Animal Reproduction Science 226 (2021) 106710

Contents lists available at ScienceDirect

Animal Reproduction Science

journal homepage: www.elsevier.com/locate/anireprosci

Morphological and ultrastructural changes in seminal coagulum of the squirrel monkey (*Saimiri collinsi* Osgood, 1916) before and after liquefaction

Patrícia da Cunha Sousa [a], Danuza Leite Leão [a], Wlaisa Vasconcelos Sampaio [a,b,*], Fábio Roger Vasconcelos [c], Sergimar Kennedy de Paiva Pinheiro [d], Emilio de Castro Miguel [d], Regiane Rodrigues dos Santos [a], Arlindo Alencar Moura [c], Sheyla Farhayldes Souza Domingues [a]

[a] *Laboratory of Biotechnology and Animal Medicine of Amazônia – BIOMEDAM, Federal University of Pará, 68.746-360, Castanhal, Pará, Brazil*
[b] *Postgraduate Program in Animal Health and Production in the Amazon, Federal Rural University of Amazônia, 66.077-530, Belém, Pará, Brazil*
[c] *Department of Animal Science, Federal University of Ceará, 60.356-000, Fortaleza, Ceará, Brazil*
[d] *Federal University of Ceará, Department of Physics/Central Analytical of the Federal University of Ceará, 60.020-181, Fortaleza, Ceará, Brazil*

ARTICLE INFO

Keywords:
Semen
Microscopy
Coagula
Extender
Neotropical primate

ABSTRACT

Studies with squirrel monkey semen are of special interest due to the large amount of coagulation that is a component of the semen, which is a problem that has to be overcome when the objective is harvesting of gametes. In the present study, there was characterization of the seminal coagulum of captive *S. collinsi*. Four samples of ejaculates were collected using electroejaculation procedures from four animals. The aim in conducting this study was to evaluate seminal coagulum of *S. collinsi* using histological and scanning electron microscopy (SEM) procedures before and after semen liquefaction in an ACP-118® extender. Seminal coagulum of *S. collinsi* was composed of a superficial plate (external), which coats the spongy seminal plasma matrix of *S. collinsi*. Additionally, there were sperm in the external and internal components of the coagulum with these gametes being isolated or grouped and with there being a heterogeneous distribution of gametes. The supplementation of semen with ACP-118® resulted in a partial dissolution of the seminal plate and spongy matrix portions of the seminal coagulum within the first hour of incubation.

1. Introduction

Semen coagulation is a common occurrence in several species of primates, including humans (Greer et al., 1968; Oliveira et al., 2016b; Settlage and Hendrickx, 1974; Valtonen-Andre et al., 2005). In primates, there is classifications of ejaculates based on four increments of coagulation (1–4), where the most liquid semen is classified as Grade 1 and the most viscous is classified as Grade 4 (Dixson and Anderson, 2002). Coagulated semen (Grade 3 or 4) does not liquefy spontaneously when in conditions outside the female reproductive tract. Even during *in vitro* procedures, the seminal coagulum of *Sapajus apella* (Lima et al., 2017; Oliveira et al., 2011) and *Saimiri collinsi* (Oliveira et al., 2016a, 2016b, 2015) partially liquefy when placed in extender solutions *in natura* or of coconut water made from powdered coconuts (ACP-118®), resulting in a suboptimal recovery of viable gametes. The effects of the liquefaction in the

* Corresponding author at: Avenida dos Universitários, s/n, Jaderlândia, 68.746-360, Castanhal, Pará, Brazil.
E-mail address: wlaisa.sampaio@gmail.com (W.V. Sampaio).

https://doi.org/10.1016/j.anireprosci.2021.106710
Received 16 May 2020; Received in revised form 19 January 2021; Accepted 20 January 2021
Available online 31 January 2021

seminal coagulum, however, are not completely understood.

There is a relationship between the rate of liquefaction and the morpho-structural characteristics of the seminal coagulum in different species (humans, rhesus monkeys and guinea pigs) (Zaneveld et al., 1974). It was reported that the coagulum comprising specific constituents formed a more viscous structure, and that the constituents of a human's seminal coagulum formed a narrow fiber-like network structure, which retained the sperm until there was induction of liquefaction. There was placement of the coagulated fraction in a physiological solution and there subsequently was histological procedures conducted for evaluation of the samples. There are very few studies focused on Neotropical non-human primates, and it is important to understand the morphological changes in the coagulated seminal fraction after partial liquefaction. This study, therefore, was conducted to characterize the morphology and ultrastructure of seminal coagulum from *S. collinsi*, as well as to verify the implications of supplementing semen of this species with ACP-118® extender.

2. Materials and methods

2.1. Study facilities

Semen collection and sample fixation were conducted at the National Primate Center (CENP, Ananindeua, Pará, Brazil). Histological analyses were conducted at the Laboratory of Plant Biology and scanning electron microscopy was conducted at the Analytical Center of Federal University of Ceará (UFC, Fortaleza, Ceará, Brazil).

2.2. Animals and experimental conditions

All experimental protocols were approved by System of Authorizationand Information in Biodiversity (SISBIO/ICMBio/MMA n^{o}. 47051-2) and by the Ethical Committee on Animal Research (CEPAN/IEC/SVS/MS/ n° 02/2015). Semen samples were obtained from four reproductively mature males of *Saimiri collinsi*, approximately 10–15 years old, maintained in captivity at the CENP (1°22′57″S and 48°22′52″W), where the climate is humid and tropical, with an average annual temperature of 28 °C. The animals were collectively housed in cages of 4.74, 1.45, and 2.26 m (length, width, and height, respectively), with there being a natural photoperiod (12-h light/12-h dark cycle). The diet consisted of fresh fruits, vegetables, milk, commercial pellet chow for primates, and cricket larvae (*Zophobas morio*) and water was available *ad libitum*.

2.3. Animal restraint and semen collection

There was physical restraint by a trained animal caretaker wearing leather gloves. After physical restraint, the animals were anesthetized using ketamine hydrochloride (15 mg/kg, intramuscular; Vetanarcol; Köning S.A., Santana de Parnaíba, São Paulo, Brazil) and xylazine hydrochloride (1 mg/kg, intramuscular; Vetanarcol; Köning S.A., Santana de Parnaíba, São Paulo, Brazil) administered by a veterinarian. Subsequently, the genital region was sanitized with mild soap (Ypê, Química Amparo, Amparo, SP, Brazil), distilled water (Quimis®, Diadema, SP, Brazil) (1:10) and gauze. The prepuce was retracted with the thumb and index fingers for more efficient cleaning of the penis with saline solution before semen collection (Oliveira et al., 2015).

Semen was collected during the same time period of the day (*i.e.*, in the morning before feeding). The semen was collected using electroejaculation procedures (Autojac-Neovet, Uberaba, Minas Gerais, Brazil), with an interval of 30 days between the times of the collections. This technique has previously been successfully utilized in *Saimiri collinsi* (Oliveira et al., 2016a, 2016b), and it does not require animal training for effective utilization. Also, the electroejaculation equipment used in this experiment emits electromagnetic waves (10–100 mA;1–12 V), which does not pose a risk to the animal's health. For conducting electroejaculation procedures, a bipolar rectal probe (12.5 cm in length and 0.6 cm in diameter) with a distal rounded end, bearing two metal plates (2 cm in length and 0.8 cm in width) on opposite sides was used. The probe was covered with a sterile lubricant jelly (KY™Jelly, Johnson & Johnson Co., Arlington, Texas, USA), placed in the rectum (~2.5 cm deep), and there was initiation of electrical stimulation. The session consisted of three periods of stimulation (7−8 min), with there being 35 electrical stimuli of increasing magnitude (12.5−100 mA) during each period, with an interval of 30 s between electrical stimulations (Oliveira et al., 2016a, 2016b). Ejaculate contents (coagulated fractions) were collected in micro-centrifuge tubes (1.5 mL). If an animal did not ejaculate after the electrical stimulation session, another electroejaculation period was attempted after an interval of at least 30 days. The physiological parameters of the animals were continuously monitored by a veterinarian throughout the procedure until there was complete recovery from anesthesia.

2.4. Preliminary semen analysis

Immediately after ejaculation, semen in a graduated microtube was placed in a water bath at 37 °C. Seminal pH was determined using a pH strip (Merk Pharmaceuticals, Darmstadt, Hesse, Germany). Appearance and consistency were evaluated subjectively by the same researcher using the following parameters: color (colorless, yellowish, or whitish), opacity (opaque or transparent), and appearance (amorphous or filamentary seminal coagulum) (Oliveira et al., 2016a, 2016b).

2.5. Semen extender

The extender solution was prepared with 5.84 g of ACP-118® (300 mOsm/kg and pH 7.8, ACP Biotechnology®, Fortaleza, Ceará,

Brazil) diluted in 50 mL of ultrapure water and stored at 37 °C. Samples of the seminal coagulum were then incubated in this solution in volumes of equal proportions (1:1) using procedures subsequently described in this manuscript. The seminal coagulum volume was evaluated according to the displacement of ACP-118® in the graduated microtube and the liquid fraction was evaluated using a pipette.

2.6. Sample fixation and transport

The semen samples (n = 4) were previously classified according to coagulated grade, and each one was fragmented into small pieces (~ 0.5 mm^3) with a scalpel blade (MYCO Medical, Apex, North Carolina, US). After that, the pieces of coagulated semen were randomly separated according to coagulation grade into two groups, the control (*in natura*) and incubated in ACP-118® (ACP Biotechnology®, Fortaleza, Ceará, Brazil) for 1 h at 37 °C for liquefaction and release of spermatozoa. After preliminary evaluation for determination of values for the sperm variables, ACP-118® extender was discarded. These samples and the control (*in natura*) were fixed using a solution of 2.5 % glutaraldehyde and 4.0 % paraformaldehyde prepared in 0.05 M cacodylate buffer, pH 7.2 (PB – Sigma Co®, St. Louis, United States) at room temperature (25 °C). The samples were transported in a thermal box to the Federal University of Ceará with the transport time being approximately 3 h.

2.7. Histological analysis

There were preparation and description of seminal coagulum of *S. collinsi* for histological analysis based on the method previously reported for human seminal plasma coagulum by Polak and Daunter (1983). The samples of seminal coagulum (control portions and ACP-118® incubated portions) were washed three times in 50 % ethanol (Rioquimica S/A, São José do Rio Preto, São Paulo, Brazil) for 15 min each time of washing, and dehydrated in increasing concentrations of ethanol (50 %, 60 %, 70 %, and 100 %) for 30 min at each concentration. Subsequently, samples were infiltrated with histresin (Leica®, Wetzlar, Hesse, Germany) and ethanol 100 % in a ratio of 1:1 for 24 h, followed by infiltration with histresin (24 h; Leica®, Wetzlar, Hesse, Germany) only. Thin sections (0.6 μm) were stained using 1% toluidine blue (Sigma-Aldrich, San Luis, Missouri, US). The glass slides were sealed with varnish comprised of acrylic resin (Acrilex, São Bernardo do Campo, São Paulo, Brazil). Sections were evaluated using a photomicroscope (Olympus UC30, Munster, North Rhine-Westphalia, Germany) utilizing a UC30 model digital camera (Olympus, Munster, North Rhine-Westphalia, Germany) and software for Cell B image analysis (Olympus, Munster, North Rhine-Westphalia, Germany).

2.8. Scanning electron microscopy

Seminal coagulum samples was washed three times (15 min for each washing) in sodium cacodylate buffer (Sigma-Aldrich, San Luis, Missouri, US) for 15 min at room temperature and were subsequently fixed for 1 h at room temperature using 1% osmium tetroxide (Sigma-Aldrich, San Luis, Missouri, US) in cacodylate buffer (0.05 M, pH 7.2; Sigma-Aldrich, San Luis, Missouri, US). The samples were dehydrated with increasing concentrations of acetone (50 %, 70 %, 90 %, and 100 %; Rioquimica S/A, São José do Rio Preto, São Paulo, Brazil), by placing for 20 min in the differing concentrations of the dehydrating agents. After dehydration, the sample was dried using hexamethyldisilazane (HDMS; Wacker Chemie AG, Burghausen, Baviera, Germany) and acetone (100 %) in equal proportions (1:1) for 1 h, and then in HMDS for 12−15 h. The dried samples were attached to aluminum supports (12.7 mm x 8 mm stubs; Shenyang Roundfin Trade Co., Ltd, Liaoning, China) with carbon adhesive tape (Agar Scientific, Essex, UK), sprayed with 20-nm gold (Q150 T Turbo-Pumped Sputter Coater / Carbon Coater; Lewes, East Sussex, UK), and then evaluated using the Quanta™ FEG 450 microscope with a digital scanner (Quanta 450-FEG, Thermo Fisher Scientific; Massachusetts, US). The preparation of seminal coagulum for SEM was conducted based on the method previously reported for human seminal plasma coagulum by Polak and Daunter (1983).

3. Results

3.1. Semen collection and preliminary analysis

The ejaculates were of a whitish or yellowish color and had an opaque appearance; the alkaline pH of the seminal ejaculate was 7.5;

Table 1
Seminal characteristics of *Saimiri collinsi* ejaculate.

Seminal parameters	
Seminal volume	
Liquid fraction	32,5 ± 15 μl (5−100 μl; min–max)
Coagulated fraction	339 ± 61 μl (15–500 μl; min–max)
Degree of seminal coagulum	Degree II and III
pH	7.5
Colour	Yellowish
Opacity	Opaque
Appearance	Amorphous

value for mean (± standard error of the mean) seminal volume was 339 ± 61 μl (15–500 μl; min–max), and the coagulation increments ranged from 1 to 3, with the coexistence of different increments of coagulation in the same ejaculate (*i.e.*, Grades 1 and 2 or Grades 1 and 3). All the samples also contained a liquid fraction. The Grade 1 coagulant was discarded due to the loss, as a result of the washing and centrifugation procedures, which was inherent in the sample processing procedures utilized. Data included in Table 1 summarize the preliminary semen analysis.

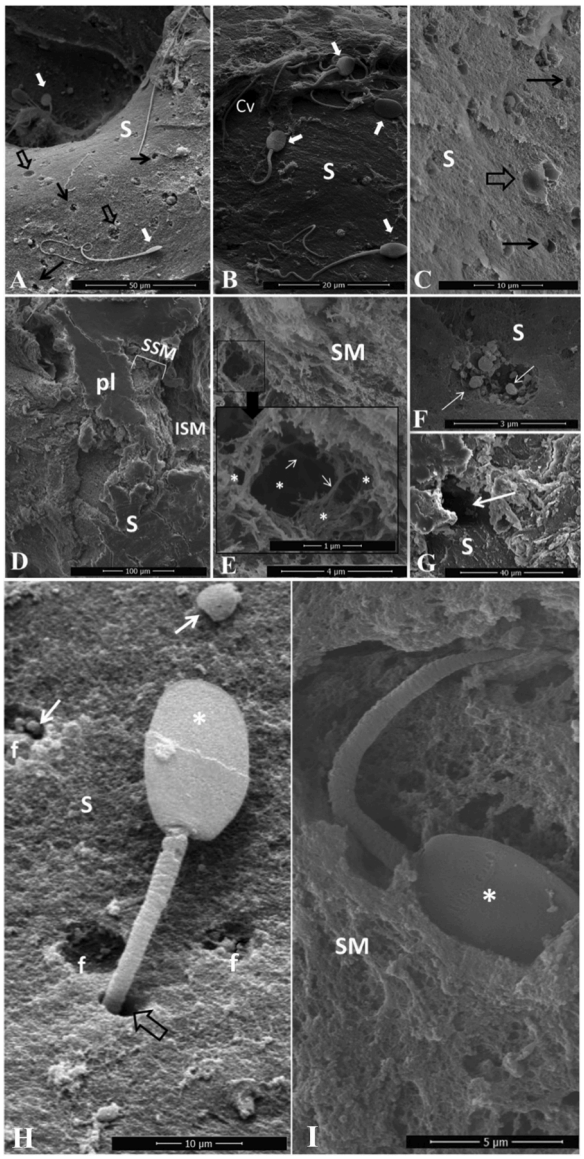

Fig. 1. Scanning electromyrographs of the *in natura* seminal coagulum of S. *collinsi*; A) Compact surface (S) of the seminal coagulum, containing vacuole (black arrows) and invaginations (hollow arrows) and some spermatozoa (white arrows); B) Surface of the coagulum (S) containing structures "caves-like" (Cv) and some spermatozoa (white arrows); C) Pores (hollow arrows) and shallow gaps (black arrows); D) Smooth plates (pl) on the surface of the coagulum (S); Spongy matrix: superficial spongy matrix (SSM) and internal spongy matrix (ISM); E) Spongy matrix (SM) constituted by fiber-like network; In the highlighted area, there is the fiber-like network of the spongy matrix (SM) with fiber-like structure of varying thickness (white arrows) forming networks of different diameters (*); F) Gap of the coagulum surface (S), filled by globular material (arrows); G) Pores (arrow) in the coagulum surface (S); H) Spermatozoon (*) attached in a pore (hollowed arrow) on the coagulum surface (S); Presence of globular material (white arrow) outside and inside gaps (f); I) Spermatozoon (*) trapped in the spongy matrix of the fiber-like network (SM).

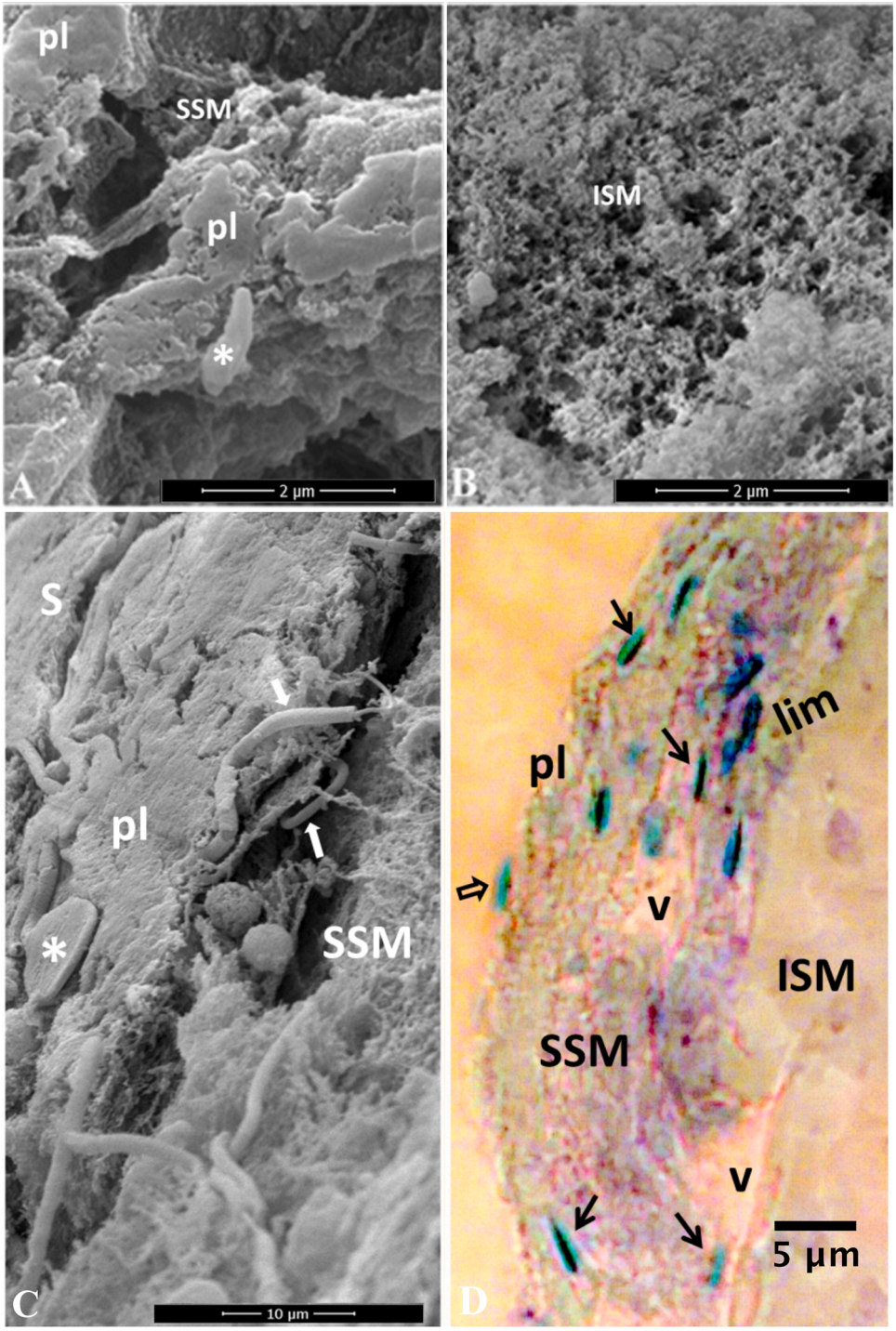

Fig. 2. Scanning electromyrographs of the *in natura* seminal coagulum of *S. collinsi*; A) Surface plate (pl) and surface sponge matrix (SSM) composed of a loose network of fiber-like contents where a spermatozoon is retained (*); B) Internal sponge matrix (ISM), consisting of a more compact arrangement of fiber-like structures Electromyrography (C) and histological images (100x objective, 1000x increase - tranversal section) (D) of the in natura seminal coagulum of *Saimiri collinsi*; C) Coagulum surface (S) the surface plate (pl) of the seminal coagulum coating a spongy surface matrix (SSM); Spermatozoa (*) adhered to the superficial plate (pl), and the presence of sperm tails (white arrows) in the superficial spongy matrix (SSM); D) Surface plate (pl) of the seminal coagulum coating a spongy surface matrix (SSM), and the "limitroph" (lim) with the internal spongy matrix (ISM); Presence of small vacuole (v) in the surface spongy matrix (SSM) and spermatozoa (arrows) distributed in the surface spongy matrix (SSM) and adhered (arrow cast) on the surface plate (pl); Magnification, 100× objective, 1000×.

3.2. Histological analysis and scanning electron microscopy of the seminal coagula from S. collinsi

3.2.1. Characterization of the seminal coagula immediately after collection

Findings as a result of conducting the histological and SEM procedures indicated there were no marked increments of coagulation when there was evaluation of Grade 2 and 3 coagulants. In the seminal coagulum of *S. collinsi*, there were two types of the spongy matrix: a more superficial (spongy surface matrix) adjacent to the surface plate, composed of a comparatively looser arrangement of coagulant contents (Figs. 1C, D and 2 A), and an internal coagulant content, the internal spongy matrix, characterized by a more compact arrangement of coagulant contents (Figs. 1D, 2 B). The surface plate was observed in the histological images as having a remarkably fine structure with a light brown or amber coloration (Fig. 2D). From the histological sections, it was also possible to observe a sheath separating the spongy surface matrix from the internal spongy matrix (Fig. 2D).

Findings with analysis using SEM procedures indicated that in undiluted seminal coagulum, there was an irregular surface with large amount of compaction (Fig. 1A), the presence of "cave-like" structures (Fig. 1B), fissures (Fig. 1B, E), pores (Fig. 1A, C, G), and shallow gaps (Fig. 1C), sometimes filled with globular material (Fig. 1F). At some sites on the surface, thin plates were observed with a smooth outer face (Fig. 1D) which covered an area with an irregular surface consisting of a disorganized network of coagulant contents composed of a network of long fiber-like structures interconnected by smaller fiber-like structures. This structure resulted in there being vacuoles with apparent variable diameters, which contributed to the spongy appearance to the seminal coagulum matrix (Fig. 1E). Spermatozoa were adhered to and dispersed on the compact surface and were also present in the vacuoles (Fig. 1H) and evenly interspersed in the superficial network of the spongy matrix constituents of the seminal coagulum (Fig. 1I).

3.2.2. Distribution and interaction of spermatozoa in fresh seminal coagula

There were spermatozoa in different components of the seminal coagula of *S. collinsi*, isolated (Fig. 3A) or agglutinated in the innermost layers (Fig. 3B) resulting in a heterogeneous distribution of these gametes. Results from conducting scanning electromicrograph procedures indicated spermatozoa of *S. collinsi* were persistently attached to the surface of the seminal coagulum. The adhesion points between spermatozoon and substrate (seminal coagulum) were distributed in regions of the head (Fig. 4A–F), intermediate piece (Fig. 4C–F), and tail (Fig. 4C–F), and along the entire length of the gamete (Fig. 4F).

3.2.3. Effects of ACP-118® on S. collinsi seminal coagulum and spermatozoa attachment

Coagulated semen samples of *Saimiri collinsi* were not fully liquefied after 1 h of incubation in ACP-118®. When the histological sections from the fresh seminal coagulum (Fig. 5A) were compared with those incubated in ACP-118® for 1 h, there were more vacuoles in the spongy matrix, with some spermatozoa inside these vacuoles (Fig. 5D, E), and there was no surface plate in the coagulum (Fig. 5B). The superficial spongy matrix was narrower (Fig. 5B) or absent (Fig. 5C), with there being sperm agglomerates in the spongy surface matrix (Fig. 6A, B).

After 1 h of incubation of the *S. collinsi* coagula in ACP-118®, in the dense surface of the seminal coagulum there were areas in which there was an absence of the surface plaque, degradation of the spongy surface matrix, and exposure of the internal spongy matrix

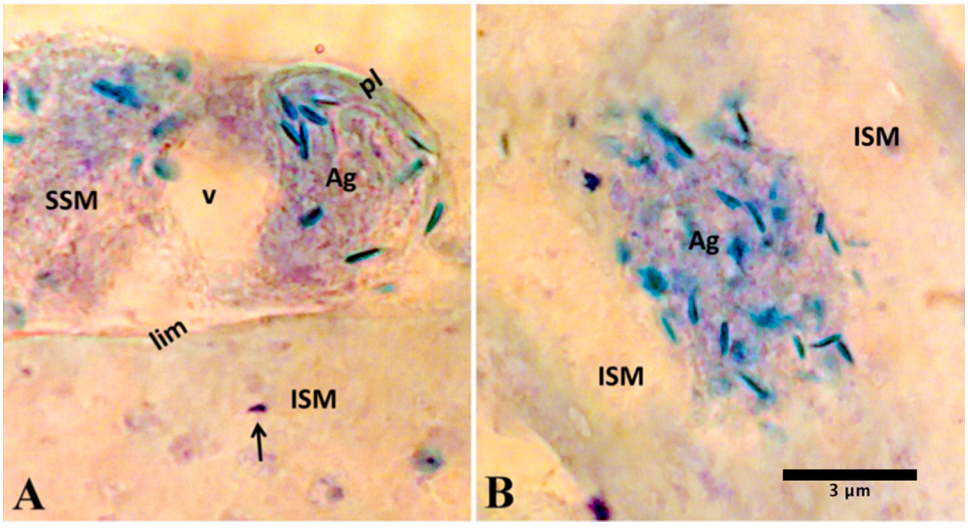

Fig. 3. Photomicrographs of transverse sections of the *in natura* seminal coagulum of *S. collinsi*; A) Isolated spermatozoon (arrow) in the internal spongy matrix (ISM) and agglutinated (Ag) in the spongy surface matrix (SSM); Empty vacuole (v) in SSM; Surface plate (pl) of the seminal coagulum coating a spongy surface matrix (SSM) and the "limitroph" (lim) with the internal spongy matrix (ISM); Presence of small vacuole (v) in the surface spongy matrix (SSM) and spermatozoa (arrows) distributed in the surface spongy matrix (SSM) and adhered (arrow cast) on the surface plate (pl); Spongy surface matrix (SSM) and the "limitroph" (lim) with the internal spongy matrix (ISM); B) Agglomerate of spermatozoids (Ag) contained in the internal spongy matrix (ISM); Magnification of all panel images, 100× objective, 1000×.

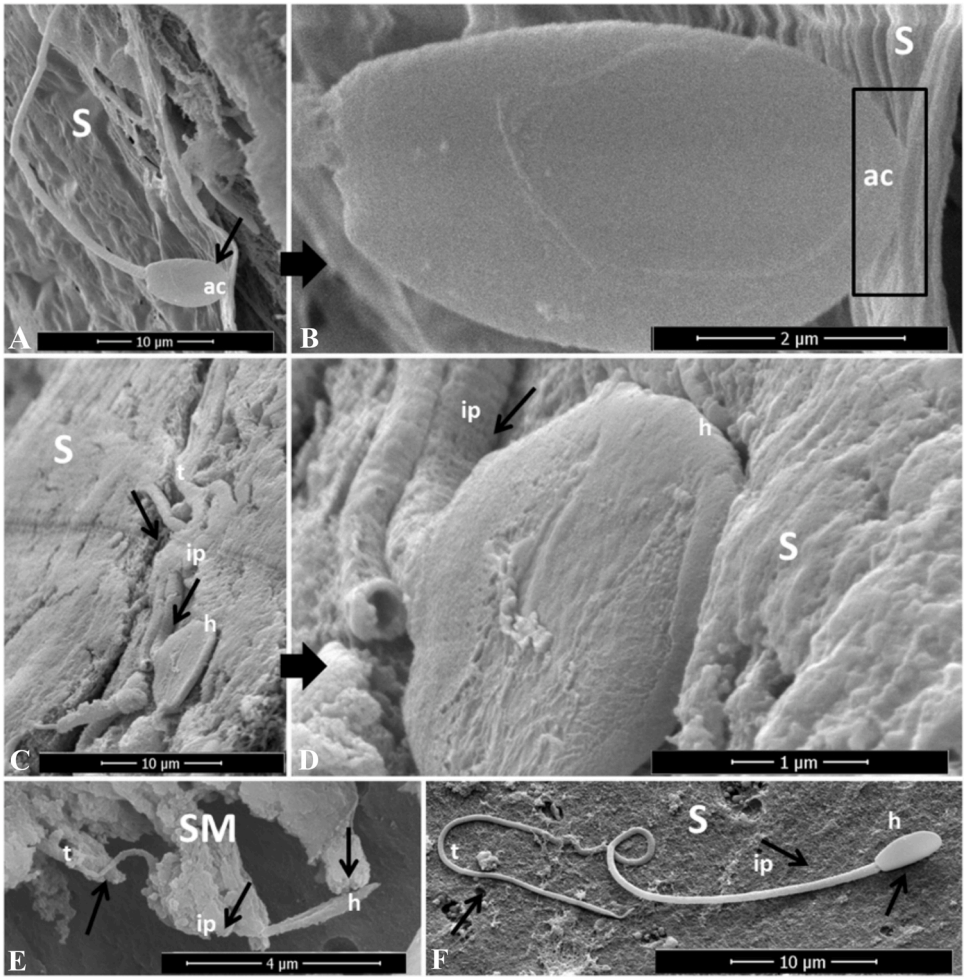

Fig. 4. Scanning electromyrographs of the seminal coagulum of *S. collinsi*, with specific points of attachment between the surface of the spermatozoa and the coagulum; A-B) Interaction (arrow) between the apical region of the acrosome sperm (ac) and the surface of the seminal coagulum (S); Spongy surface matrix (SSM) and its "limitroph" (lim) with the internal spongy matrix (ISM); C) Interaction (arrow) between head (h), intermediate piece (ip) and tail (t) of the spermatozoon with the surface (S) of the coagulum; D) Detail of the interaction (arrow) of the region of the head (h) and intermediate peace (ip) of the spermatozoon with the substrate (S); E) Connection points (arrow) of the seminal coagulum in the region of the spongy matrix (SM) with different regions of the spermatozoon: head (h), intermediate piece (ip) and tail (t); F) Connection points (arrow) of the surface (S) of the seminal coagulum to different regions of the spermatozoon: head (h), intermediate piece (ip) and tail (t).

(Fig. 7A). In the surface coating plate, there was an obvious state of degradation (Fig. 7B, C), where there were agglomerated spermatozoa located in the superficial spongy matrix (Fig. 7C).

4. Discussion

Immediately after collection, seminal coagula from *Saimiri collinsi* that were classified with Grades 2 and 3 had a semisolid consistency based on results from histological and SEM evaluations. Seminal coagulum was characterized as a being spongy with a stratified capsule, consisting of a compact surface containing some widely dispersed spermatozoa. Within the inner layers of the coagulum, spermatozoa were in greater concentrations, located in the fibrous-like network that is less dense than that at the surface of the coagulum. Dixson and Anderson (2002) reported that ejaculates might have specific increments (or score) of coagulation depending on the mating characteristics of members of each primate species. It was previously reported that the ejaculates of *S. collinsi, S. vanzolinii, S. cassiquiarensis,* and *S. macrodon* could coagulate at increments ranging from 1 to 4 from least to greatest extent of coagulation with there being the possibility of different increments of coagulation in the same seminal sample (Oliveira et al., 2016a, 2016b, 2015). There are similar characteristics of seminal coagulants in a Neotropical primate of the genus *Sapajus* (*S. apella*) (Lima et al., 2017) and *Leontopithecus chrysomelas* (Arakaki et al., 2020), but without the presence of seminal fluid fraction (Grade 1).

In some Neotropical species, the mechanical fragmentation of the seminal coagulum is a common practice for acceleration of the process of dissolution of the semen (Oliveira et al., 2016a, 2016b, 2011). With use of this procedure, there is complete dissolution of

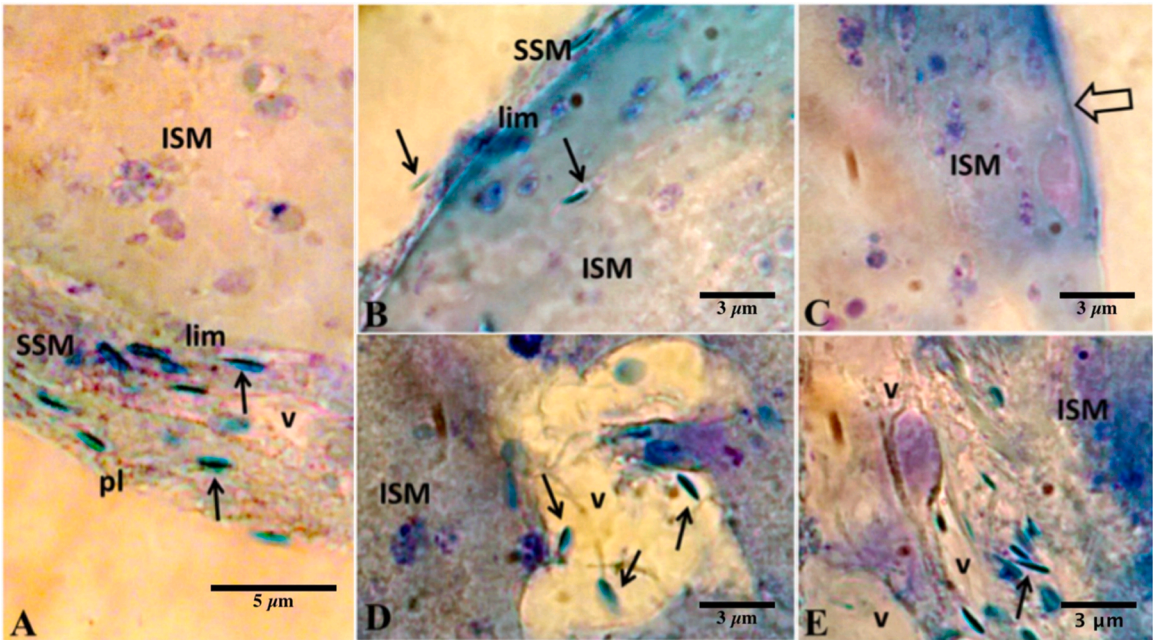

Fig. 5. Photomicrographs of sections of the undiluted (fresh) seminal coagulum of *S. collinsi* and after being diluted in ACP-118® at 37 °C for 1 h; A) Fresh seminal coagulum with all the structural components: superficial plate (pl), superficial spongy matrix (SSM), adjacent lamina (lim) between superficial spongy (SSM) and internal (ISM) spongy matrices; Presence of spermatozoa (arrow) and small vacuity (v) in superficial spongy matrix (SSM); B) Diluted seminal with absence of surface plate and narrowing of the superficial sponge matrix (SSM); Thin spongy surface matrix (SSM) and "limitroph" (lim) with internal spongy matrix (ISM); C) Diluted seminal with absence of the spongy surface matrix (hollowed arrow); D) Diluted seminal with large vacuoles (v) in the internal spongy matrix (ISM), with spermatozoa in the lumen (arrows); E) Diluted seminal plasma with increase in the number of vacuoles (v) in the internal spongy matrix (ISM), and spermatozoa (arrow); Magnification on all panel images, 100× objective, 1000×.

the seminal coagulum of *S. apella* by the second hour of incubation period in a solution of *in natura* coconut water at 37 °C, and this method has subsequently been used to facilitate the partial seminal coagulum liquefaction of *S. collinsi* (Oliveira et al., 2015). In *Sapajus apella*, there was liquefaction of the seminal coagulum using digestive enzymes, such as trypsin or hyaluronidase. The use of proteolytic enzymes can prevent total liquefaction and induce damage to sperm membranes, thereby decreasing the fertilization capacity of the sperm (Hernández-López et al., 2002; Paz et al., 2006).

In the present study, spermatozoa remained adhered to the surface of the seminal coagulum, even after washing and centrifugation of the samples which was performed for conducting the morphological analyses. This also occurred in the seminal coagulum in the rhesus monkey, resulting in it being suggested that there was possible adhesions between the spermatozoa and the seminal coagulum (Zaneveld et al., 1974). The interactions of spermatozoa with other substrates, such as the uterine tube epithelium, for example, are considered important for maintenance of the fertility of the sperm cells prior to release from the tissue from which the sperm are adhered (Fan et al., 2006). In some primates, such as *Callithrix* sp. (Valtonen-Andre et al., 2005), rhesus monkeys (Ulvsback and Lundwall, 1997), and humans (De Lamirande and Lamothe, 2010), there is a semenogelin that has been identified as the main structural protein component of coagulated semen. This protein has the property of inhibiting sperm motility (De Lamirande, 2007; Robert and Gagnon, 1995; Yoshida et al., 2008), and is responsible for temporarily immobilizing sperm in the seminal coagulum. Semenogelin, due to its low isoelectric point (pI 9.5) (De Lamirande, 2007; Robert and Gagnon, 1999), has the capacity to bind to acidic proteins present in spermatozoa (Bjartell et al., 1996; Lijla et al., 1989; Yoshida et al., 2009; Zalensky et al., 1993). Silva et al. (2013) reported that there has been co-evolution of the capacity of proteins of the seminal coagulum and the surface of the sperm to bind tightly. It is suggested that the sperm-agglutinant seminal plasma bond that occurred with *S. collinsi* is a fundamental evolutionary process in the reproduction of this species, as well as other primates.

The results from conducting scanning electromicrography procedures indicate there is a general structural organization of the seminal coagulum of squirrel monkeys (*S. collinsi*) comparable to that already described for rhesus monkeys and humans (Zaneveld et al., 1974). In humans, however, there are differences in the constituents of the coagulum matrix, with the fiber-like structures being smaller, thinner, and of a more uniform network. The arrangement of these fiber-like structures is associated with the morphological differences between the ejaculates of primates.

In humans, when there is liquefaction of seminal coagulant, there are changes in the structure of the fibrous network, such that there is rupturing of the fiber-like structures, which make the coagulum matrix more irregular in structure, thereby resulting in a looser structure of the coagulum, with a gradual release of spermatozoa (Zaneveld et al., 1974). Comparatively, the seminal coagulum of rhesus monkeys is more viscous (Zaneveld et al., 1974) and has an intriguing dynamic, because it has the "sponge-like" characteristic,

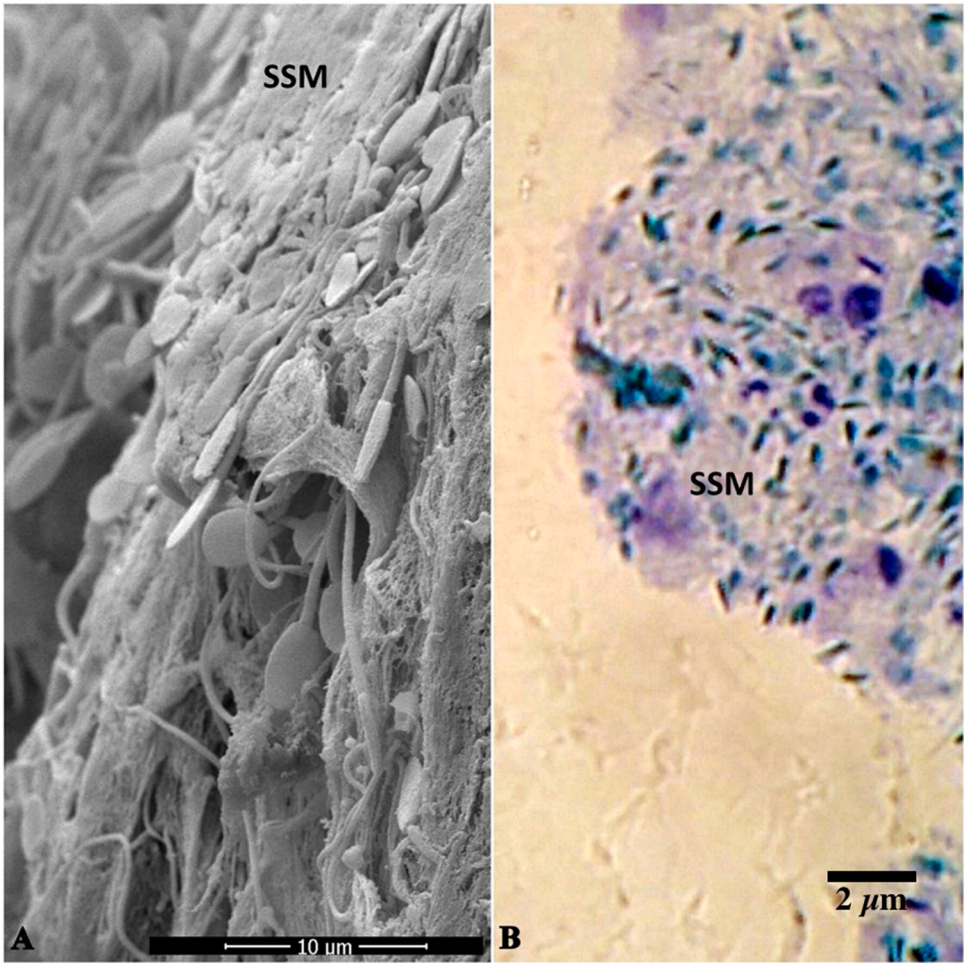

Fig. 6. Scanning electromyrography and histological photomicrography, after the seminal coagulum of *S. collinsi* was incubated with ACP-118® extender for 1 h at 37 °C; A-B) Sperm agglomerate released from the superficial spongy matrix (SSM); Note the absence of the surface plate in both images; Magnification, 100× objective, 1000×.

thus retaining an exudate rich in spermatozoa with these sperm being subsequently released as a result of spontaneous contraction of the coagulum (Van Pelt, 1970). When this occurs, there is no true liquefaction of the coagulum, because the "sponge-like" coagulum structure is not disrupted, and when the coagulum is in contact with the exudate rich in spermatozoa for long periods, this exudate tends to be reabsorbed by the coagulum (Settlage and Hendrickx, 1974; Van Pelt, 1970). The biological reasons for the existence of structural and functional characteristics of seminal coagulum in primates are not well defined due to the lack of information on the basic reproductive physiology of most species in this group of mammals.

Human spermatozoa are present in the more internal regions of the seminal coagulum (Polak and Daunter, 1983). In contrast, spermatozoa from *S. collinsi* were heterogeneously distributed and were present in isolated portions within the seminal coagulum. This finding indicates that during ejaculation, the spermatozoa from the epididymis are mixed in the liquid matrix and, in the process of coagulation, this confines the gametes to isolated clusters. The reproductive advantage(s) to the species with these semen characteristics remain to be elucidated. Aspects such as these should certainly be explored when the results from studies are considered where there have been found to be associations between gamete morphology (Leão et al., 2020), reproductive physiology, evolution, and behavior, involving *S. collinsi* of both sexes.

To improve efficacy of breeding biotechnology practices, comprehensive knowledge of seminal coagulum morphology is extremely relevant, especially when substances are formulated and used *in vitro* for the purpose of liquefying the coagulum and recovering the spermatozoa. Solutions using coconut water are among those that have been evaluated in previous studies (Lima et al., 2017; Arakaki et al., 2017; Oliveira et al., 2016b, 2015). In studies where the ACP-118® is the extender evaluated, there have been assessments of the effects on sperm quality based on values for the sperm variables and time of partial coagulum liquefaction (Leão et al., 2015; Oliveira et al., 2016b, 2015), however, there have not been determinations of the physical or structural effects of this extender on coagulated ejaculates of primates. In *S. collinsi*, after 1 h in ACP-118® at 37 °C, the seminal coagulum was partially liquefied. The effects of ACP-118® on the surface plate degradation of the seminal coagulum, and the presence of vacuoles in the interstitium that contain no

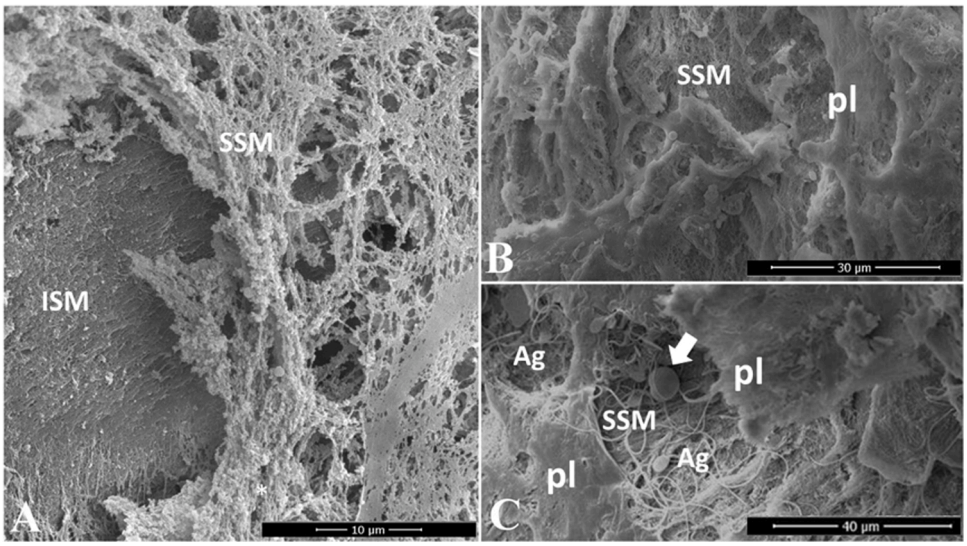

Fig. 7. Scanning electromyrographs of the *S. collinsi* seminal coagulum after incubation in ACP-118® for 1 h at 37 °C; A) Seminal coagulum without superficial plate with the superficial spongy matrix (SSM) and internal (ISM); B) Surface plate (pl) in degraded state, with the spongy matrix (SSM) of the seminal coagulum; C) Surface sponge matrix (SSM) containing sperm agglomerates (Ag), after surface plate degradation (pl); Globular material (full arrow).

sperm, indicate that this diluent has a degradation effect both on the surface and in the more internal portions of the seminal coagulum. Groups of spermatozoa in the vacuoles, termed as "open pores", were also observed in the fresh seminal coagulum of rhesus monkeys (Settlage and Hendrickx, 1974). These vacuoles were numerous in the semen samples of *S. collinsi* incubated in ACP-118®. It, therefore, is presumed that supplementation of semen with this extender leads to an induction of these changes resulting in dissolution of the spongy matrix of the seminal coagulum, thereby allowing the release of the gametes into these vacuoles. It, however, remains unknown which enzymes are involved in degradation of the semen coagulant.

5. Conclusion

Results from the present study indicate seminal coagulum of *Saimiri collinsi* resembled a capsule, consisting of a smooth and compact surface plate, where occasional firm adherence of the spermatozoa. This plate coats a spongy matrix that contains among the seminal coagulant constituents a greater concentration of gametes heterogeneously distributed in the interstitium. The supplementation of semen with the extender ACP-118® resulted in some dissolution of the spongy matrix of the seminal coagulum which could consequently result in the release of spermatozoa from the seminal coagulant. Not only does seminal coagula dissolution need to occur, but there needs to be maintenance of spermatozoa viability after incubation, which has been previously reported to occur when there was supplementation of semen with ACP-118® (Leão et al., 2015). The results from the present study, therefore, provide important information regarding the optimum use of semen collected from males of the *Saimiri* genus. There could be subsequent application of the information from the present study when gametes are used when conducting reproduction biotechniques, including formation of biobanks and multiplication of Neotropical non-human primate species.

Funding

This research was supported by the Coordination for the Improvement of Higher Education Personnel (Capes, Brazil, Finance code 001) and the National Council for Scientific and Technological Development (CNPq, Brazil, Projeto Universal 01-2016/ Processo n°: 421649/2016-0).

Declaration of Competing Interest

The authors report no declarations of interest.

Acknowledgments

This study was supported by the National Primate Center (CENP), CAPES (Brazil), Federal University of Pará, and Department of Physics/Central Analytical of the Federal University of Ceará.

References

Arakaki, P.R., Carvalho, F.Mde, Castro, P.H.Gde, Muniz, J.A.P.C., Valle, Rdel Rdo, 2017. Collection, evaluation, and coagulum dissolution of semen from Goeldi's monkey, *Callimico goeldii*. Folia Primatol. 88, 334–343.

Arakaki, P.R., Losano, J.D.de A., Salgado, P.A.B., Pereira, R.J.G., 2020. Seasonal effects on testes size and sustained semen quality in captive golden-headed lion tamarins, *Leontopithecus chrysomelas*. Anim. Reprod. Sci. 218, 106472.

Bjartell, A., Malm, J., Moller, C., Gunnarsson, M., Lundwall, A., Lilja, H., 1996. Distribution and tissue expression of semenogelin I and II in man as demonstrated by in situ hybridization and immunocytochemistry. J. Androl. 17, 17–26.

De Lamirande, E., 2007. Semenogelin, the main protein of the human semen coagulum, regulates sperm function. Sem. Thromb. Hemost. 33, 60–68.

De Lamirande, E., Lamothe, G., 2010. Levels of semenogelin in human spermatozoa decrease during capacitation: involvement of reactive oxygen species and zinc. Hum. Reprod. 25, 1619–1630.

Dixson, A.L., Anderson, M.J., 2002. Sexual selection, seminal coagulation and copulatory plug formation in primates. Folia Primatol. 73, 63–69.

Fan, J., Lefebvre, J., Manjunath, P., 2006. Bovine seminal plasma proteins and their relatives: a new expanding superfamily in mamals. Gene 375, 63–74.

Greer, W.E., Roussel, J.D., Austin, C.R., 1968. Prevention of coagulation in monkey semen by surgery. J. Reprod. Fertil. 15, 153–155.

Hernández-López, L., Parra, G.C., Cerda-Molina, A.L., Pérez-Bolaños, S.C., Sánchez, V.D., Mondragón-Ceballos, R., 2002. Digestion by trypsin enhances assessment of sperm parameters in the black-handed spider monkey (*Ateles geoffroyi*). Lab. Primate Newsl. 41, 4–6.

Leão, D.L., Miranda, S.A., Brito, A.B., Lima, J.S., Santos, R.R., Domingues, S.F.S., 2015. Efficacious long-term cooling and freezing of *Sapajus apella* semen in ACP-118®®. Anim. Reprod. Sci. 159, 118–123.

Leão, D.L., Sampaio, W.V., Sousa, P.C., Moura, A.A.A., Oskan, I.C., Santos, R.R., Domingues, S.F.S., 2020. Micromorphological and ultrastructural description of spermatozoa from squirrel monkeys (*Saimiri collinsi* Osgood, 1916). Zygote 1–5.

Lijla, H., Abrahamsson, P.A., Lundwall, A., 1989. Semenogelin, the predominant protein in human semen. Primary structure and identification of closely related proteins in the male accessory sex glands and on the spermatozoa. J. Biol. Chem. 264, 894–1900.

Lima, J.S., Leão, D.L., Oliveira, K.G., Brito, A.B., Sampaio, W.V., Santos, R.R., Domingues, S.F.S., 2017. Seminal coagulation and sperm quality in different social contexts in captive tufted capuchin monkeys (*Sapajus apella*). Am. J. Primatol. 79, e22643.

Oliveira, K.G., Miranda, S.A., Leão, D.L., Brito, A.B., Santos, R.R., Domingues, S.F.S., 2011. Semen coagulum liquefaction, sperm activation and cryopreservation of capuchin monkey (*Cebus apella*) semen in coconut water solution (CWS) and TES–TRIS. Anim. Reprod. Sci. 123, 75–80.

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, 743–749.

Oliveira, K.G., Santos, R.R., Leao, 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, 283–289.

Oliveira, K.G., Santos, R.R., Leao, 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, 879–887.

Paz, R.C.R., Teixeira, R.H.F., Guimarães, M.A.B.V., 2006. Avaliação das características seminais de macacos-pregos (*Cebus apella*) mantidos em cativeiro, antes e após vasectomia bilateral. Braz. J. Vet. Res. Anim. Sci. 43 (4), 561–567.

Polak, B., Daunter, B., 1983. Scanning electron microscopy and histological examination of human seminal plasma coagulum. Andrologia 15, 452–462.

Robert, M., Gagnon, C., 1995. Sperm motility inhibitor from human seminal plasma: association with semen coagulum. Hum. Reprod. 10, 2192–2197.

Robert, M., Gagnon, C., 1999. Semenogelin I: a coagulation forming multifunctional seminal vesicle protein. Cell. Mol. Life Sci. 55, 944–960.

Settlage, D.S.F., Hendrickx, A.G., 1974. Electroejaculation technique in *Macaca mulatta* (Rhesus monkeys). Fertil. Steril. 25, 157–159.

Silva, D.J.R., Hamil, K.G., O'Rand, M.G., 2013. Interacting proteins on human spermatozoa: adaptive evolution of the binding of semenogelin I to EPPIN. PLoS One 8 (12), e82014.

Ulvsback, M., Lundwall, A., 1997. Cloning of the semenogelin II gene of the rhesus monkey. Duplications of 360 bp extend the coding region in man, rhesus monkey and baboon. Eur. J. Biochem. 245, 25–31.

Valtonen-Andre, C., Olsson, A.Y., Nayudu, P.L., Lundwall, A., 2005. Ejaculates from the common marmoset (Callithrix jacchus) contain semenogelin and beta-microseminoprotein but not prostate-specific antigen. Mol. Reprod. Dev. 71, 247–255.

Van Pelt, L.F., 1970. Intraperitoneal insemination of *Macaca mulatta*. Fertil. Steril. 21, 159–162.

Yoshida, K., Kawano, N., Yoshiike, M., Yoshida, M., Iwamoto, T., Morisawa, M., 2008. Physiological roles of semenogelin I and zinc in sperm motility and semen coagulation on ejaculation in humans. Mol. Hum. Reprod. 14, 151–156.

Yoshida, K., Krasznai, Z.T., Krasznai, Z., Yoshire, M., Kawano, N., Yoshida, A.N., Morisawa, M., Toth, Z., Bazsane, Z.K., Marian, T., Iwamoto, T., 2009. Functional implications of membrane modification with semenogelins for inhibition of sperm motility in humans. Cell Motil. Cytoskeleton 66, 99–108.

Zalensky, A.O., Yau, P., Brenerman, J.W., Bradmury, E.M., 1993. The abundant 19-kilodalton protein associated with human sperm nuclei that is related to seminal plasma alpha-inhibin. Mol. Reprod. Dev. 36, 164–173.

Zaneveld, L.J.D., Tauber, P.F., Port, C., Propping, D., Schumacher, G.F.B., 1974. Scanning electron microscopy of the human, guinea-pig and rhesus monkey seminal coagulum. J. Reprod. Fertil. 40, 223–225.