Theriogenology 84 (2015) 743–749

Contents lists available at ScienceDirect

Theriogenology

journal homepage: www.theriojournal.com

Seminal characteristics and cryopreservation of sperm from the squirrel monkey, *Saimiri collinsi*

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ARTICLE INFO

Article history:
Received 9 January 2015
Received in revised form 20 April 2015
Accepted 22 April 2015

Keywords:
Squirrel monkey
Semen
Freezing
Coconut water
Glycerol

ABSTRACT

The Neotropical nonhuman primate squirrel monkey (*Saimiri* sp.) is one of the most commonly used species in research in several areas of knowledge. However, little progress has been reported in respect to techniques for preservation of their gametes. Thus, the main objectives of this study were (1) to describe testicular and seminal aspects of a new species, *Saimiri collinsi*, (2) to preserve semen of this species by cooling or freezing using ACP-118 (powdered coconut water), and (3) to test two glycerol (GLY) concentrations (1.5% or 3%) for semen freezing in the presence of ACP-118. The experimental group started with 14 captive males, but only 11 were suitable to collect ejaculates containing sperm. After anesthesia, both testes were evaluated: length, width, height, and testicular circumference. Semen was collected by electroejaculation and evaluated, followed by dilution, cooling, and freezing. Seminal parameters and sperm motility, vigor, plasma membrane integrity, and normal morphology were evaluated after each step; functionality was also checked in fresh and frozen–thawed sperm. Sperm motility, plasma membrane integrity, and normal sperm in cooled semen ($n = 11$) were 44.1 ± 34.0, 63.1 ± 15.6, and 73.8 ± 19.8, respectively, with vigor ranging of 2 to 3. Sperm motility, plasma membrane integrity, normal and functional sperm in frozen semen ($n = 5$) were 0.6 ± 1.3 (1.5% and 3% GLY); 4.4 ± 4.9 (1.5% GLY) and 6.6 ± 7.2 (3% GLY); 86.8 ± 3.0 (1.5% GLY) and 88.8 ± 5.1 (3% GLY); 13.3 ± 11.9 (1.5% GLY) and 14.3 ± 13.5 (3% GLY), respectively, and vigor 0 for both 1.5% and 3% GLY. No significant difference between GLY concentrations was observed. We concluded that electroejaculation was efficient for semen collection of *S collinsi* and tested the cooling protocol that allowed to recover a satisfactory percentage (63%) of membrane intact sperm. However, the freezing protocol was not appropriate to sperm preservation.

1. Introduction

Neotropical nonhuman primates (NHP) have been facing serious conservation problems due to a drastic decrease in their population or even extinction of species, sometimes before being characterized [1,2]. To overcome or control this genetic loss, it is crucial to preserve and to restore preservation areas. In the meanwhile, preserving germplasm of NHP appears as an alternative to safeguard genetic material for future support in maintaining biodiversity [3].

Cryopreservation of male gametes has been described for different NHP species [4–16], with glycerol (GLY) being the most used permeable cryoprotectant at variable concentrations (2.5%–14%) and TES-TRIS as the extender (Table 1). Besides TES-TRIS, a coconut water solution for semen coagulum liquefaction appears as a promising extender [16]. A standardized powdered coconut water (ACP-118; ACP

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http://dx.doi.org/10.1016/j.theriogenology.2015.04.031

Table 1
Commonly used extenders and glycerol concentrations for the cryopreservation of nonhuman primate semen.

Species	Extender	Cryoprotectant
Callithrix jacchus	TES-TRIS [4]	3% Glycerol
Cercopithecus aethiops	Sodium glutamate [5]	14% Glycerol
Erythrocebus patas	Sodium glutamate [5]	14% Glycerol
Macaca fascicularis	TRIS [6]	5% Glycerol
	TES-TRIS [7]	3% Glycerol
	TRIS [8]	6% Glycerol
Macaca fuscata	TES-TRIS [9]	5% Glycerol
Macaca mulatta	Sodium glutamate [5]	14% Glycerol
	TES-TRIS [10]	12% Glycerol
	TES-TRIS [10]	3% Glycerol
	TRIS [11]	5% Glycerol
Macaca speciosa	Sodium glutamate [5]	14% Glycerol
Papio anubis	Sodium glutamate [12]	Egg yolk
	TES-TRIS [4]	3% Glycerol
Pan troglodytes	Sodium glutamate [5]	14% Glycerol
	TES-TRIS [13]	5% Glycerol
	TES-TRIS [13]	2.5% Glycerol
	TES-TRIS [4]	3% Glycerol
Saimiri boliviensis	TES-TRIS [14]	8% Glycerol and egg yolk
Saimiri sciureus	Lactose [15]	4% Glycerol and egg yolk
Sapajus apella	TES-TRIS [16]	3.5% Glycerol and egg yolk
	CWS [16]	2.5% Glycerol and egg yolk

Abbreviations: CWS, coconut water solution; TES: 2-[(2-hydroxy-1,1-bis(hydroxymethyl)ethyl)amino]ethanesulfonic acid; TRIS, Tris(hydroxymethyl)aminomethane.

Biotecnologia, Fortaleza, Ceará, Brazil), which contains vitamins, minerals, amino acids, carbohydrates, growth factors, phytohormones, and saturated fatty acids [17], has been successfully used as a semen extender in other animal species such as dogs [18,19], stallions [20], fish [17,21], agoutis [22], and collared peccary [23]. Therefore, we considered it as a candidate to be tested in the NHP *Saimiri collinsi*.

Among the NHP species, the squirrel monkey (*Saimiri* sp.) has been used in laboratory studies for more than 40 years, resulting in vast information on their physiology and reproduction [24], but little is known in respect to gametes preservation. After a recent taxonomic review through molecular studies [25,26], *S collinsi* has been recognized as a new species. Up to now, *S sciureus* and *S boliviensis* are the most studied species [27,28], from which semen obtained by electroejaculation (EEJ) and penile vibratory stimulation (PVS) was characterized [5,15,29–33] (Table 2). Only two studies have described semen cryopreservation for these two species [14,15]. No seminal description or semen preservation techniques are available for *S collinsi*.

We aimed to describe seminal characteristics of the new species, *S collinsi*, to test cooling and freezing protocols using ACP-118 as the extender and to test two GLY concentrations (1.5% or 3.0%) for the cryopreservation of *S collinsi* semen.

2. Materials and methods

2.1. Animals and semen collection

This study was conducted with institutional approval from Ministério do Meio Ambiente, Sistema de Autorização e Informação em Biodiversidade—SISBIO/ICMBio/MMA n° 31542-2 and by the Ethical Committee in Animal Research of Instituto Evandro Chagas (n° 0010/2011/CEPAN/IEC/SVS/MS).

The males were originated from the Marajó Archipelago (0°58′S and 49°34′W) and were maintained in captivity at the National Primate Center (CENP), Ananindeua, Brazil (1°22′58″S and 48°22′51″W) [34]. The climate is humid tropical, with an average annual temperature of 28 °C. The experimental group (n = 14 males; ~15 years) was selected by physical characteristics, clinical parameters such as complete hemogram and blood biochemical analyses. Animal body weight, external genitalia, and andrologic examination, i.e., inspection and palpation of the testes to verify size, consistency, symmetry, and mobility, were also used to select animals.

Animals were collectively housed in cages of 4.74 × 1.45 × 2.26 m (length, width, and height, respectively), under natural photoperiod (12 hours of light and 12 hours of dark). The diet consisted of fresh fruits, vegetables, milk, commercial pellet chow for primates, and cricket larvae (*Zophobas morio*); water was available *ad libitum*.

Physical restraint was performed by a trained animal caretaker wearing leather gloves. Semen was collected at the same period of the day, i.e., in the morning before feeding. After physical restraint, animals were anesthetized with ketamine hydrochloride (15 mg/kg, intramuscular;

Table 2
Mean (±standard deviation) or range values of seminal volume (μL), sperm concentration ($\times 10^6$ sperm/mL), plasma membrane integrity (%), sperm motility (%), and normal sperm morphology (%) in fresh semen collected from *Saimiri sciureus* and *S boliviensis*.

Species	Seminal volume	Sperm concentration	Sperm plasma membrane integrity	Sperm motility	Normal sperm Morphology
S sciureus[a]	50.8–81.3 [29]	106	NI	NI	NI
	100 [30]	0.295 ± 13	79 ± 2	NI	NI
	400 [5]	205.9	NI	52	NI
	NI [31]	153 ± 96	36.8 ± 19	35 ± 11.8	55.8
	159.5 ± 57 [15]	427.3 ± 160.6	NI	65.9 ± 15.4	NI
	80–300 [32]	NI	NI	40–80	<51
S boliviensis[a]	205 ± 25 [33]	2.8 ± 1.7	NI	44.1 ± 11.4	NI
S boliviensis[b]	436 ± 90 [33]	77.1 ± 20.4	NI	80.6 ± 4.3	NI

Abbreviation: NI: not informed.
[a] Semen was collected by penile electroejaculation.
[b] Semen was collected by penile vibrostimulation.

Vetanarcol; Köning S.A., Avellaneda, Argentina) and xylazine hydrochloride (1 mg/kg, intramuscular; Köning S.A.) by a veterinarian. Achieved total anesthetic effect, the animals were placed in dorsal recumbence; both testes were evaluated, and length (cranial–caudal), width (medial–lateral), height (dorsal–ventral), and total testicular circumference were measured. Genital region was then sanitized with a mild soap and distilled water (1:10) and gauze. Prepuce was retracted with the thumb and index fingers for an efficient cleaning of the penis.

Males were stimulated by EEJ (Autojac; Neovet, Uberaba, Brazil) with a rectal probe of 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 width) on opposite sides [29]. The probe was smeared with a sterile lubricant jelly (KY Jelly, Johnson & Johnson Co., Arlington, TX, USA), introduced in the rectum (~2.5 cm deep), and electrical stimuli were delivered. The stimulation session consisted of three series (7–8 minutes), composed of 35 increasing electrical stimuli (12.5–100 mA) with an interval of 30 seconds between series [16]. If a male was unable to ejaculate after the session, no further attempts were made to collect semen, and intervals between semen collections were at least 30 days. A veterinarian monitored continually the animals during as well as after recovering from anesthesia.

2.2. Semen extenders

Two extenders were prepared containing A and B fractions. A-fraction consisted of 5.84-g ACP-118 (ACP Biotecnologia) diluted in 50-mL ultrapure water. B-fraction was constituted by 60% A-fraction plus 40% egg yolk. Final concentration of egg yolk was 20%. Osmolarity of A and B fractions was 300 and 353 mOsm/L, respectively. Egg yolk was obtained at the University Farm School, from chicken (*Gallus gallus domesticus*) eggs laid not more than 12 hours.

2.3. Seminal evaluation

One and a half milliliter conical Eppendorf tubes containing the semen were placed in a water bath at 37 °C immediately after ejaculation. Volumes of liquid and coagulated fractions were evaluated in a graduated tube, with the aid of a pipette. Appearance and consistency were assessed subjectively, i.e., color (colorless, yellowish, or whitish), opacity (opaque or transparent), and appearance (amorphous or filamentary seminal coagulum). All evaluations were performed under a light microscope (Nikon, Tokyo, Japan), at a magnification × 100. Sperm vigor was subjectively evaluated on a scale of 0 to 5 [15]. In brief, no motility was considered 0, slight movement with greater than 75% of sperm showing vibration only was represented by 1, moderate forward movement in about greater than 50% of sperm was represented by 2, forward movement in about 70% of sperm was represented by 3, and when ~90% or greater than 95% of sperm presented very active forward movement, scales 4 and 5 were used. 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 [3]. To measure the percentage of progressive forward motility, 10 μL of semen was placed in a prewarmed (37 °C) glass slide with a coverslip and 200 sperm were counted, as described by Oliveira et al. [16]. Sperm concentration was determined in a Neubauer chamber after dilution of 1-μL semen in 99-μL formalin solution 10%. Sperm morphology and integrity of plasmatic membrane were evaluated by a smear prepared adding 5 μL of eosin–nigrosin stain (Vetec, Rio de Janeiro, Brazil) to 5 μL of semen on a prewarmed (37 °C) glass slide. Morphologic defects detected in sperm were classified as primary or secondary [16]. Sperm membrane functionality was assessed by the hypo-osmotic swelling test after dilution of 5 μL of semen in 45 μL of hypo-osmotic solution (108 mOsm/L). Seminal pH was measured with a pH strip (Merck Pharmaceuticals, Darmstadt, Germany). Semen was assessed directly after collection (fresh), after dilution and before cooling (precooled), after cooling, and after freezing in the presence of 1.5% or 3% GLY.

2.4. Semen cooling and freezing

After evaluation, fresh semen was diluted in A-fraction (1:1), placed in a water bath at 37 °C until a sufficient volume of liquid fraction for cooling (above 50 μL) was obtained; this procedure took approximately 1 hour. This limiting time of dissolution was chosen to preserve sperm motility. Seminal samples were diluted in B-fraction (1:1) and microscopically evaluated. Only samples (n = 11) presenting at least motility of 20%, vigor of 3, 50% of sperm membrane integrity, and 30% of normal sperm morphology were cooled. Samples were cooled in covered microtubes, following a curve of 37 °C to 4 °C within 1.5 hours. After cooling, sperm was evaluated as described previously. Cooled semen samples (n = 5) that displayed the least 5% motile sperm, sperm vigor of 2 or more, plasma membrane integrity of 40%, and with a minimum of 30% sperm with normal morphology were then cryopreserved. For this, cooled semen was divided into two equal aliquots with a range in sperm concentration of 13 to 29 × 10^6 sperm/mL. To one aliquot was added GLY to a final concentration of 1.5% and to the other aliquot to a final concentration of 3% GLY. This cryoprotectant was added directly to the semen in three steps, with 30 seconds interval each. Thereafter, aliquots were drawn into 0.12-mL plastic straws (IMV, L'Aigle, France), sealed with a metal bead and stored horizontally in vapor of liquid nitrogen (−60 °C) for 20 minutes, and then plunged into liquid nitrogen (−196 °C). After 1 month storage, straws were kept in a water bath (37 °C) for 30

Table 3
Mean (±standard deviation) of length, width, height, volume of right and left testicles and total testicular circumference of *Saimiri collinsi* (n = 13 males).

Testicle	Length (cm)	Width (cm)	Height (cm)	Volume (cm^3)	Total volume (cm^3)	Total circumference (cm)
Right	1.86 ± 0.21	1.23 ± 0.11	1.16 ± 0.05	1.40 ± 0.26	2.96 ± 0.63	7.38 ± 0.42
Left	1.83 ± 0.24	1.34 ± 0.14	1.18 ± 0.09	1.55 ± 0.42		

Table 4
Mean (±standard deviation) values of ejaculates (number), sperm motility (%) and vigor (grade), sperm plasma membrane integrity (PMI; %), normal sperm morphology (NSM; %), sperm concentration ($\times 10^6$sperm/mL), and seminal pH of fresh semen collected.

Animal	Ejaculates	Motility	Vigor	PMI	NSM	Concentration	pH
AAA	1	100	5	75	81	98	7.0
AHR	1	45	3	49	68	—	6.5
AIM	2	70 ± 28.3	5	86.5 ± 13.4	67 ± 8.5	50 ± 7.1	7.5
BAA	2	50 ± 56.6	4	67.5 ± 17.7	80.5 ± 0.7	—	7.5
BAD	1	85.0	5	90	75	68	7.0
BAH	2	80 ± 14.1	4	83.5 ± 16.3	69 ± 0.9	53 ± 15	8.0
BAJ	2	80 ± 14.1	5	72.5 ± 10.6	66 ± 0.6	69 ± 8.6	7.5
BAO	1	50	3	54	77	49	7.8
BBC	1	80	5	88	61	102	7.0
BEX	2	95 ± 27.1	5	83.2 ± 16.7	61 ± 6.0	87	7.5
BEZ	2	90 ± 14.1	5	90 ± 14.1	78	72 ± 9.6	7.5

Data from each male (n = 11).

seconds [10], and the thawed semen was microscopically evaluated.

2.5. Statistical analysis

All data are expressed as the mean ± standard deviation (SD) and analyzed by the StatView 5.0 program (SAS Institute Inc., Cary, NC, USA), except vigor, which was expressed as mode. The effect of storage temperature on sperm motility, plasma membrane integrity, morphology, and functionality was evaluated by ANOVA. Comparisons between cryopreservation steps were performed using Fisher's test. The same effects on vigor were evaluated using the Kruskal–Wallis test. $P < 0.05$ was considered as statistically significant.

3. Results

3.1. Testicular biometry and semen analysis

Selected males for this study (n = 14) were healthy, as confirmed by their complete hemogram and blood biochemical analyses (Supplementary Tables 1 and 2). Mean (±SD) body weight was 886 ± 113 g (620–1115 g; min–max). All animals presented testes with normal consistency, symmetry, and mobility. However, one male was diagnosed with unilateral cryptorchidism, and hence, it was removed from the experiment, despite the fact that measurements of its testis were equivalent to the average of the sample group. Testicular biometry of the 13 remaining males is presented in Table 3.

From the 13 males, one did not ejaculate. A total of 32 semen collection trials (at least two attempts in each of the 12 males) were performed resulting in 20 ejaculates (62.5%). From the 20 ejaculates, three did not contain sperm and one animal presenting azoospermia was excluded. Therefore, 17 ejaculates were used in the present study (see details in Table 4). Ejaculation was almost always initiated by a liquid fraction often partially or totally coagulated after 10 seconds. Mean (±SD) collected volume of liquid and coagulated seminal fractions were 51.8 ± 49.5 μL (5–200 μL) and 304 ± 283.6 μL (10–1100 μL), respectively. Liquid and coagulated fractions were transparent or opaque and colourless, whitish, or yellowish. It was observed a wide variation in appearance and constitution of ejaculates between collections regardless the

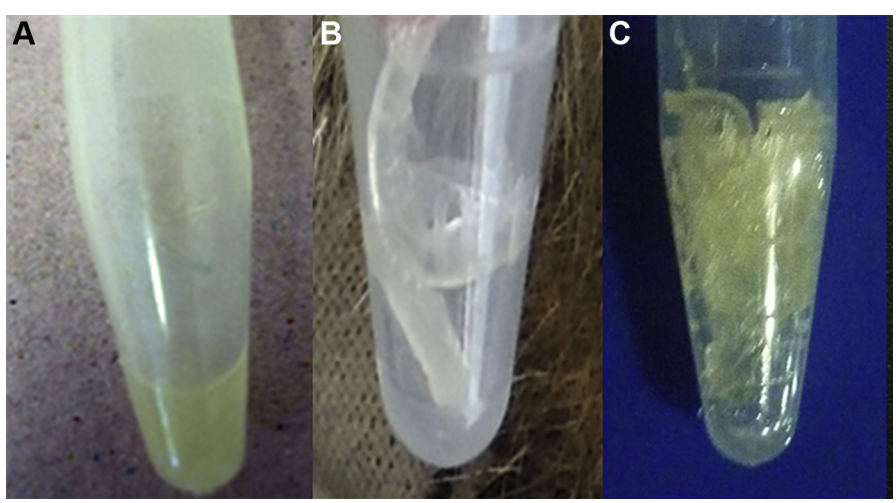

Fig. 1. Representative images of opaque liquid fraction (A), filamentary (B), and amorphous (C) fractions of semen collected from *Saimiri collinsi* by electroejaculation.

male. Coagulated fraction presented filamentary or amorphous appearance (Fig. 1). Microscopic parameters of collected fresh semen are depicted in Table 4.

3.2. Semen cooling and freezing

From the 20 ejaculates, 11 (55%) provided semen presenting sufficient quality to be cooled, i.e., at least sperm motility of 20%, vigor of 3, 50% of sperm membrane integrity, and 30% of normal sperm morphology. From the 11 cooled ejaculates, five (46%) provided semen presenting quality to be frozen, i.e., at least sperm motility of 5%, vigor of 2, 40% of sperm membrane integrity, and 30% of normal sperm morphology. Sperm motility and plasma membrane integrity were not affected during semen dilution (pre-cooled semen) when compared to fresh semen, but this procedure decreased ($P < 0.01$) vigor. Cooling, on the other hand, resulted in decreased ($P < 0.01$) sperm motility, vigor, and plasma membrane integrity when compared to fresh ($P < 0.001$) semen. Independently on the GLY concentration (1.5% or 3%), all the evaluated parameters (sperm motility, vigor, plasma membrane integrity, and functionality) were negatively affected ($P < 0.001$) by freezing, except the percentages of morphologically normal sperm that remained the same in all steps (Fig. 2). The most common observed sperm morphologic changes were coiled and strongly coiled tail (Table 5).

4. Discussion

Despite the studies on *S sciureus* and *S boliviensis* [14,15,29–33], this is the first report describing seminal and andrologic parameters of *S collinsi*. Mean body weight was similar to that of other *Saimiri* species [35], but the total

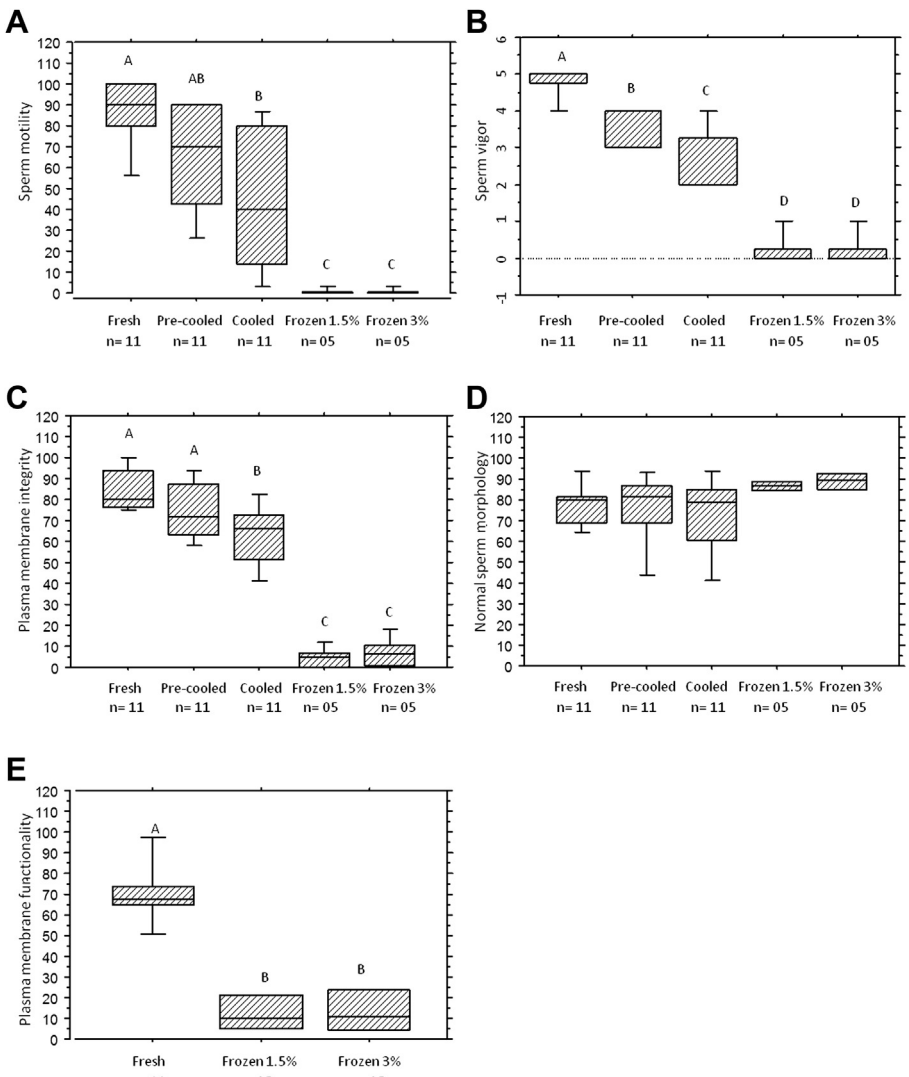

Fig. 2. Sperm parameters of fresh (n = 11), precooled (n = 11), cooled (n = 11), and frozen semen using 1.5% (n = 5) or 3% (n = 5) glycerol. (A) Sperm motility, (B) sperm vigor, (C) plasma membrane integrity, (D) normal sperm morphology, and (E) plasma membrane functionality (evaluated only in fresh and frozen–thawed sperm). [A–D]Different uppercase letters indicate significant differences among groups within each parameter ($P < 0.05$).

Table 5
Mean (±standard deviation) percentages of normal sperm and sperm morphology (major and minor pathologic defects) in fresh (n = 11 males) and frozen (n = 5 males) semen of *Saimiri collinsi* in 1.5% or 3% glycerol (GLY).

Morphology	Fresh	Frozen	
		1.5% GLY	3.0% GLY
Normal	74.7 ± 7.8	87 ± 1.4	89.5 ± 2.1
Major pathologic defects			
Strongly coiled tail	8.3 ± 13.6	5 ± 7.1	5.5 ± 7.8
Pear-shaped defect	0.33 ± 0.6	0	0
Minor pathologic defects			
Coiled tail	9.3 ± 5.5	5 ± 7.1	2.5 ± 3.5
Bent tail	7 ± 1.7	3 ± 1.4	2.5 ± 2.1
Axial tail	0.3 ± 0.6	0	0

testicular volume of *S collinsi* (2.96 ± 0.63 cm^3; mean ± SD) was greater than that in *S sciureus* (0.95–1.79 cm^3; min–max) [36].

Anesthetic and EEJ protocol applied during this experiment allowed semen collection, although only 55% of the ejaculates were of sufficient quality that they were then cooled. In a study by Yeoman et al. [33], PVS was found to improve sperm recovery compared to EEJ in *S boliviensis*. However, the use of PVS requires animal conditioning, which means its application in captive males. Although the present study was performed using captive *S collinsi*, our main goal was to develop a protocol to be applied under field conditions for free-living animals. In this case, semen collection must be performed without conditioning and, hence, by EEJ under anesthesia.

Microscopic characteristics of fresh semen was similar to those previously described for semen of *S sciureus* collected by EEJ [31] and of *S boliviensis* collected by EEJ and PVS [33], regarding plasma membrane integrity, sperm motility, morphology, and concentration. Seminal volume collected in this work was similar to that previously reported in *S sciureus* [5,15,30,32] and *S boliviensis* [33]. Seminal pH was similar as reported for *Ateles geoffroyi* [37]. There is no available report on the seminal pH in other *Saimiri* species.

Differently from *S sciureus* and *S boliviensis*, from whose collected semen (EEJ or PVS) coagulates immediately after ejaculation [29,33], we observed coagulation only 10 seconds after *S collinsi* semen collection, with part of the ejaculate remaining liquid. No relationship between ejaculate constitution and individual was observed. The time spent for incubation (maximum 60 minutes) with the extender did not affect sperm motility but appeared to contribute with an impairment of sperm vigor as previously observed in *Sapajus apella* [16]. Although a decrease in seminal quality after cooling was observed, the values of parameters are still acceptable and this procedure appears as a tool for the short-term storage of semen from *S collinsi*.

Unfortunately, the present freezing procedure was unable to preserve sperm quality. It is difficult to point out the exact cause of this failure once we were testing ACP-118 for the first time, but some explanations can be proposed. Sperm concentration in the frozen samples in the present study was ranging of 13 to 29 × 10^6 sperm/mL, which is low when compared to other NHP species submitted to a similar procedure. For instance, cryopreserved semen from common squirrel [15] and rhesus [38] monkeys often contains a sperm concentration of 50 to 100 × 10^6 sperm/mL, from which motility appears preserved after thawing. In the present study, centrifuging the semen to obtain a greater sperm concentration was leading to semen coagulation. Because of the low sperm concentration obtained, it was not possible to include extra treatments or experiments to compare ACP-118 with the routine extender TES-TRIS. This challenge should be solved in future studies. In some investigations with primate sperm, GLY was diluted in a part of the used medium before being added to the semen for cooling [4,6,10,16,39–41]. In the present study, GLY was added after cooling, based on a study with *Sapajus apella* sperm, where prolonged exposure to GLY was detrimental to sperm quality [16]. Maybe this also had affected the success of the present procedure.

4.1. Conclusions

In conclusion, EEJ was efficient for semen collection of squirrel monkeys and the tested cooling protocol allowed the recovery of a satisfactory percentage of sperm with intact plasma membrane. The possibility to preserve semen from *S. collinsi* at low temperatures, even for a short period, facilitates transport and handling of sperm from this Neotropical NHP. Although the present freezing protocol using ACP-118 was not appropriate for the preservation of *S. collinsi* sperm, future studies including the improvement of the sperm concentration and the adequate moment of cryoprotectant addition to the medium might help to develop an efficient freezing protocol for semen from these species.

Acknowledgments

This study was supported by the National Primate Center Centro nacional de primatas (CENP), Coordenação de aperfeiçoamento de pessoal de nível superior (CAPES) (Brazil), and Federal University of Pará (UFPA).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.theriogenology.2015.04.031

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Supplementary Table 1
Hemogram results from *Saimiri collinsi* males (n = 13).

Parameter	Results	Reference values
Hematocrit (%)	43.2 ± 3.6	44.00 ± 0.6
Red blood cells ($\times 10^6$/mL)	7.2 ± 0.4	7.1 ± 0.1
Hemoglobin (g/dL)	13.9 ± 1.2	13.8 ± 0.2
Mean corpuscular volume (fL)	59.9 ± 2.8	61.9 ± 0.6
Mean corpuscular hemoglobin (pg)	19.1 ± 1.1	19.4 ± 0.2
Mean corpuscular hemoglobin concentration (%)	31.8 ± 1.0	31.5 ± 0.2
White blood cells ($\times 10^3$/mL)	11.0 ± 4.1	10.5 ± 0.6
Platelets (m/mm^3)	268 ± 93	—
Basophils (%)	0.44 ± 0.7	0.0 ± 0.2
Eosinophils (%)	1.9 ± 0.9	1.0 ± 0.2
Neutrophils (%)	51.5 ± 11.7	35.0 ± 3.2
Lymphocytes (%)	44.1 ± 11.2	61.0 ± 3.1
Monocytes (%)	2.1 ± 1.6	2.0 ± 0.3

Values are expressed as the mean ± standard deviation.
Source: Kakoma I, James MA, Jackson W, Bennett G, Ristic M. 1985. Hematologic values of normal Bolivian squirrel monkeys (*Saimiri sciureus*): a comparison between wild-caught and laboratory-bred male animals. *Folia Primatologica*, 44:102–107.

Supplementary Table 2
Biochemical analysis of plasma from *Saimiri collinsi* males (n = 13).

Parameters	Results	Reference values
Glucose (mg/dL)	108 ± 42.4	103 ± 30.3
Blood urea nitrogen (mg/dL)	48.8 ± 37.7	38.7 ± 10
Cholesterol (mg/dL)	188.3 ± 43.0	151 ± 64.7
Triglycerides (mg/dL)	72.5 ± 40.4	74.9 ± 32.7
Creatinine (mg/dL)	0.6 ± 0.1	0.9 ± 0.2
Total bilirubin (mg/dL)	<0.10	0.8 ± 0.6
Phosphatase (U/L)	249 ± 308	358 ± 175
Glutamic oxaloacetic transaminase (U/L)	190 ± 56.6	185 ± 95.3
Glutamic pyruvic transaminase (U/L)	201 ± 75.4	184 ± 110
Total protein (g/dL)	6.5 ± 0.7	6.9 ± 1.0
Calcium (mg/dL)	9.7 ± 0.9	9.6 ± 0.9
Albumina (g/dL)	3.7 ± 0.3	4.2 ± 0.6
Carbon dioxide	21	11.1 ± 3.9
Potassium	3.5	5.7 ± 1.0
Thyroxine-binding globulin	2.8 ± 0.5	—
Very low density lipoprotein	17.3 ± 7.6	—
Ammonia	46.5 ± 55.9	—
Phosphorus	5.3 ± 0.2	—
Magnesium	2.7 ± 0.3	—
Iron	154 ± 94.1	—

Values are expressed as the mean ± standard deviation.
Source: KCCMR. Michale E. Keeling Center for Comparative Medicine and Research. [Internet]. Texas: The University of Texas MD. Anderson Cancer Center. [cited 2014 March 2]. Available from: http://www.mdanderson.org/education-and-research/departments-programs-and-labs/programs-centers-institutes/michale-e-keeling-center-for-comparative-medicine-and-research/animal-resources/squirrel-monkey-diagnostic-reference-values.html.