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Extender supplementation with catalase maintains the integrity of sperm plasma membrane after freezing–thawing of semen from capuchin monkey

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Summary

We aimed to evaluate the effect of supplementation of ACP-118® extender with the antioxidant catalase (10 and 50 µg/ml) on *Sapajus apella* sperm motility, vigour, and plasma membrane integrity during the processes of seminal liquefaction, cooling, and freezing. Catalase did not affect any of the evaluated parameters after semen dilution or cooling. Cryopreserved sperm in the presence of 50 µg/ml catalase presented a plasma membrane integrity similar to that fresh sperm, however.

Keywords: Antioxidant, Cell membrane, Monkey, Semen

Introduction

Sapajus apella presents a high degree of seminal coagulation, which does not liquefy spontaneously after ejaculation (Dixson & Anderson, 2002). Hence, sperm from *S. apella* suffer stress during coagulum liquefaction, followed by the stress caused by the freezing process (Oliveira *et al.*, 2011; Leão *et al.*, 2015). Although seminal freezing has been described in *S. apella* using TES–TRIS and coconut-derivate extenders, sperm parameters such as motility and vigour decrease significantly after seminal coagulum liquefaction, cooling, freezing, and thawing, sometimes being almost null (Oliveira *et al.*, 2011; Leão *et al.*, 2015). Similar effects have been observed in other species of neotropical primates such as *Saimiri collinsi* (Oliveira *et al.*, 2015, Oliveira *et al.*, 2016a,b), *S. vanzolinii*, *S. cassiquiarensis* and *S. macrodon* (Oliveira *et al.*, 2016ab). It is known that the use of extenders will dilute the concentration of natural antioxidants present in the seminal plasma (Agarwal *et al.*, 2006), and cryopreservation leads to an increased production of reactive oxygen species (ROS) (Aitken & Baker, 2006). Consequently, a redox system imbalance causes the impairment of sperm's capacity to fertilize due the injury of the plasma membrane (Taylor *et al.*, 2009), and decrease in sperm motility (Kefer *et al.*, 2009). Catalase is an antioxidant present in seminal plasma and plays a role in counteracting oxidative stress (Mora-Esteves & Shin, 2013). The beneficial effect of catalase on sperm viability and motility has been reported for human (Moubasher *et al.*, 2013). In non-human primates the use of catalase has been investigated in the rhesus macaque (*Macaca mulatta*) (Dong *et al.*, 2009; McCarthy & Meyers, 2011), and was found to decrease lipid peroxidation (McCarthy & Meyers, 2011), and to improve post-thaw sperm motility (Dong *et al.*, 2009). Our aim was to evaluate the effect of semen extender supplementation with catalase at two different concentrations (10 and 50 µg/ml) on sperm morphology, motility, vigour, and plasma membrane integrity during the processes of seminal liquefaction, cooling, and freezing–thawing.

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Materials and Methods

This study was approved by the Ethical Committee in Animal Research (no. 013/2009/CEPAN/IEC/SVS/MS) and by the System of Authorization and Information in Biodiversity (SISBIO/ICMBio/MMA no. 34009-3). Six *S. apella* males were provided by the National Primate Center (Ananindeua, Brazil). Length, width, height, circumference and volume of both testes were measured. Animals were stimulated using a rectal electro-ejaculation (EEJ) procedure (Oliveira *et al.*, 2011). Two extenders were prepared, representing the A and B fractions. The A fraction was used for liquefaction of the seminal coagulum and consisted of 5.84 g ACP 118® (ACP Biotecnologia®, Fortaleza, Ceará, Brazil) diluted in 50 ml of ultrapure water. The B fraction was used for freezing and was composed of 60% A fraction, with a final concentration of 20% egg yolk, and 3% glycerol (Sigma Chemical Corporation, St. Louis, MO, USA). Both A and B fractions were either or not supplemented with the antioxidant enzyme catalase (Sigma), as follows: (i) fractions A and B without catalase (Control); (ii) fractions A and B with 10 μg/ml catalase; or (iii) fractions A and B with 50 μg/ml catalase. Both concentrations were reached after semen dilution in the extenders. After collection, semen coagulum was divided into three equal parts, each one was diluted (1:1) in fraction A of the tested treatments (Control, or 10 μg/ml or 50 μg/ml catalase) and maintained at 37°C in a water bath. Seminal coagulum in each extender was mechanically dissociated (Oliveira *et al.*, 2011) until a volume of >100 μl of liquid fraction was obtained. Samples were cooled down from 37°C to 4°C within 90 min (–0.4°C/min). An equal volume of precooled material (4°C) corresponding to the B fraction from each treatment was added in three steps interspersed with 10 min breaks. The spermatozoa were drawn into 0.12 ml plastic straw (IMV, L'Aigle, France) and sealed with metal beads. Straws were placed horizontally on a rack 10 cm above the surface of liquid nitrogen. Twenty minutes later, they were submerged directly into liquid nitrogen for 1-week storage. For thawing, straws were kept in a water bath (37°C) for 30 s, and thawed semen was evaluated microscopically. Samples were evaluated for sperm motility, vigour, plasma membrane integrity, and morphology. All evaluations were performed under a light microscope (Nikon, Tokyo, Japan) at ×100 magnification. Sperm vigour was evaluated on a scale of 0 to 5 (Oliveira *et al.*, 2011). Sperm motility was evaluated with the help of an optical microscope, and expressed as the percentage of cells actively moving (Dong *et al.*, 2008). For this, 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 (Dong *et al.*, 2008).

Sperm plasma membrane integrity and morphology, including morphologic defects, were evaluated as described previously (Oliveira *et al.*, 2011). Sperm motility, plasma membrane integrity and morphology were compared using analysis of variance (ANOVA) and vigour was compared using the Kruskal–Wallis test. A P-value < 0.05 was considered to be statistically significant. When an animal provided more than one ejaculate, the mean value was used for statistical analysis as a unit, and not all the values individually from a single animal.

Results

All the males ($n = 6$) used in this study were healthy during the experiments and presented symmetrical testes with normal consistency and mobility. Mean (±SD) body weight was 3.915 ± 3 kg. Testicular biometry is presented in Table S1. In total, 26 semen collection trials (at least two attempts in each of the six males) were performed resulting in seven ejaculates from four males (Table 1). Before liquefaction, the seminal coagulum presented a yellowish colour and was opaque. Mean (±SD) collected volume of coagulated semen was 410 ± 129 μl (250–600 μl; min–max). After liquefaction some seminal microclots were still present, but without interference with semen manipulation and cooling or freezing procedures. No significant effect of liquefaction or cooling on the evaluated semen parameters was observed (Table 2). However, freezing–thawing led to a significant decrease in sperm motility and vigour, as well as impaired plasma membrane integrity. Exposure to catalase during dilution and cooling did not improve sperm motility, or plasma membrane integrity. Similar results were observed when sperm motility and vigour were evaluated after freezing–thawing. However, cryopreservation solution supplementation with 50 μg/ml catalase maintained plasma membrane integrity similar to that of fresh sperm (Table 2). Liquefaction, cooling, and freezing–thawing did not affect sperm morphology, independently of extender supplementation with catalase (Table 3).

Discussion

The limited capacity of storing antioxidant enzymes due to small cytoplasm, combined with a

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Table 1 Mean (±SD) data of ejaculates (number), sperm motility (%), vigour (grade), and sperm plasma membrane integrity (PMI; %)

Animal	Ejaculates	Group	Motility	Vigour	PMI
I	1	Control	90	5	71
II	1	Control	90	5	90
III	1	Control	50	4	51
IV	4	Control	72.5 ± 23	4	65 ± 21
I	1	Catalase 10 µg/ml	90	5	78
II	1	Catalase 10 µg/ml	90	5	58
III	1	Catalase 10 µg/ml	20	4	51
IV	4	Catalase 10 µg/ml	74 ± 23	5	51 ± 26
I	1	Catalase 50 µg/ml	90	5	60
II	1	Catalase 50 µg/ml	90	5	80
III	1	Catalase 50 µg/ml	0	0	27
IV	4	Catalase 50 µg/ml	72 ± 23	4	69 ± 24

Table 2 Motility, vigour, and plasma membrane integrity (PMI) of *Sapajus apella* sperm

		Motility	Vigour	PMI
Diluted	Control	77 ± 19^{A}	5 ± 1^{A}	60 ± 22^{A}
	Catalase 10 µg/ml	69 ± 33^{A}	5 ± 1^{A}	59 ± 19^{A}
	Catalase 50 µg/ml	63 ± 43^{A}	4 ± 2^{A}	39 ± 12^{A}
Cooled	Control	59 ± 41^{A}	3 ± 2^{A}	60 ± 22^{A}
	Catalase 10 µg/ml	54 ± 42^{A}	3 ± 2^{A}	59 ± 20^{A}
	Catalase 50 µg/ml	51 ± 38^{A}	3 ± 2^{A}	39 ± 12^{A}
Frozen–thawed	Control	6 ± 12^{B}	1 ± 1^{B}	11 ± 19^{B}
	Catalase 10 µg/ml	4 ± 8^{B}	1 ± 1^{B}	14 ± 15^{B}
	Catalase 50 µg/ml	7 ± 14^{B}	1 ± 1^{B}	15 ± 29^{A}

[A,B]Different uppercase letters indicate differences between means comparing diluted, cooled and freezing–thawing within the same group; $P < 0.05$.

Table 3 Mean (± SD) percentages of morphologically normal sperm and sperm with pathologic defects

	Diluted[a]			Cooled			Frozen–thawed		
Sperm	Control	Catalase		Control	Catalase		Control	Catalase	
morphology		10 µg/ml	50 µg/ml		10 µg/ml	50 µg/ml		10 µg/ml	50 µg/ml
Normal spermatozoa	77 ± 4	82 ± 3	81 ± 3	78 ± 4	85 ± 4	82 ± 3	84 ± 6	88 ± 2	88 ± 2
Primary abnormalities									
Strongly coiled tail	5 ± 1	4 ± 1	2 ± 1	6 ± 2	2 ± 0	3 ± 1	3 ± 1	5 ± 1	3 ± 1
Secondary abnormalities									
Coiled tail	11 ± 3	10 ± 2	10 ± 2	10 ± 2	6 ± 2	9 ± 2	9 ± 4	5 ± 1	6 ± 1
Bent tail	7 ± 1	4 ± 1	7 ± 1	7 ± 1	6 ± 2	4 ± 1	4 ± 1	2 ± 1	3 ± 1

[a]After liquefaction.

rich membrane with unsaturated fatty acids makes sperm susceptible to oxidative stress and to lipid peroxidation (Aitken & Baker, 2006; Agarwal *et al.*, 2014). Thus, supplementation of the extender with antioxidant has been reported as an alternative to protect the sperm against ROS during semen cryopreservation. Usually the targets are the plasma membrane integrity and sperm motility (Aitken & Baker, 2006; Moubasher *et al.*, 2013). No effect was observed when extenders were supplemented with

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catalase during liquefaction and cooling. This can be simply because no expressive deleterious effects were observed during these procedures, without a need to include catalase. Catalase is already naturally found in cells, and the stress levels occurred during the present liquefaction and cooling processes did not require exogenous catalase. Catalase was efficient to maintain sperm motility after the cryopreservation process. Differently from our findings, the use of catalase (200 IU/ml) was associated with an improved human sperm viability, increase in percentage of progressive motility, as well as decrease in DNA damage, when compared with ejaculates cryopreserved without catalase (Moubasher *et al.*, 2013). In this study, all treatments had partially recovered post-thaw sperm motility in *S. apella*, differently from the data reported by Oliveira *et al.* (2011) who described absence of motility after cryopreservation in TEST and *in natura* coconut water extenders. This discrepancy may be due the extenders used by Oliveira *et al.* (2011), as well as the cryopreservation protocol performed within 4.8 hours, while in the present study all handling took 3.4 h, a factor that influence ROS production (Kothari *et al.*, 2010). Considering that supplementation with catalase was effective in maintaining the plasma membrane integrity in frozen–thawed sperm, this antioxidant may have minimized the effects of lipid peroxidation on *S. apella* sperm membrane (Mccarthy & Meyers, 2011). However, it is not advised to simply supplement extender with catalase, and other strategies or antioxidants should improve not only sperm membrane integrity, but motility post-thaw.

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Supplementary material

To view supplementary material for this article, please visit https://doi.org/10.1017/S0967199416000447

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