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Nanocarried antioxidants in freezing extenders for boar spermatozoa

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Abstract

Post-thawing cryoinjuries in boar spermatozoa due to oxidative stress may be reduced by adding nanoencapsulated antioxidants to freezing extenders. This study evaluated post-thawing kinetics, structural and biochemical functions of boar spermatozoa frozen with extenders including resveratrol and vitamin E loaded into polymeric nanocapsules. Resveratrol was added at 0 (control), 5, 10, 20, 40 and 80 μg/ml, whereas Vitamin E was added at 0 (control), 50, 100, 200 and 400 μg/ml. Both antioxidants were tested in free and nanoencapsulated presentations. In contact with empty nanocapsules, some sperm kinetics parameters were impaired compared to the control ($p < .05$), whereas lipoperoxidation declined ($p < .05$). With inclusion of 40 μg/ml nanoencapsulated resveratrol, some sperm kinetics parameters were improved ($p < .01$), but sperm motility, structural and biochemical functions did not differ from the control ($p > .05$). No improvement in sperm quality occurred with inclusion of vitamin E, although sperm kinetics with 400 μg/ml nanoencapsulated vitamin E was reduced compared to the control ($p < .01$). Inclusion of 40 μg/ml nanoencapsulated resveratrol benefitted boar sperm kinetics after thawing, but no improvement resulted from inclusion of vitamin E.

KEYWORDS

boar spermatozoa, cryopreservation, resveratrol, vitamin E

1 | INTRODUCTION

In swine, most artificial inseminations (AI) are routinely conducted with cooled spermatozoa (Waberski et al., 2019), since AI with frozen spermatozoa yields inferior performance (Yeste, 2016). That may result from injuries in spermatozoa attributed to cold shock during freezing and thawing. Also, boar spermatozoa have high content of unsaturated fatty acids in their plasma membrane and limited intracellular antioxidant mechanisms, being prone to oxidative stress due to lipoperoxidation and to the production of reactive oxygen species (ROS) (Cerolini et al., 2000). Thus, inclusion of antioxidants in extenders for sperm freezing can mitigate oxidative stress.

One of such antioxidants would be vitamin E, which is a natural component of spermatozoa's antioxidant system (Lenzi et al., 1996). Positive effects of vitamin E on post-thawing quality have been reported for spermatozoa of men (Taylor et al., 2009), bulls (Nasiri et al., 2012) and boars (Peña et al., 2003; Satorre et al. 2007). Other potentially efficient antioxidant is resveratrol, a molecule found in grapes which has anti-inflammatory properties, but low stability and solubility (Vitaglione et al., 2005). Positive effects of resveratrol were described for human (Branco et al., 2010), bull (Bucak et al., 2015) and buffalo spermatozoa (Longobardi et al., 2017), but negative effects were reported for ram spermatozoa (Silva et al., 2012). Data for boar spermatozoa revealed no effect on post-thawing quality, but positive effects on in vitro oocyte penetration (Gadani et al., 2017).

Nanocarriers may boost the availability of antioxidants included in media, as reported for resveratrol and vitamin E in studies testing anticancer therapies (Shao et al., 2009; Gorain et al., 2018 respectively). Improved post-thawing quality of rooster spermatozoa was observed with extenders including nanoparticles containing vitamin E (Safa et al., 2016) and resveratrol (Najafi et al., 2019). Nevertheless, antioxidants loaded into nanocarriers have not yet been tested for frozen boar spermatozoa. The aim of this study is to evaluate the effects of the inclusion of nanoencapsulated resveratrol and vitamin E in the freezing extender on kinetics, structural and biochemical parameters of boar spermatozoa after thawing.

2 | MATERIALS AND METHODS

2.1 | Preparation of formulations

Except when stated differently, the chemicals used in this study were from Sigma Chemical Company. The nanocapsules were prepared by interfacial deposition of the Eudragit® S100 polymer [poly (methacrylic acid methacrylate)] (Evonik Health Care). Two solutions were formulated in 26 ml acetone: one with 50 mg resveratrol; the other with 465 mg vitamin E. Both received 94 mg Eudragit® S100, 72 mg Sorbitan monooleate (Span 80, Medquímica) and 300 mg medium chain triglycerides (Fragon), to compose the organic phase, which was slowly injected under agitation into the aqueous phase (water plus polysorbate 80 Tween 80®). The resulting solution was concentrated to remove the acetone using a rotatory evaporator (Fisatom) under reduced pressure, until reaching a white-milky appearance with a volume of 50 ml (either 9.3 mg/ml vitamin E or 1.0 mg/ml resveratrol). Both antioxidants were also tested in a free presentation, as a solution of 0.05% DMSO in sterile water with the same volume of the nanoencapsulated form. Empty nanocapsules were prepared similarly.

The contents of vitamin E and resveratrol in the formulations were analysed by spectrophotometry, as described by Detoni et al. (2012). From a mother solution of each antioxidant in acetonitrile, a calibration curve was developed, containing five points defined according to the interval of concentrations for each molecule. The absorbances were read with a 305 wavelength. Then, 200 μl of each formulation was diluted in acetonitrile. The molecules were recovered by adding known concentrations of the standard solution to the formulations. The macroscopic characteristics of the nanoencapsulated formulations were within expected standards (Table S1). The diameter and the Zeta potential of the nanocapsules were determined by dynamic light scattering using Zetasizer® (Malvern Panalytical). The pH was determined using a pH meter (Hanna Instruments®).

2.2 | Semen freezing and thawing

The sperm donors were five sexually mature boars of the same genetic line, which were housed in individual pens and fed 2.8 kg/d of a commercial diet (6.0% crude protein and 3,200 kcal/kg). Access to water was *ad libitum*. Seven sperm samples were collected per boar through the gloved hand method at weekly intervals. The gel fraction of the ejaculates was separated by a disposable filter, so only the sperm-rich portion was processed (Corcini et al., 2012).

Samples with sperm motility and normal morphology of at least 80% were extended in Beltsville Thawing Solution (BTS) (Pursel & Johnson, 1975), with 3×10^9 viable spermatozoa/ml. The samples were stabilised at 22°C for 2 hr, kept at 17°C for 3 hr and centrifuged at 800×*g* for 10 min. After the supernatant was removed, the resulting pellet was resuspended in 2/3 of the final volume of the extender (11% lactose and 20% egg yolk, v/v).

Empty nanocapsules were added to the cooling extender at 0 (control), 10, 20, 30, 40 and 50%, in a room with no direct light incidence. Antioxidants were added in both the free and the nanoencapsulated presentations. Resveratrol was added at 0 (control), 5, 10, 20, 40 and 80 μg/ml, and vitamin E was added at 0 (control), 50, 100, 200 and 400 μg/ml.

Diluted samples were placed in a cold chamber at 5°C for 90 min. The remaining volume (1/3) was added to the tubes to complete the freezing extender: 89.5% of the cooling extender; 1.5% Equex-Paste® (Nova Chemical Sales); and 9% glycerol (v/v). Samples with 1.0×10^9 spermatozoa/ml were packed in 0.5 ml straws and placed at 5 cm above the vapour of liquid nitrogen. After 20 min, samples were immersed in liquid nitrogen and stored.

Thawing was done in water bath at 37°C for 20 s. A 100 μl portion was diluted in 900 μl BTS containing 3.0 mg BSA/ml. Then, a 1.0 ml aliquot was split in two portions: one (0.8 ml) was incubated at 37°C for 10 min for evaluation of spermatozoa kinetics; the other (0.2 ml) was cooled at 5 °C for evaluations of structural and biochemical functions.

2.3 | Evaluations of spermatozoa kinetics

These evaluations were done with a computerised system (SpermVision, Minitube) using 3.0 μl of diluted spermatozoa, previously heated at 38°C for 10 min. The evaluated parameters were total motility; progressive motility; distance in a straight and in a curved line (DSL and DCL respectively); distance average path (DAP); velocity in a straight (VSL) and in a curved line (VCL); velocity average path (VAP); linearity (LIN); straightness (STR); amplitude of lateral head displacement (ALH); wobble (WOB); and beat cross frequency (BCF).

2.4 | Evaluations of spermatozoa structure and biochemical functions

These evaluations were conducted by flow cytometry (Attune®, Thermo Fisher Scientific) using blue (Argonium 488 nm) and purple lasers (UV 450 nm), in samples with 10 μl of spermatozoa plus 20 μl Hoechst 33342 and 500 μl PBS/EDTA, at 37°C for 10 min.

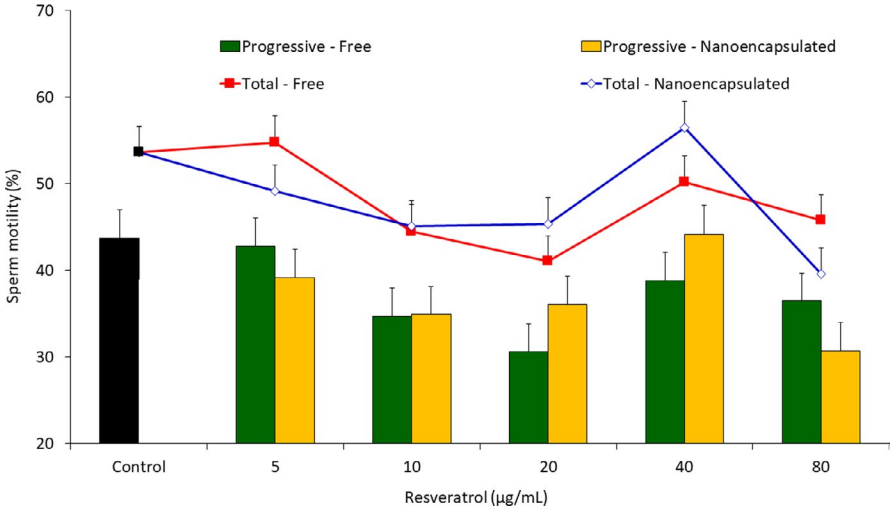

FIGURE 1 Total and progressive motility after thawing for boar spermatozoa processed with extender including resveratrol at distinct presentations and concentrations*. *Means did not differ ($p < .05$)

Membrane functionality was evaluated using a conjugate of 20 µM carboxyfluorescein diacetate (CFDA) and 7.5 µM propidium iodide (PI). Functional membranes (stained with CFDA) showed green fluorescence. Nonfunctional membranes (permeable to IP) showed either red fluorescence or simultaneous green and red fluorescence (Gillan et al., 2005).

Mitochondrial functionality was determined using 100 nM rodamine 123 (rh123) and 7.5 µM IP. Spermatozoa with highly functional mitochondria were stained with intense green fluorescence in the intermediate piece, whereas those with low mitochondrial functionality exhibited weak green fluorescence (Gillan et al., 2005).

Acrosomal status was evaluated using 7.5 µM IP and a conjugate of fluorescein isothiocyanate and lecithin peanut (FITC-PNA). As FITC-PNA only binds to the external acrosome membrane, intact acrosomes were impermeable and remained unstained. Damaged acrosomes were stained with IP, presenting red fluorescence (Petrunkina et al., 2005).

Spermatozoa DNA fragmentation was determined with acridine orange and expressed by the median DNA fragmentation index (Myromslien et al., 2019). Orange fluorescence marked denatured DNA, whereas intact DNA was stained with green fluorescence (Jenkins et al., 2015).

The production of ROS was assessed using 7.5 µM IP and 2.0 µM 2'7 'dichlorofluorescein diacetate (DCF). Spermatozoa producing ROS showed green fluorescence (Dominguez-Rebolledo et al., 2011), and those with integer membranes were unstained.

Lipoperoxidation was evaluated using the C11-BODIPY fluorophore (5 µM). When C11-BODIPY is incorporated to the membranes, it causes an irreversible reaction in response to the attack of free radicals, staining lipoperoxidised spermatozoa with a green fluorescence. Red fluorescence was shown when there was no lipoperoxidation (Hagedorn et al., 2012).

2.5 | Statistical analyses

Since all responses of interest lacked normality according to the Shapiro–Wilk test, the data were normalised though transformations (logarithmic, power and arcsine), depending on each variable. Analyses of variance models including the effect of the boars, considered five treatments for empty nanocapsules, 11 treatments for resveratrol and 9 treatments for vitamin E. Comparisons of means were done by the Tukey test. To allow interpretation, all data were shown in their original scales. All statistical analyses were conducted with Statistix® (2013).

3 | RESULTS

3.1 | Empty polymeric nanocapsules

Generally, spermatozoa kinetics declined ($p < .05$) as the concentration of empty nanocapsules increased (Table S2). Inclusion of the lowest tested concentration (10%) did not affect kinetics parameters ($p > .05$), except for total motility, which was lower compared to the control. With inclusion of empty nanocapsules at any concentration, lipoperoxidation was reduced compared to the control ($p < .05$). All other structural and biochemical parameters were unaltered ($p > .05$).

3.2 | Resveratrol

For both presentations of resveratrol, total and progressive post-thawing motility did not differ from the control ($p > .05$), at any concentration (Figure 1). Some kinetics parameters (VCL, VSL, VAP, DCL, DSL, DAP, ALH and BCF) were improved compared to the control ($p < .01$), with inclusion of 40 µg/ml nanoencapsulated resveratrol (Table 1). Though, LIN, WOB and STR did not differ from the control ($p > .05$). (Table 1).

Inclusion of resveratrol had no effect ($p > .05$) in spermatozoa structural and biochemical functions, regardless of the presentation and the concentration (Table 2).

TABLE 1 Post-thawing kinetics parameters for boar spermatozoa processed with extender including resveratrol at distinct presentations and concentrations

Resveratrol	DAP	DCL	DSL	VAP	VCL	VSL	LIN	WOB	STR	ALH	BCF
Free (μg/ml)											
Control	22.8^{a,b,c}	39.3^{a,b,c}	16.5^{a,b,c}	51.9^{a,b,c}	90.4^{a,b,c}	38.0^{a,b,c}	0.421	0.579	0.728	2.99^{a,b,c}	31.4^{a,b,c}
5	24.7^{a,b,c}	42.7^{a,b,c}	18.4^{a,b,c}	56.7^{a,b,c}	97.5^{a,b,c}	41.9^{a,b,c}	0.430	0.578	0.740	3.04^{a,b,c}	33.5^{a,b,c}
10	23.2^{a,b,c}	40.8^{a,b,c}	17.3^{a,b,c}	52.8^{a,b,c}	92.3^{a,b,c}	39.6^{a,b,c}	0.422	0.568	0.740	2.83^{a,b,c}	32.7^{a,b,c}
20	23.3^{a,b,c}	40.1^{a,b,c}	17.2^{a,b,c}	52.7^{a,b,c}	91.4^{a,b,c}	39.5^{a,b,c}	0.432	0.578	0.743	2.97^{a,b,c}	32.6^{a,b,c}
40	23.4^{a,b,c}	40.3^{a,b,c}	17.4^{a,b,c}	53.3^{a,b,c}	91.4^{a,b,c}	39.8^{a,b,c}	0.429	0.579	0.736	3.00^{a,b,c}	32.7^{a,b,c}
80	22.5^{a,b,c}	39.5^{a,b,c}	16.4^{a,b,c}	51.4^{a,b,c}	89.7^{a,b,c}	37.4^{a,b,c}	0.410	0.570	0.726	2.83^{a,b,c}	31.7^{a,b,c}
Nanoencapsulated (μg/ml)											
5	23.8^{a,b,c}	41.0^{a,b,c}	17.9^{a,b,c}	54.0^{a,b,c}	94.0^{a,b,c}	41.0^{a,b,c}	0.440	0.588	0.742	3.09^{a,b,c}	32.8^{a,b,c}
10	23.9^{a,b,c}	41.5^{a,b,c}	17.3^{a,b,c}	54.7^{a,b,c}	94.6^{a,b,c}	39.6^{a,b,c}	0.419	0.575	0.723	3.10^{a,b,c}	32.1^{bc}
20	22.3^{a,b,c}	38.4^{a,b,c}	16.5^{a,b,c}	50.8^{a,b,c}	87.2^{a,b,c}	38.0^{a,b,c}	0.431	0.580	0.73	2.86^{a,b,c}	31.7^{a,b,c}
40	25.6^{a,b,c}	44.0^{a,b,c}	18.8^{a,b,c}	58.4^{a,b,c}	100.9^{a,b,c}	43.2^{a,b,c}	0.422	0.579	0.736	3.15^{a,b,c}	34.1^{a,b,c}
80	23.1^{a,b,c}	40.5^{a,b,c}	16.9^{a,b,c}	52.8^{a,b,c}	92.4^{a,b,c}	38.0^{a,b,c}	0.425	0.567	0.731	3.07^{a,b,c}	32.0^{a,b,c}
SEM	0.6	1.2	0.4	1.4	2.7	0.7	0.007	0.004	0.001	0.07	0.4

Abbreviations: ALH, amplitude of head lateral displacement (μm); BCF, beat cross frequency (Hz); DAP, distance average path; DCL, distance in a curved line (μm); DSL, distance in a straight line (μm); LIN, linearity (VSL/VCL, %); STR, straightness (VSL/VAP, %); VAP, velocity average path (μm/s); VCL, velocity in a curved line (μm/s); VSL, velocity in a straight line (μm/s); WOB, wobble (%).

^{a,b,c}Means ± *SEM* with distinct superscripts differ on columns by at least $p < .01$.

TABLE 2 Post-thawing structural and biochemical parameters for boar spermatozoa processed with extender including resveratrol in distinct presentations and concentrations*

Resveratrol	Membrane functionality (%)	Acrosome integrity (%)	Mitochondrial functionality (%)	DNA fragmentation index	Lipoperoxidation (%)	Reactive oxygen species (UF × 10^7)
Free (μg/ml)						
Control	58.1	66.1	3.44	0.042	26.2	19,120.6
5	81.4	59.9	2.53	0.036	23.4	10,216.0
10	75.4	68.2	2.64	0.032	25.9	14,992.9
20	47.6	70.4	2.35	0.044	26.3	12,593.9
40	87.6	63.8	3.03	0.041	24.3	11,167.0
80	55.5	68.3	3.44	0.039	28.1	21,447.6
Nanoencapsulated (μg/ml)						
5	69.7	66.3	2.53	0.043	24.2	9,459.4
10	54.8	73.3	2.64	0.042	26.3	12,987.0
20	59.1	69.7	2.48	0.041	25.2	18,245.0
40	73.3	62.5	2.47	0.040	24.1	10,931.2
80	79.9	72.3	3.15	0.039	28.1	12,633.9
SEM	11.9	7.3	0.97	0.004	3.2	6,984.9

* Means ± *SEM* did not differ ($p > .05$).

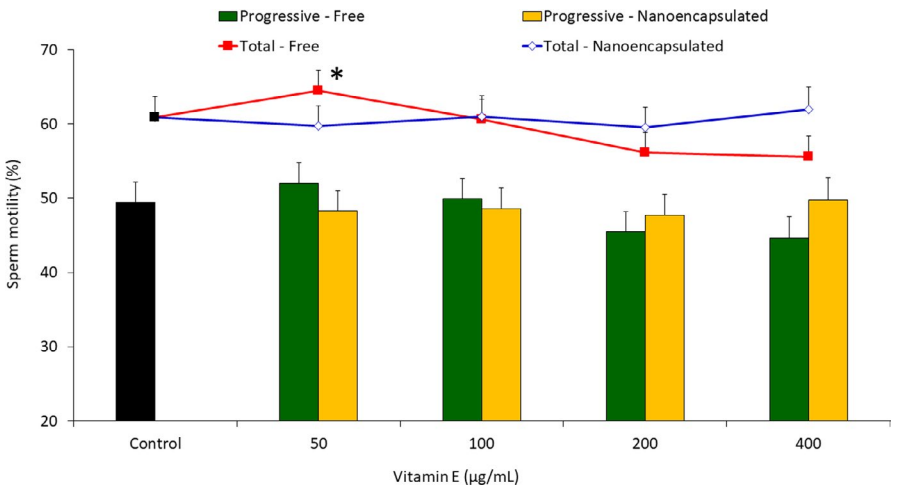

FIGURE 2 Total (a) and progressive (b) motility after thawing for boar spermatozoa processed with extender including vitamin E at distinct presentations. *Total motility with 50 μg/ml free vitamin E is greater than with both 200 and 400 μg/ml free vitamin E ($p = .0223$)

3.3 | Vitamin E

Total and progressive spermatozoa motility in treatments including any concentration of vitamin E did not differ compared to the control ($p > .05$), although total motility in treatments with free 50 μg/ml vitamin E was greater ($p = .0223$) than with 200 and 400 μg/ml (Figure 2). Other kinetics parameters were unaffected ($p > .05$) by inclusion of free vitamin E at any concentration and of nanoencapsulated vitamin E at concentrations equal to or lower than 200 μg/ml (Table 3). In treatments with 400 μg/ml nanoencapsulated vitamin E, VCL, VSL, VAP, DCL, DSL, DAP and BCF were reduced ($p < .01$).

No effects of inclusion of free and nanoencapsulated vitamin E were observed on spermatozoa biochemical functions and structure ($p > .05$, Table 4).

4 | DISCUSSION

This is the first study to report improvement in various parameters of post-thawing kinetics (VCL, VSL, VAP, DCL, DSL, DAP, ALH and BCF) for boar spermatozoa stored in an extender including 40 μg/ml nanoencapsulated resveratrol. Although total and progressive motility were numerically greater compared to the control, the differences were not significant. Inclusion of nanocarried resveratrol at the same concentration was previously related to improved quality of roster spermatozoa after thawing (Najafi et al., 2019). However, in such study, total and progressive motility were the only improved kinetics parameters, along with membrane functionality, mitochondrial activity and total antioxidant capacity. Nonetheless, in the present study, the inclusion of resveratrol in the free presentation was not related to improved sperm quality.

TABLE 3 Post-thawing kinetics parameters for boar spermatozoa processed with extender including vitamin E at distinct presentations and concentrations

Vitamin E	DAP	DCL	DSL	VAP	VCL	VSL	LIN	WOB	STR	ALH	BCF
Free (μg/ml)											
Control	28.3^{a,b}	51.7^{a,b}	20.6^{a,b}	64.0^{a,b}	116.3^{a,b}	45.9^{a,b}	0.384	0.545	0.701	3.38	32.8^{a,b}
50	29.0^{a,b}	51.8^{a,b}	20.6^{a,b}	65.0^{a,b}	117.9^{a,b}	46.7^{a,b}	0.396	0.556	0.712	3.30	33.9^{a,b}
100	29.1^{a,b}	52.3^{a,b}	20.7^{a,b}	65.8^{a,b}	117.9^{a,b}	47.0^{a,b}	0.385	0.546	0.701	3.36	33.6^{a,b}
200	27.6^{a,b}	51.1^{a,b}	19.5^{a,b}	63.2^{a,b}	115.5^{a,b}	45.3^{a,b}	0.382	0.545	0.698	3.24	32.9^{a,b}
400	27.1^{a,b}	49.4^{a,b}	19.0^{a,b}	61.2^{a,b}	111.2^{a,b}	43.1^{a,b}	0.382	0.542	0.702	3.27	32.8^{a,b}
Nanoencapsulated (μg/ml)											
50	28.2^{a,b}	52.3^{a,b}	19.8^{a,b}	64.4^{a,b}	116.5^{a,b}	44.8^{a,b}	0.380	0.545	0.695	3.35	32.8^{a,b}
100	29.1^{a,b}	53.7^{a,b}	20.6^{a,b}	65.9^{a,b}	121.1^{a,b}	46.6^{a,b}	0.382	0.542	0.700	3.42	33.6^{a,b}
200	27.8^{a,b}	50.6^{a,b}	19.5^{a,b}	62.2^{a,b}	114.0^{a,b}	44.1^{a,b}	0.382	0.542	0.702	3.24	33.3^{a,b}
400	25.0^{a,b}	44.8^{a,b}	17.4^{a,b}	57.1^{a,b}	103.0^{a,b}	39.4^{a,b}	0.381	0.556	0.683	3.26	30.6^{a,b}
SEM	0.8	1.6	0.2	1.8	3.5	1.4	0.005	0.004	0.007	0.05	0.6

Abbreviations: ALH, amplitude of head lateral displacement (μm); BCF, beat cross frequency (Hz); DAP, distance average path; DCL, distance in a curved line (μm); DSL, distance in a straight line (μm); LIN, linearity (VSL/VCL, %); STR, straightness (VSL/VAP, %); VAP, velocity average path (μm/s); VCL, velocity in a curved line (μm/s); VSL, velocity in a straight line (μm/s); WOB, wobble (%).

^{a,b} Means ± *SEM* with distinct superscripts differ by at least $p < .01$.

That was also reported for cooled boar spermatozoa, along with adverse effects on subsequent embryo viability (Torres et al., 2021). Positive effects of resveratrol on post-thawing sperm quality were reported for spermatozoa of men (Nashtaei et al., 2018); bulls (Bucak et al., 2015) and rams (Silva et al., 2012), with no effect on oxidative stress, even though resveratrol may be efficient on reducing lipoperoxidation, as reported for men spermatozoa (Collodel et al., 2011). That would occur due to the lipophilicity of resveratrol, which enables its incorporation to the lipid bylayer of membranes, inhibiting the production of free radicals and helping to keep membrane functionality (Halliwell, 2015). However, in such studies, resveratrol was not loaded into nanocarriers. In the present study, with inclusion of 40 μg/ml nanoencapsulated resveratrol, structural and biochemical traits after thawing, including lipoperoxidation and production of ROS, were maintained at levels similar to those of the control. As nanocarriers optimise the availability of biomolecules (Feugang et al., 2019), those findings suggest that nanoencapsulation of 40 μg/ml resveratrol may compensate its natural instability and poor solubility (Vitaglione et al., 2005), resulting in acceptable post-thawing sperm quality, even with no evident improvement on indicators of oxidative stress.

Although nanocapsules have been used to deliver molecules in studies with distinct cellular models (Komninou et al., 2016; Silva et al., 2017), in the present study, some detrimental effects were observed for spermatozoa in contact with empty nanocapsules in the extender. On the other hand, the inclusion of empty nanocapsules in the extender, even at the lowest tested concentration, was related to reduced lipoperoxidation. Therefore, those potentially cytotoxic effects may have been compensated by the improved availability of the antioxidants in the nanoencapsulated presentation, as post-thawing sperm quality was equivalent to the control at some concentrations, and improved, with inclusion of 40 μg/ml nanoencapsulated resveratrol. Therefore, the tested nanocarrier may continue to be tested in future studies, not only to deliver antioxidants, but also to carry other molecules.

Some beneficial effects of vitamin E on post-thawing sperm quality were expected, since vitamin E is a component of spermatozoa's antioxidant mechanisms (Lenzi et al., 1996), with lipophilic action on the fatty acids present in the liposoluble portion of membranes (Breininger et al., 2011). Nonetheless, no improvement on post-thawing sperm quality was observed in the present study in treatments including vitamin E. Although lack of effect of vitamin E in post-thawing boar sperm motility has been reported (Satorre et al., 2007), positive effects of 100–200 μM vitamin E were described for cooled boar spermatozoa (Peña et al., 2003). Such contradictory reports may reflect the greater severity of the cryoinjuries that occur in spermatozoa during freezing and thawing compared to those that occur during cooling, which may limit the efficiency of their intracellular antioxidant systems. Conversely, in the present study, inclusion of 400 μg/ml nanoencapsulated vitamin E was related to some negative effects on spermatozoa kinetics, such as decreased velocity, travelled distances and beat cross frequency. As extenders including 5 μg/ml nanocarried vitamin E

TABLE 4 Post-thawing structural and biochemical functions for boar spermatozoa processed with extender including vitamin E at distinct presentations and concentrations*

Vitamin E	Membrane functionality (%)	Acrosome integrity (%)	Mitochondrial functionality (%)	DNA fragmentation index	Lipoperoxidation (%)	Reactive oxygen species (UF × 10^7)
Free (μg/ml)						
Control	10.3	0.34	14.3	0.019	75.6	3,515.4
50	15.7	0.45	37.0	0.019	75.8	3,321.6
100	8.6	0.52	16.3	0.018	73.5	3,337.0
200	8.2	0.33	30.8	0.019	75.7	3,596.9
400	10.1	0.24	15.4	0.019	75.8	3,260.0
Nanoencapsulated (μg/ml)						
50	9.7	0.21	27.8	0.015	75.2	3,384.6
100	15.2	0.45	9.9	0.018	75.3	3,436.0
200	15.7	0.36	15.9	0.019	75.8	3,127.5
400	9.3	0.70	34.9	0.018	75.8	3,402.0
SEM	3.2	0.17	8.2	0.001	1.7	230.9

* Means ± *SEM* did not differ ($p > .05$).

have been previously related to improved post-thawing quality of roster spermatozoa (Safa et al., 2016), our findings suggest that increased concentrations of vitamin E may be cytotoxic. That is reinforced by the fact that total sperm motility in treatments with 50 μg/ml free vitamin E and some kinetics parameters in treatments with 50–100 μg/ml (in both presentations) were greater than with 200 and 400 μg/ml. Thus, a concentration of vitamin E efficient to improve the quality of boar spermatozoa after thawing is yet to be identified.

5 | CONCLUSIONS

Inclusion of 40 μg/ml nanoencapsulated resveratrol in the freezing extender benefitted post-thawing kinetics of boar spermatozoa, but inclusion of resveratrol in the free presentation did not improve sperm quality. No beneficial effects on post-thawing boar sperm quality were observed with inclusion of vitamin E, in both the free and the nanoencapsulated presentations.

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CONFLICT OF INTEREST

The authors have no conflict of interest to disclose.

DATA AVAILABILITY STATEMENT

The data available in the supplementary material support the findings of this study.

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