSION

The individual males analyzed in the present investigation differed in the intensity and duration of their fattened condition. Thus, only by evaluating biometric parameters (weight, axilla, and arm skinfolds) and developing the FI were we able to accurately identify the fatted

TABLE 3 Body biometric and seminal parameters and degrees of seminal coagulation (mean, *SD*, and *SE*) of squirrel monkeys (*Saimiri collinsi*) during 14 months: comparisons between social context

Parameters	Social context Only male exp. gr. (*n* = 3)	Mixed exp. gr. (*n* = 7)	
Weight (g)	957.30 ± 117.40 (18.1)	911.90 ± 191 (19.3)	W = 3735.5 *p* = 0.0004
Axilla skinfold (mm²)	2.39 ± 1.07 (0.16)	2.20 ± 0.63 (0.06)	W = 2978 *p* = 0.940
Arm skinfold (mm²)	2.80 ± 1.13 (0.17)	2.70 ± 0.92 (0.09)	W = 2936 *p* = 0.911
Success in EJJ attempts (%)	83.30 ± 37.70 (5.8)	80.60 ± 39.70 (4.01)	W = 3017 *p* = 0.708
Presence of sperm in the semen (%)	80 ± 40.60 (6.86)	70.9 ± 45.72 (5.14)	W = 2138 *p* = 0.312
Testicular Volume (cm³)	2.7 ± 0.94 (0.14)	2.24 ± 0.97 (0.09)	W = 3636 *p* = 0.002
Seminal Volume (µl)	191.4 ± 173.4 (29.30)	174.7 ± 150.7 (17)	W = 2004.5 *p* = 0.963
pH	7.53 ± 0.54 (0.05)	7.54 ± 0.56 (0.06)	W = 9733.5 *p* = 0.992
Degree I (%)	91.43 ± 28.40 (4.80)	84.8 ± 36.12 (4.06)	W = 2104 *p* = 0.334
Degree II (%)	37.14 ± 49.02 (8.30)	41.7 ± 49.63 (5.60)	W = 1948.5 *p* = 0.646
Degree III (%)	31.43 ± 47.10 (7.90)	37.97 ± 48.84 (5.50)	W = 1922 *p* = 0.506
Degree IV (%)	8.47 ± 28.40 (4.80)	16.46 ± 37.31 (4.20)	W = 1903.5 *p* = 0.268

TABLE 4 Body biometric and seminal parameters and degrees of seminal coagulation (mean, *SD*, and *SE*), of squirrel monkeys (*Saimiri collinsi*) during 14 months: comparisons between ages

Parameters	Age (years) 5–6 (*n* = 5)	10–12 (*n* = 2)	15 (*n* = 3)	
Weight (g)	945.9 ± 213.9 (25.6)	929.5 ± 152.4 (28.8)	888.2 ± 84.9 (13.1)	H = 0.1 *p* = 0.953
Axilla skinfold (mm²)	2.3 ± 0.65 (0.07)	2.5 ± 1.18 (0.22)	2.04 ± 0.65 (0.1)	H = 4.27 *p* = 0.118
Arm skinfold (mm²)	2.9 ± 1 (0.12)	3.1 ± 1.16 (0.22)	2.2 ± 0.52 (0.08)	H = 19.17 *p* < 0.0001
Success in EJJ attempts (%)	77.14 ± 42.3 (5.06)	89.3 ± 31.5 (5.95)	83.3 ± 37.72 (5.82)	H = 2.08 *p* = 0.354
Presence of sperm in the semen (%)	72.22 ± 45.21 (6.15)	72 ± 45.83 (9.17)	77.14 ± 42.6 (7.2)	H = 0.31 *p* = 0.857
Testicular Volume (cm³)	2.25 ± 1.08 (0.13)	2.03 ± 0.36 (0.07)	2.8 ± 0.94 (0.14)	H = 15.84 *p* = 0.0003
Seminal Volume (µl)	165.1 ± 154.6 (21)	234.4 ± 166.5 (33.3)	163.7 ± 150.7 (25.5)	H = 4.26 *p* = 0.119
pH	7.45 ± 0.55 (0.1)	7.6 ± 0.52 (0.1)	7.62 ± 0.54 (0.1)	H = 2.64 *p* = 0.267
Degree I (%)	83.3 ± 37.62 (5.12)	88 ± 33.2 (6.63)	91.43 ± 28.4 (4.8)	H = 1.24 *p* = 0.537
Degree II (%)	35.2 ± 48.2(6.56)	52 ± 51 (10.2)	40 ± 49.7 (8.4)	H = 1.99 *p* = 0.369
Degree III (%)	44.4 ± 50.16 (6.83)	36 ± 49 (9.8)	22.86 ± 42.6 (7.2)	H = 4.26 *p* = 0.119
Degree IV (%)	16.67 ± 37.62 (5.12)	12 ± 33.2 (6.63)	11.43 ± 32.3 (5.46)	H = 0.59 *p* = 0.745

condition. This was true even for animals whose variation in body condition was not visually perceptible, evidencing that all males experienced this phenomenon to some degree.

In our study, fattening mainly occurred in March, April, and May, suggesting that this is the seasonal fattening period for *S. collinsi* in captivity, where all management conditions are maintained at constant levels throughout the year. They are kept under similar photoperiod, humidity, and temperature conditions as those found in eastern Amazonia, where this species is naturally distributed. In nature, the mating season of this species extends from July to August and coincides with male seasonal fattening (Stone, 2014).

Previous observations have suggested that male fattening in squirrel monkeys could be a product of pre-copulatory selection, including female choice and male–male competition (*S. oerstedii*, Boinski, 1987, 1992; *S. collinsi*, Stone, 2014). However, the evidence gathered in the present study suggests that fattening in *S. collinsi* may also have implications for post-copulatory sexual selection in the form of sperm competition. Female squirrel monkeys copulate with more than one male, which might lead to significant variations in fertilization success (Dixson, 1995, 2018). Early investigations on squirrel monkey reproduction reported an association between the activation of testicular spermatogenesis and fattening (DuMond &

Hutchinson, 1967; Belt & Cavazos, 1971; Nadler & Rosenblum, 1972).

Larger testes that are heavier in proportion to male body weight tend to be found among species with polygamous mating systems in multimale–multifemale groups (Baker & Shackelford, 2018; Dixson, 1995), even when the effect of seasonality is removed. Therefore, in species with mating systems that favor sperm competition, testicles still tend to be larger and heavier regardless of the level of reproductive seasonality (Harcourt et al., 1995). As a result of intense mitotic and meiotic activity, the lumen of the seminiferous tubules becomes completely occupied by cells at all stages of spermatogenesis and consequently increases in diameter, as previously reported in *S. sciureus* (DuMond & Hutchinson, 1967; Belt & Cavazos, 1971). This leads to faster rates of spermatogenesis and sperm production (Amann & Schanbacher, 1983; Kappler, 1997), resulting in increased testicular volume (Schlatt & Ehmcke, 2014), as found in the present study for males in the fatted condition (Table 2).

Increased seminal volume in fatted males can be a response to testicular activity, since the testicles, in addition to producing sperm, are also responsible for the production of semen components and hormones (Møller, 1988). These act in the sexual glands, stimulating the production and secretion of seminal fluid required to suspend and maintain sperm, and for buffering the vaginal pH after ejaculation (Dixson, 1998). This can also be a response to the increased need for semen coagulation. Among the components of the seminal fluid, seminogelins, proteins produced by the seminal vesicles, are important for the formation of the seminal coagulum, of which the consistency varies from a non-gelatinous fluid state (grade I) to the maximum solidification state (Grade IV) (Dixson & Anderson, 2002). This coagulum plays a central role in post-copulatory sexual selection (Dixson, 2018) as an evolutionary mechanism in response to sperm competition (Dixson & Anderson, 2002). In polygamic mating systems, where sperm competition is intense, such as in squirrel monkeys (Dixson & Anderson, 2002), seminal coagulation tends to produce a solid consistency semen, corresponding to Grades III and IV. We observed that seminal coagulum of Grades II and III were obtained more frequently from males in the fatted state, while Grade I was more frequently observed for males in the non-fatted state (Table 2). Seminal coagulum can develop a physical barrier that prevents the intrusion of the next penis, blocking the vaginal orifice of females of various species, a structure usually known as copulatory plug (Dunham & Rudolf, 2009; McCreight et al., 2011), a structure never observed in squirrel monkeys. However, in many species, the seminal coagulum does not form copulatory plugs, but can still play an important role in post-copulatory sexual selection via sperm competition (Flores-Herrera et al., 2012; Kinoshita et al., 2021; Lima et al., 2017; Sousa et al., 2021). There are many reproductive advantages related to this coagulum (Dixson, 1998, 2012, 2018; Hernández-López et al., 2008; Lima et al., 2017; Miller & Kurzrok, 1932; Suarez & Pacey, 2006); it promotes the probability of fertilization, and consequently, reproductive success. In addition to our results (Table 2), recent studies on these aspects of *S. collinsi* showed that the seminal coagulum alkalinity may be responsible for neutralizing vaginal acidity and conferring protection to the sperm. Thus, allowing sperm to remain in the female reproductive tract for longer periods, until the most suitable time for fertilization (Oliveira et al., 2016a, 2016b). Therefore, during the fattening period, the higher frequency of coagulation degrees II and III, identified in the present study, can reveal an adaptation providing an advantage to gametes, increasing their chance of fertilization in periods when sperm competition increases.

As observed in our results (Tables 3 and 4), age and social context are not significant sources of variation for both the morphological and seminal traits. Except arm skinfold, body weight, and testicular volume, the other main morphological and seminal characteristics involved in alterations in reproductive potential were not affected by social context and age. Reductions in body mass with the aging of primate species (Hämälĺinen et al., 2014; Kirkwood, 1985) were observed in this study as the loss of muscular tissue and increase in arm skinfold of older males, similar to that observed for other primate species (Alberts et al., 2013). Apparently, the animals were not in the age of reproductive senescence, since no reduction in testicular volume was found as the animals aged. Actually, testicular volume showed a trend to increase in older males, and also observed in all male groups. However, in both cases, the increase in testicular volumes was not reflected in the seminal volumes, as observed in fatted males (Table 2) as well as other seminal parameters, thus, the link between morphological and seminal traits that would guarantee a possible influence on postcopulatory sexual selection patterns was not significant.

Males of species that face a high probability of sperm competition are more likely to masturbate (Thomsen et al., 2003). Copulation frequency is influenced by attractive female sexual stimuli, and in nonhuman primates, in the context of captivity or living in the wild, masturbation is considered a compensatory act, given the frequent lack of mating opportunities during sexual arousal (Harrison, 1980). In captivity, this may occur when the males are housed without females (e.g., *Macaca arctoides*: Nieuwenhuijsen et al. 1986, 1987), especially when there is visual contact with unattainable females in a neighboring cage (e.g., *M. arctoides*: Linnankoski et al., 1993; *M. fuscata*: Thomsen et al., 2003). The animals in our study, regardless of the social context of the experimental groups, maintained continuous visual, auditory, and olfactory contact with the other animals in the CENP facility, which may have favored an increase in masturbation, and consequently, interfered with ejaculations during the fatted state, resulting in a similar effect for all experimental groups. Thus, as an unexpected result, we found that males in the fatted condition had more azoospermic samples.

Studies have shown that the frequency of ejaculation and the interval between ejaculations can affect semen characteristics (Chenoweth & Lorton, 2014; Welliver et al., 2016). The male refractory period, which follows ejaculation, is one of the factors that leads to the absence of erection and expulsion of the seminal fluid (Chenoweth & Lorton, 2014; Welliver et al., 2016). The male refractory period is believed to occur through a negative feedback mechanism in which there is a delay in the expulsion of seminal fluid by seminal vesicles (Turley & Rowland, 2013). Factors such as repeated copulations or frequent semen collection can result in a decreased concentration or even the absence of sperm due to the depletion of extragonadal reserves in the epididymis (Amann & Schanbacher, 1983; Dixson, 1995; Torres et al., 2016; Welliver et al., 2016). The opposite can also occur when males are sexually inactive, or if they less frequently copulate and they tend to show higher sperm concentrations (Chenoweth & Lorton, 2014; Welliver et al., 2016). We observed a negative response from the fatted male in obtaining semen by electroejaculation together with an increase in the frequency of azoospermic ejaculates. There was a high probability that the electroejaculation occurred in the refractory period of the individual or in a period shortly after copulation, which may have negatively affected the animal's response to electroejaculation. This may have affected the level of sperm present in the semen when the individual was in the fatted state (Table 2). In future studies, maintaining males in a state of sexual rest before semen collection could be a solution to generate more accurate information on the fluctuations in seminal parameters.

5 | CONCLUSION

We developed a fattening index that allowed a more comprehensive understanding of the seasonal morphological changes in *S. collinsi* males, and the subsequent identification of individuals that were in the fatted and non-fatted states. The index developed here can be useful for future research aiming to explore how sexual selection acts upon these species. There was a clear seasonal period of fattening observed in the social groups, and all animals manifested this phenomenon to different degrees. Our results indicated that the fattening condition is associated with higher seminal quality in the form of higher seminal volume, neutral seminal pH, and firmer ejaculate coagulation. We found that *S. collinsi* males in the fatted condition had a larger testicular volume compared with non-fatted males, with a direct relationship with the increase in spermatogenic activity. We observed an increase in the frequency of ejaculates with coagulation degrees II and III among fatted males. These factors could be seen as advantages for the protection of sperm in the female reproductive tract. Our results suggest that fattening in squirrel monkey males may also be related to sperm competition, and that fatted males have important advantages in the production and survival of sperm, with increased fertilization probability. In fatted males, azoospermic ejaculate was more frequent, probably due to the increased frequency of ejaculation. However, our results do not explain why males have to produce such an expansive advertisement of their reproductive potential. The fattening phenomenon has many implications in the sexual selection of squirrel monkeys, and they are yet to be completely understood. Nevertheless, our results corroborate the idea that, in *S. collinsi*, the fatted male condition is indeed related to sexual selection, and we found evidence that suggests it may be somehow also expressed by a post-copulatory component, sperm competition.

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CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

AUTHOR CONTRIBUTIONS

Wlaisa Vasconcelos Sampaio: conceptualization (equal); data curation (equal); formal analysis (equal); investigation (equal); methodology (equal); writing – original draft (equal); writing—review & editing (equal). Danuza Leão Conceptualization-Equal, Data curation-Equal, Investigation-Equal, Methodology-Equal, Writing-original draft-Equal, Writing-review & editing-Equal. Patrícia Sousa Conceptualization-Equal, Data curation-Equal, Investigation-Equal, Methodology-Equal, Writing-original draft-Equal, Writing-review & editing-Equal. **Helder Lima de Queiroz**: conceptualization (equal); investigation (equal); supervision (equal); writing—original draft (equal); writing—review & editing (equal). **Sheyla Domingues**: conceptualization (equal); funding acquisition (equal); investigation (equal); project administration (equal); resources (equal); supervision (equal); writing—original draft (equal); writing—review & editing (equal).

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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