

Coffea arabica Pulp as a Natural Antioxidant to Improve Cryopreserved Brown Swiss Bull Semen Quality: A Pilot Study

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Abstract

Cryopreservation induces oxidative stress in bovine semen, compromising sperm quality. This pilot study evaluated the antioxidant potential of *Coffea arabica* pulp (CAP), an agro-industrial by-product, on refrigerated and cryopreserved Brown Swiss bull semen. Ten ejaculates were diluted in Optixcell® and supplemented with CAP (0, 1, or 2 mg/ml). Sperm motility and kinematics (CASA), membrane integrity (HOST), and acrosomal integrity (Coomassie blue) were assessed. CAP at 1 mg/ml (T1) significantly improved progressive motility (81.2%) in cryopreserved samples compared with the control (62.6%, $p < 0.05$). Kinematic parameters (VAP, VSL) were also higher in T1 than in the control ($p < 0.05$). Acrosomal integrity was improved in T2 (88.8%, $p < 0.05$), while membrane functionality increased in refrigerated semen with T1 ($p < 0.05$). These findings indicate that CAP may act as a natural antioxidant to enhance semen cryopreservation outcomes. Further studies with larger sample sizes and fertility trials are warranted.

INTRODUCTION

Cryopreservation is a key technique for preserving genetic resources in cattle; however, it induces oxidative stress that compromises sperm function. Reactive oxygen species (ROS) can damage sperm membranes and DNA, ultimately reducing fertility¹. Supplementation of semen extenders with antioxidants has been shown to mitigate the harmful effects of ROS, thereby improving post-thaw semen quality². Natural antioxidants such as plant extracts and by-products are increasingly being investigated due to their bioactive compounds and sustainability³. *Coffea arabica* pulp (CAP), a by-product rich in phenolic compounds, exhibits strong antioxidant activity, yet its potential application in bovine semen cryopreservation remains unexplored. The aim of this study was therefore to evaluate the effect of CAP supplementation on sperm quality during refrigeration and cryopreservation of Brown Swiss bull semen.

MATERIALS AND METHODS

Ten ejaculates from a Brown Swiss bull were collected using an artificial vagina, diluted in Optixcell®, and divided into three groups: control (0 mg/ml CAP), T1 (1 mg/ml CAP), and T2 (2 mg/ml CAP). Samples were refrigerated (4°C, 16 h) and cryopreserved in liquid nitrogen. Semen quality was assessed post-refrigeration and post-thaw using computer-assisted sperm analysis (CASA), the hypo-osmotic swelling test (HOST) for membrane functionality, and Coomassie blue staining for acrosomal integrity. Data were analyzed using ANOVA followed by Tukey's post hoc test, with significance set at $p < 0.05$.

RESULTS AND DISCUSSION

As expected, progressive motility decreased after cryopreservation, consistent with previous reports showing that freezing and thawing compromise sperm viability and kinematics¹. However, supplementation with CAP at 1 mg/ml (T1) maintained significantly higher progressive motility (81.2%) compared with the control (62.6%, $p < 0.05$), suggesting a protective antioxidant effect (Table 1). Similar

improvements in motility and velocity parameters have been observed when other natural antioxidants were added to semen extenders ^{1,4}.

Kinematic parameters such as VAP and VSL are key predictors of sperm transport efficiency in the female reproductive tract. Their improvement in CAP-treated groups is in line with studies demonstrating that antioxidants stabilize sperm membranes and enhance flagellar function during cryopreservation ². As shown in Table 1, CAP supplementation preserved higher VAP and VSL values after thawing compared to the control.

Membrane integrity, as evaluated by HOST, was also significantly improved in refrigerated semen supplemented with CAP (Fig. 1). This agrees with findings from ³ who reported that plant-derived compounds can preserve lipid and protein composition of sperm membranes under capacitation and stress conditions.

In addition, acrosomal integrity was significantly higher in the T2 group (88.8%) compared with the control, suggesting a dose-dependent protective effect (Fig. 2). Similar protective effects of selenium and plant-based antioxidants on the acrosome have been previously reported in avian and bovine models ⁴.

Although this study was limited by the use of a single bull, the results provide preliminary evidence that *Coffea arabica* pulp supplementation can mitigate cryodamage in bovine semen. Moreover, valorization of CAP contributes to sustainable livestock production by transforming an agro-industrial by-product into a functional additive, consistent with current trends in sustainable animal reproduction research ³.

Table 1

Effect of the addition of CAP on sperm kinetic parameters in refrigerated and cryopreserved samples.

Seminal Parameters		Refrigerated			Cryopreserved		
		Treatment with CAP (g)			Treatment with CAP (g)		
		0	1	2	0	1	2
Sperm Motility	Total Motility (MT%)	98.05 ± 3.92 ^a	99.21 ± 1.05 ^a	99.13 ± 1.23 ^a	92.39 ± 4.51 ^a	95.84 ± 3.78 ^a	95.87 ± 4.26 ^a
	Progressive Motility (MP%)	91.21 ± 8.94 ^a	92.81 ± 5.97 ^a	83.68 ± 14.41 ^a	62.63 ± 11.96 ^b	81.19 ± 15.62 ^a	70.48 ± 15.49 ^{ab}
Kinetic Parameters	VCL (µm/s)	179.29 ± 30.71 ^a	168.72 ± 35.66 ^a	161.95 ± 25.31 ^a	148.75 ± 20.36 ^a	163.64 ± 21.90 ^a	152.92 ± 14.77 ^a
	VAP (µm/s)	102.58 ± 16.48 ^a	100.17 ± 19.51 ^a	91.90 ± 12.30 ^a	80.11 ± 7.19 ^b	93.45 ± 13.00 ^a	86.51 ± 7.84 ^{ab}
	VSL (µm/s)	82.52 ± 13.60 ^a	81.28 ± 16.23 ^a	74.58 ± 10.64 ^a	64.50 ± 6.21 ^b	75.74 ± 10.76 ^a	70.49 ± 6.94 ^{ab}
	STR (%)	80.45 ± 0.40 ^a	81.94 ± 3.60 ^a	80.96 ± 1.88 ^a	80.32 ± 0.88 ^a	80.81 ± 1.19 ^a	81.26 ± 1.32 ^a
	LIN (%)	48.06 ± 3.84 ^a	50.58 ± 5.41 ^a	49.35 ± 6.46 ^a	46.53 ± 3.18 ^a	47.78 ± 1.73 ^a	47.99 ± 2.73 ^a
	WOB (%)	59.41 ± 4.73 ^a	61.98 ± 5.82 ^a	60.48 ± 6.56 ^a	57.68 ± 4.13 ^a	56.78 ± 4.31 ^a	57.70 ± 3.88 ^a
	ALH (µm)	4.31 ± 0.66 ^a	3.93 ± 0.89 ^a	3.92 ± 0.63 ^a	3.78 ± 0.53 ^a	3.99 ± 0.42 ^a	3.89 ± 0.34 ^a
	BCF (hz)	13.48 ± 2.26 ^a	15.05 ± 2.89 ^a	14.45 ± 2.70 ^a	12.66 ± 1.56 ^a	14.41 ± 1.95 ^a	14.07 ± 2.11 ^a

CONCLUSION

Coffea arabica pulp improved progressive motility, membrane functionality, and acrosomal integrity in cryopreserved Brown Swiss bull semen, particularly at 1 mg/ml. CAP represents a promising natural antioxidant for bovine semen cryopreservation. Larger-scale studies are required to validate these results and determine its impact on fertility.

Declarations

ETHICAL APPROVAL

All experimental procedures involving animals were conducted in accordance with the ethical standards of the institutional committee for animal care and use (Universidad Nacional Toribio Rodríguez de

Mendoza de Amazonas) and following the guidelines established by Peruvian Law No. 30407 on Animal Protection and Welfare, with approval number CIEI-N° 0071.

DATA AVAILABILITY

The datasets generated and analyzed during the current study are available from the corresponding author upon reasonable request.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Figures

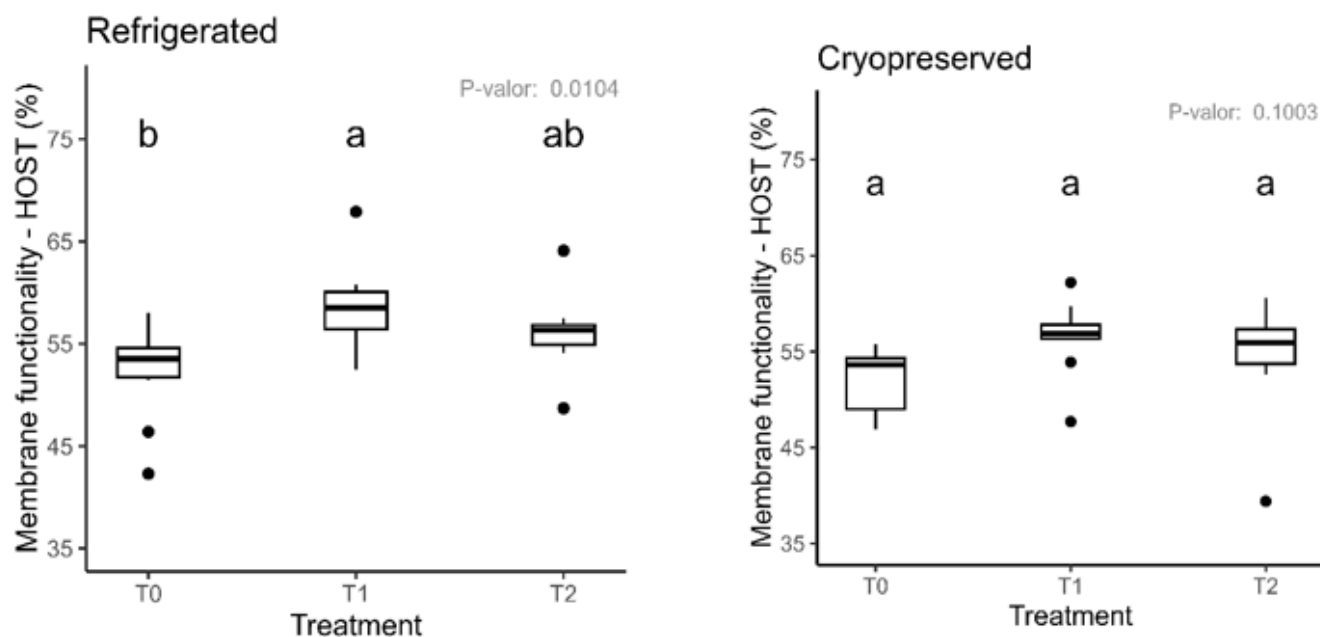


Figure 1

Boxplot - Functionality of Bovine Sperm Membrane – HOST.

Note: Treatment 0 (T0): Control (without CAP); Treatment 1 (T1): CAP 1 mg/ml; and Treatment 2 (T2): CAP 2 mg/ml. Means with the same letter indicate no significant differences according to the refrigerated and cryopreserved samples.

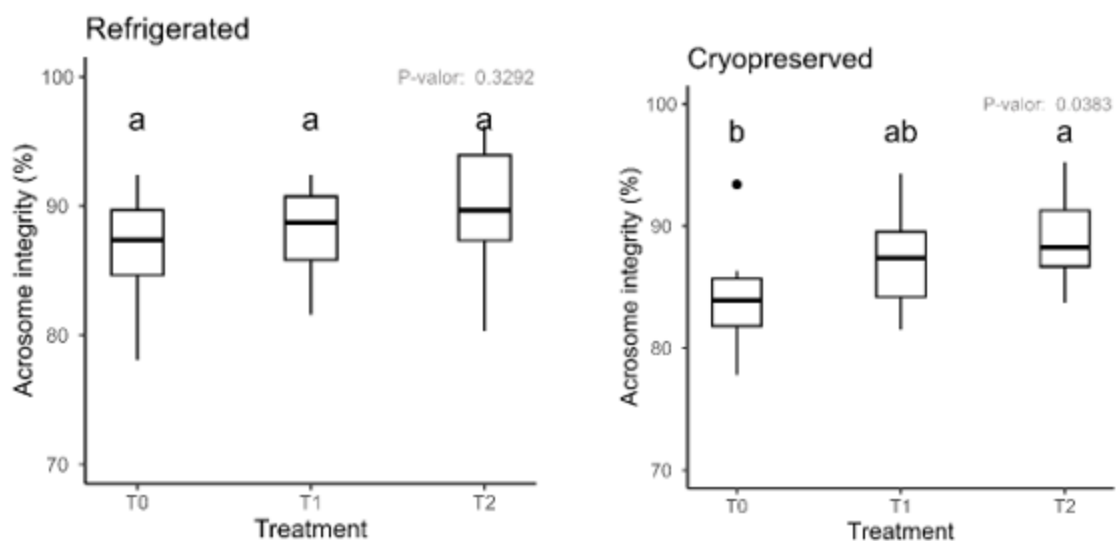


Figure 2

Acrosomal integrity - Coomassie blue 0.22%

Note: Treatment 0 (T0): Control (without CAP); Treatment 1 (T1): CAP 1 mg/ml; and Treatment 2 (T2): CAP 2 mg/ml. Means with the same letter indicate no significant differences according to the refrigerated and cryopreserved samples.

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