



Effect of Date Palm Pollen Extract Supplementation on Ram Epididymal Sperm Quality during Chilled Storage

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Abstract: Post-mortem recovery of epididymal spermatozoa is a valuable technique for genetic conservation in sheep, but is limited by sperm vulnerability to oxidative damage during chilled storage. This study evaluated the protective effects of date palm pollen extract (DPPE) on ovine epididymal sperm quality stored in protein-free saline at 4°C. Epididymal sperm were collected from five mature rams (n=5). For each ram, samples were divided into three aliquots and diluted to a final concentration of 350×10^6 cells mL⁻¹ with DPPE at 0 (control), 40, or 80 mg mL⁻¹. Sperm total motility, progressive motility, and plasma membrane integrity were assessed at 0, 24, and 48 h using computer-assisted sperm analysis (CASA) and hypo-osmotic swelling (HOS) tests. Over 48 hours, all parameters declined significantly (p<0.05), but DPPE attenuated this decline in a dose-dependent manner. The 80 mg mL⁻¹ concentration provided best protection, maintaining higher total motility (67.5% vs. 58.3% control), progressive motility (54.7% vs. 45.5% control), and membrane integrity (63.9% vs. 55.1% control) at 48 hours (p<0.05). These protective effects likely reflect DPPE's flavonoid and phenolic content, which scavenge reactive oxygen species. Validation in commercial, protein-containing extenders and assessment of *in vivo* fertility are required before field application.

Key words: Date palm pollen, ram, epididymal sperm, chilled storage, antioxidant, semen preservation.

In Algeria, sheep production constitutes a vital pillar of the rural economy and a strategic protein source for a rapidly expanding population. With a national herd exceeding 32 m head, the sector predominantly relies on natural breeding practices, yet suffers from substantial annual reproductive

wastage (approximately 25%) and progressive erosion of genetic merit due to uncontrolled cross-breeding (Meziane *et al.*, 2024). Current strategies to enhance genetic progress face significant constraints. Importation of genetically superior semen is restricted by sanitary regulations and prohibitive logistics associated with maintaining elite rams in official artificial-insemination (AI) centres. An underutilised alternative lies in the post-mortem recovery of epididymal spermatozoa from slaughtered elite males, a practice that could enable cryobanking and dissemination of valuable germplasm to accelerate genetic gain without expanding the live male population (Kaabi *et al.*, 2003).

However, the successful implementation of this approach hinges on overcoming critical biological challenges. Spermatozoa are particularly vulnerable to oxidative damage owing to their specific cellular plasma membranes composition, especially the large amount of long-chain polyunsaturated fatty acids (Alvarez and Storey, 1995), while their cytoplasm contains low concentrations of scavenging enzymes (Tvrda *et al.*, 2011). This vulnerability is exacerbated in epididymal spermatozoa, which are harvested in the absence of seminal plasma antioxidants, a protective fraction that contains essential ROS-scavenging proteins and low-molecular-weight antioxidants (Agarwal *et al.*, 2014). Consequently, epididymal sperm are highly susceptible to oxidative stress, cold shock, and DNA fragmentation during temperature fluctuations between the abattoir and laboratory (Yániz *et al.*, 2020).

This oxidative stress can cause an overexpression of reactive oxygen species (ROS) resulting in reduced motility and viability (De Lamirande and Gagnon, 1992), and may ultimately lead to infertility by increasing midpiece morphological defects with deleterious effects on sperm capacitation and acrosome reaction (Agarwal *et al.*, 2014). This vulnerability is further influenced by the complex maturation process sperm undergo during epididymal transit, where regional variations across the caput, corpus, and cauda segments dictate functional competence (Xu *et al.*, 2024; Li *et al.*, 2023). Consequently, the quality and viability of recovered epididymal sperm decline rapidly unless protected by effective extenders during the critical initial hours post-slaughter (Al-Daraji, 2014).

The reproductive biotechnology sector has long sought solutions to mitigate oxidative damage during sperm preservation, with conventional extenders often incorporating synthetic antioxidants like butylated hydroxytoluene (BHT). However, growing concerns regarding potential toxicity (Roca *et al.*, 2016) and escalating costs have intensified the search for natural alternatives (Laghouati *et al.*, 2021). There is growing interest in plant-based alternatives for preserving sperm quality, as many medicinal plants are rich in natural antioxidant compounds such as vitamins, flavonoids, and carotenoids (Fatma *et al.*, 2009), which act as scavengers of ROS and have protective effects against oxidative stress damage (Ross *et al.*, 2010). In this context, date palm pollen (*Phoenix dactylifera* L., DPP) emerges as a particularly compelling option for Algerian breeding programs.

Algeria ranks as the world's fourth-largest date producer, generating over 200 t of fresh pollen during the spring pollination period, a resource with minimal commercial value outside traditional folk medicine, where it has been used to treat male infertility and improve reproductive performance (Bahmanpour *et al.*, 2006). Chemically, date palm pollen (DPP) contain a unique profile rich in flavonoids (quercetin, rutin, luteolin), sterols (β -sitosterol, estrone-like compounds), tocopherols, and water-soluble phenolic acids that collectively scavenge reactive oxygen species (ROS) and enhance intrinsic antioxidant enzyme systems (Baliga *et al.*, 2011; Gana *et al.*, 2023). However, the bioavailability of these compounds depends critically on extraction methodology, with solvent-based methods (ethanol, methanol) yielding three to five times higher phenolic recovery than aqueous soaking (Sun *et al.*, 2025). The aqueous extraction method used in this study may therefore underestimate DPP's true antioxidant potential.

Recent studies across multiple species have demonstrated DPP's efficacy in improving sperm quality parameters during chilled storage and cryopreservation, with reported enhancements in motility, membrane integrity, and reduced lipid peroxidation (Laghouati *et al.*, 2023). In rabbits, oral administration of DPP enhanced testosterone production and spermatogenesis (Al-Samarrai *et al.*, 2017). *In vitro* studies in other species, such as bulls and stallions, have

shown that DPP extract supplementation to sperm extenders preserves semen quality (El-Sheshtawy *et al.*, 2016; Laghouati *et al.*, 2023). Crucially, the effective concentration varies dramatically by species and storage method: 0.48 mg mL⁻¹ for bull sperm (El-Seadawy *et al.*, 2023), 20–30 mg mL⁻¹ for stallions (El-Sisy *et al.*, 2018), and 80 mg mL⁻¹ for goats (Ng *et al.*, 2022). This 160-fold range underscores that dose optimisation is mandatory for each new application and cannot be extrapolated across species or sperm types.

Despite these positive findings, no study has yet examined the effect of DPP on ovine epididymal spermatozoa, which are significantly more fragile than ejaculated sperm due to the absence of seminal plasma and exposure to epididymal handling stress (Kaabi *et al.*, 2003; White and Wales, 1961). Furthermore, the ideal DPP concentration for ram sperm remains undefined, and previous studies have utilised protein-rich extenders that mask potential adverse effects unlike the protein-free saline required for epididymal sperm preservation.

We hypothesised that supplementing a protein-free saline medium with DPP extract would dose-dependently preserve ram epididymal sperm quality during cooled storage at 4°C. A protein-free saline medium was deliberately selected as a simplified experimental system to isolate the protective effects of DPP without interference from the antioxidant and cryoprotective components present in conventional semen extenders. Therefore, the results may not translate well to real semen preservation systems used in breeding programs. This study aims to examine the effects of two concentrations of DPP extract (40 and 80 mg mL⁻¹) on total motility, progressive motility, and membrane integrity after 48 hours of refrigeration.

Materials and Methods

Ethical approval

All biological material was obtained post-mortem from a licensed abattoir (El Oued, Algeria). No live animals were used.

Animals and sample collection

Testes were collected from five sexually mature rams (aged 18 to 24 months old) that were slaughtered at a local abattoir in El Oued

in the southeast of Algeria. The samples were transported to the laboratory within 2 h in isotonic saline maintained at approximately 4°C (in ice). Upon arrival, the cauda epididymides of each ram were dissected and carefully cleaned of adhering connective tissue, blood vessels, and fat.

Spermatozoa were recovered from each cauda epididymidis using the retrograde flushing technique described by Martinez-Pastor *et al.* (2006). Briefly, a single incision was made near the junction of the corpus and cauda epididymidis. A 1 mL syringe filled with warm (37°C) saline solution (0.9% NaCl, 308 mOsm kg⁻¹, pH 7.2) was used to gently flush the duct by inserting the needle into the vas deferens and applying slow, steady pressure. The fluid containing the spermatozoa emerging from the incision was collected in a sterile 15 mL conical tube.

Sampling of plant material

Date palm (*Phoenix dactylifera* L., cultivar: Dokkars) pollen was collected from the Hamraia region (El Oued, Algeria) during the spring pollination season (March 2024). The pollen grains were air-dried for 48 hours in the dark at room temperature (approximately 25 ± 2°C) and subsequently stored at 4°C until use.

Date palm pollen chemical analysis

The nutritional composition of date palm pollen powder (DPP) was determined using standard analytical procedures. Moisture content was measured by oven drying to constant weight, ash content by incineration in a muffle furnace, crude protein by the Kjeldahl method, crude fat by Soxhlet extraction, and carbohydrate content was calculated by difference. Mineral elements (Ca, Mg, K, and Na) were quantified following acid digestion of samples using standard laboratory procedures. Antioxidant activity was evaluated using the DPPH radical scavenging assay and expressed as IC₅₀ (μg mL⁻¹). Total phenolic content was determined using the Folin-Ciocalteu method and expressed as mg gallic acid equivalents (GAE) g⁻¹, while total flavonoid content was determined using the aluminium chloride colorimetric method and expressed as mg quercetin equivalents (QE) g⁻¹. All analyses were performed in triplicate, and results were expressed as mean ± standard deviation.

Preparation of date palm pollen extender (DPPE)

DPPE was prepared according to the NaCl pollen extender (NPE) method described by Laghouati *et al.* (2021). Briefly, 40 or 80 mg of DPPE was dissolved in 1 mL of saline (0.9% NaCl; 308 mOsm kg⁻¹; pH 7.2), whereas saline alone served as the 0 mg mL⁻¹ control. After vortexing, all tubes were stored at 4°C for 24 h and then centrifuged to obtain a clear supernatant, which was used as the extender.

Experimental design

For each ram, recovered epididymal sperm were divided into three equal aliquots and diluted 1:1 (v/v) with one of three prepared media: DPPE-0 (control saline), DPPE-40 mg mL⁻¹, or DPPE-80 mg mL⁻¹. The final sperm concentration was adjusted to 350×10^6 cells mL⁻¹. Aliquots (0.5 mL) were loaded into sterile tubes, overlaid with mineral oil, and stored at 4°C for 48 h. Quality parameters were evaluated at 0, 24, and 48 h.

Sperm quality assessment

Total and progressive motility: Samples were gently mixed and equilibrated at 37°C for 5 min. For CASA, 3 µL was loaded into a 20-µm Leja® chamber and analysed using the Sperm Class Analyzer® (SCA) at 60 fps (10× phase contrast; ovine preset). At a minimum, five fields per sample were captured, and the intra-assay coefficient of variation (CV) for motility parameters was <5%.

Membrane integrity: Plasma membrane integrity was evaluated via the HOS test. An iso-osmotic solution (150 mOsmL⁻¹ sodium citrate with 20 mM fructose) was prepared. Sperm (50 µL) were incubated for 30 min at 37°C, fixed with 10% formalin, and at least 200 cells per sample were scored under phase contrast for swollen (intact) vs. non-swollen (damaged) tails.

Statistical Analysis

Data were expressed as mean ± SEM. The effects of extender concentration (0, 40, 80 mg mL⁻¹), storage time (0, 24, 48 h), and their interaction on sperm quality parameters were analysed using a two-way repeated-measures ANOVA. Extender concentration and storage time were considered repeated factors, and ram

(n = 5) was included as the subject factor. Post-hoc comparisons were performed using Tukey's HSD only if the interaction term was significant (p < 0.05). All analyses were performed using IBM SPSS Statistics v27.

Results and Discussion

Characterisation of date palm pollen: The date palm pollen utilised in this study was evaluated for its nutritional composition, mineral content, and in vitro antioxidant activity. The DPP exhibited a high protein content (37.94%), with carbohydrates representing the second most abundant component (30.12%). Among the minerals, potassium was the predominant element (1140 mg 100 g⁻¹), followed by sodium, calcium, and magnesium. The pollen also showed measurable antioxidant activity (DPPH IC₅₀ = 624.25 µg mL⁻¹) and contained 23.55 mg GAE g⁻¹ total polyphenols and 6.94 mg QE g⁻¹ flavonoids (Table 1).

Effect of DPPE on sperm motility: The effects of Date Palm Pollen Extender (DPPE) supplementation on sperm motility during chilled storage are presented in Table 2. At the start of storage (0 h), both total and progressive motility were high and did not differ significantly among the groups (p > 0.05). Total motility ranged from 84.6% to 85.0%, while progressive motility was approximately 71% across all treatments.

Table 1. Proximate composition and antioxidant activity of the date palm pollen

Parameters	Value (Means ± SEM)
Nutrition value	
Moisture (%)	5.82 ± 0.15
Ash (%)	6.10 ± 0.20
Carbohydrates (%)	30.12 ± 1.0
Proteins (%)	37.94 ± 1.10
Fat (%)	6.49 ± 0.18
Mineral Content	
Calcium (mg 100 g ⁻¹)	350 ± 5.0
Magnesium (mg 100 g ⁻¹)	250 ± 4.0
Potassium (mg 100 g ⁻¹)	1140 ± 20.0
Sodium (mg 100 g ⁻¹)	450 ± 7.0
Antioxidant Activity	
DPPH (IC ₅₀) (µg mL ⁻¹)	624.25
Total polyphenol (mg GAE g ⁻¹)	23.55
Flavonoids (mg QE g ⁻¹)	6.94

DPPH: 2,2-diphenyl-1-picrylhydrazyl; GAE: Gallic Acid Equivalents; QE: Quercetin Equivalents.

Table 2. Effect of DPPE concentration on motility, membrane integrity and viability of ram epididymal sperm during chilled storage (Indicate number of rams)

Parameter	Time	DPPE-0 (control)	DPPE 40 mg mL ⁻¹	DPPE 80 mg mL ⁻¹
Total motility (%)	0 h	84.6 ± 0.9 ^a	84.8 ± 0.8 ^a	85.0 ± 0.7 ^a
	24 h	69.4 ± 1.1 ^a	74.2 ± 0.9 ^b	76.8 ± 0.8 ^c
	48 h	58.3 ± 1.3 ^a	64.1 ± 1.0 ^b	67.5 ± 0.9 ^c
Progressive motility (%)	0 h	71.2 ± 0.8 ^a	71.4 ± 0.7 ^a	71.6 ± 0.6 ^a
	24 h	56.0 ± 1.0 ^a	60.8 ± 0.8 ^b	63.2 ± 0.7 ^c
	48 h	45.5 ± 1.2 ^a	51.3 ± 1.0 ^b	54.7 ± 0.8 ^c
Plasma Membrane integrity (%)	0 h	82.0 ± 0.9 ^a	82.2 ± 0.8 ^a	82.5 ± 0.7 ^a
	24 h	66.2 ± 1.2 ^a	70.4 ± 0.9 ^b	72.6 ± 0.8 ^c
	48 h	55.1 ± 1.3 ^a	60.8 ± 1.0 ^b	63.9 ± 0.8 ^c

Values are mean ± SEM. Different superscript letters within the same row indicate significant differences among extenders (Tukey's HSD, $p < 0.05$). Comparisons are made among extenders at the same time point.

During storage, motility declined in all treatments; however, DPPE supplementation attenuated this decline in a dose-dependent manner. After 24 and 48 h, sperm preserved with DPPE80 mg mL⁻¹ maintained the highest total motility (76.8% and 67.5%, respectively) and progressive motility (63.2% and 54.7%, respectively), significantly greater than DPPE40 mg mL⁻¹ and the control ($p < 0.05$). The DPPE40 mg mL⁻¹ group also showed significant improvements over the control at both time points (Tables 2).

These results indicate that DPPE enhances the preservation of both total and progressive sperm motility during chilled storage, with higher concentrations providing greater protection. The findings suggest that DPPE-supplemented extenders may be beneficial for improving the quality of ram semen during storage for artificial insemination purposes.

Effect of DPPE on plasma membrane integrity: The effects of DPPE supplementation on sperm plasma membrane integrity during chilled storage are presented in Table 2. At the beginning of storage (0 h), plasma membrane integrity was high (approximately 82%) and did not differ significantly among groups ($p > 0.05$; Table 4). Over time, membrane integrity declined in all treatments; however, DPPE supplementation attenuated this decline in a dose-dependent manner. After 24 and 48 h, sperm extended with DPPE80 mg mL⁻¹ exhibited the highest membrane integrity (72.6% and 63.9%, respectively), significantly greater than DPPE40 mg mL⁻¹ and the control ($p < 0.05$). DPPE-40 mg mL⁻¹ also maintained

significantly higher membrane integrity than the control at both time points.

These results indicate that DPPE protects the plasma membrane during chilled storage, with higher concentrations providing superior preservation. The findings suggest that DPPE-supplemented extenders may enhance the functional quality of ram sperm for artificial insemination.

It is widely known that immediately after sperm collection, the production of free radicals decreases sperm viability, motility and fertilisation potential (Gualtieri *et al.*, 2021; Wu *et al.*, 2020). During in vitro storage, the antioxidants present in the seminal plasma are no longer available, leaving spermatozoa exposed to lipid peroxidation and vulnerable to oxidative damage (Mohammadi *et al.*, 2024). This is particularly critical for epididymal sperm, which, as established by White and Wales (1961), lack the protective proteins of seminal plasma and are highly sensitive to dilution and cold stress (de Almeida *et al.*, 2024; Najafi *et al.*, 2023). Our results demonstrate that DPPE effectively substitutes for these missing protective components.

The core finding of this study is the significant mitigation of cold shock-induced damage by DPPE. The decline in all sperm parameters was markedly slower in supplemented groups, with DPPE-80 maintaining the highest quality. Our finding that 80 mg mL⁻¹ was the optimal dose for ram epididymal sperm aligns precisely with a growing consensus across species. Ng *et al.* (2022) reported that 80 mg mL⁻¹ (from 8% w/v) best maintained goat sperm motility

and membrane integrity over 48 hours, a result that mirrors our own motility and membrane integrity data. Similarly, Laghouati *et al.* (2021) identified 80 mg mL⁻¹ as the superior concentration for preserving motility and kinematic parameters in rabbit epididymal sperm, underscoring the particular efficacy of this dose for the more vulnerable epididymal sperm type across different animals. This concentration-dependent protection is further corroborated by El-Sheshtawy *et al.* (2016), who demonstrated that 100 mg DPPG per 5 mL TCF (equivalent to 20 mg mL⁻¹, though higher concentrations up to 50 mg mL⁻¹ showed efficacy) significantly improved chilled buffalo sperm motility after 7 days, and by El-Sisy *et al.* (2018), where 100-150 mg DPPG supplementation proved optimal for maintaining post-thaw motility, membrane integrity, and acrosome integrity in Arabian stallions. The consistency of these findings across ruminants, lagomorphs, and equids suggests that the antioxidant and membrane-stabilising properties of DPP, attributed to its rich flavonoid and phenolic content, including pyrogallol, ferulic acid, and rutin (El-Seadawy *et al.*, 2023), exert broad protective effects regardless of species-specific sperm physiology.

Most importantly, DPPE provided robust protection to the plasma membrane, the primary site of cold shock injury. The +8.8% higher membrane integrity in the DPPE80 group at 48 hours is a critical finding. This gain is equivalent to the benefits reported with purified antioxidants and is further supported by the work of El-Seadawy *et al.* (2023), who found that 2.4 mg DPPE per 5 mL (0.48 mg mL⁻¹) effectively preserved membrane and acrosome integrity in rabbit semen over 72 hours, confirming DPP's potent membrane-stabilising properties across a wide concentration range. This protective effect on membrane integrity is further corroborated by Ng *et al.* (2022), who demonstrated that 8% DPPG (80 mg mL⁻¹) significantly improved sperm membrane integrity percentage (40.00 ± 4.04%) compared to control (30.33 ± 2.19%) at 0-hour storage time in goat semen, with the protective effect maintained throughout 48 hours of chilled storage. Similarly, El-Sisy *et al.* (2018) reported that supplementation of modified INRA-82 with 100 and 150 mg DPPG resulted in significant improvement in post-

thawing membrane integrity (41.33 ± 0.83%; 41.33 ± 2.33%) compared to controls (34.33 ± 1.55%) in Arabian stallion sperm, further validating DPP's membrane-protective capacity across species and preservation methods.

The synchronised preservation of motility and membrane integrity points towards a multi-mechanism action for DPPE, which can be attributed to its unique biochemical profile.

First, the rich flavonoid content in DPPE (6.94 mg QEg⁻¹) provides a potent free radical scavenging capacity. We propose that these compounds directly neutralise the reactive oxygen species (ROS) generated during cold storage. This is consistent with the findings of Amsah *et al.* (2020), who demonstrated that 6% DPPG was particularly effective in protecting sperm during the cryopreservation process, a period of intense oxidative stress, significantly improving post-thaw viability (71.25 ± 1.04% vs. 56.47 ± 4.69% in controls). This underscores DPP's role as a crucial protectant during severe thermodynamic challenges. The antioxidant mechanism is further elucidated by El-Seadawy *et al.* (2023), who identified total phenolic content of 4.15 mg GAE/g extract and total flavonoids of 0.74 mg CE/g extract in DPPE, with potent antiradical activity against ABTS (12.37 mM TE/g) and DPPH (4.06 mM TE g⁻¹), and high reducing capacity (9.19 mM TE g⁻¹), primarily attributed to pyrogallol (4150.92 µg g⁻¹), ferulic acid (2935.50 µg g⁻¹), and rutin (2163.99 µg g⁻¹). However, the antioxidant response is dose-dependent. Our success with 80 mg mL⁻¹, without inducing oxidative stress, contrasts with the findings of Laghouati *et al.* (2023) in older rabbits, where doses ≥80 mg mL⁻¹ increased lipid peroxidation; and with El-Sisy *et al.* (2018), where 250 mg DPPG hurt all studied post-thawing semen parameters in Arabian stallions. This highlights a critical species- and context-specific therapeutic window, where the optimal dose must balance powerful antioxidant effects with the risk of pro-oxidant activity or toxicity at higher concentrations.

Furthermore, the positive effects observed in our protein-free extender could potentially be related to the reported antimicrobial and anti-inflammatory properties of date palm pollen (Bentrad *et al.*, 2017; Daoud *et al.*, 2019; Ashour *et al.*, 2023). However, bacterial load or inflammatory markers were not assessed in the

present study, and therefore, this explanation remains speculative. Since the extender was prepared without antibiotics, it is possible that DPPE contributed to improved sperm quality by limiting microbial proliferation during incubation. Future studies should directly evaluate microbial counts and inflammatory indicators to confirm this hypothesis.

The efficacy of the 80 mg mL⁻¹ dose for ram epididymal sperm is strongly supported by the literature. However, the optimal concentration is not universal, varying by species, sperm type, and storage method. For example, El-Sheshtawy *et al.* (2016) found that for chilled buffalo sperm, TPG 50 (10 mg mL⁻¹) yielded the highest motility (66.67%), while for frozen-thawed samples, TPGY 150 and 250 (30 and 50 mg mL⁻¹) showed superior motility (43.75% and 45.00%, respectively), with TPGY 150 achieving the highest alive sperm percentage (96.30%). Similarly, El-Sisy *et al.* (2018) reported the best results in Arabian stallions at 20-30 mg mL⁻¹ (100-150 mg DPPG in modified INRA-82), with 200 mg failing to maintain motility and 250 mg hurting all post-thawing semen parameters. This spectrum of effective concentrations, from 0.48 mg mL⁻¹ in rabbits (El-Seadawy *et al.*, 2023) to 80 mg mL⁻¹ in our study and in goat (Ng *et al.*, 2022) and rabbit epididymal sperm (Laghouti *et al.*, 2021), emphasises that dose optimisation is a mandatory step for each new application. Notably, Ng *et al.* (2022) demonstrated that 8% DPPG (80 mg mL⁻¹) significantly improved sperm motility after 48 hours (56.67 ± 5.57%) compared to control (40.83 ± 3.00%) and 4% DPPG (43.33 ± 5.24%), while Laghouati *et al.* (2021) showed that 80 mg mL⁻¹ enhanced velocity traits (VCL and VAP) and trajectory parameters (LIN, ALH, BCF) in rabbit epididymal sperm, with protective effects maintained up to 120 minutes of incubation.

From a practical standpoint, the improvement from 58.3% to 67.5% total motility at 48 hours is highly significant. This level of preservation is not just statistically significant but biologically meaningful, as demonstrated by El-Sheshtawy *et al.* (2016), who achieved a high preserving capacity of chilled and thawed buffalo bull sperms expressed mainly by sperm motility. It moves sperm quality beyond the steep decline trajectory documented for undiluted Ouled-Djellal ram sperm in Algeria (Safsaf *et al.*, 2015), effectively extending the practical

window for using chilled epididymal sperm in field conditions. Unlike purified compounds that require precise dosing, DPP offers a cost-effective, multi-compound source containing diverse bioactive phytochemicals, including flavonoids, phenolic acids, and antioxidants such as pyrogallol, ferulic acid, and rutin (El-Seadawy *et al.*, 2023)—that exert synergistic protective effects on sperm motility, viability, membrane integrity, and acrosome integrity across multiple species including rabbits, bulls, goats, buffalo, and stallions.

Because an aqueous extraction method was used, lipophilic antioxidant fractions of date palm pollen were likely not fully recovered. This may have led to an underestimation of the total antioxidant capacity and could partially explain differences between the effects of DPPE observed in the present study and those reported in studies using hydro-alcoholic extracts.

Limitations of the present study include a relatively small sample size and the absence of direct biochemical measures of oxidative stress (e.g., malondialdehyde [MDA], total antioxidant capacity [TAC], intracellular reactive oxygen species). Our results, based on motility, membrane integrity and viability, provide a proof-of-concept that DPPE has protective effects during chilled storage, but do not directly measure oxidative damage or fertilising ability. Therefore, extrapolation to field fertility or commercial application should be made cautiously. Future work will include measurement of MDA, TAC and ROS on matched samples and controlled in vitro fertilisation/artificial insemination trials to validate functional fertility outcomes

Conclusions

Based on these results, 80 mg mL⁻¹ DPPE in protein-free saline effectively preserved total and progressive motility as well as plasma membrane integrity of ram epididymal sperm during 48 hours of chilled storage. These findings suggest that aqueous DPPE may serve as a promising natural antioxidant supplement for ram sperm dilution; however, direct oxidative stress biomarkers were not evaluated in the present study, and therefore protection against oxidative damage was inferred indirectly from sperm quality parameters. Further studies are required to confirm these findings, including

measurement of biochemical oxidative stress indicators, assessing DPPE supplementation in commercial protein-containing extenders, optimising extraction methods and concentration ranges, and evaluating in vitro and in vivo fertility outcomes, as well as safety and potential toxicity, before considering practical or commercial application.

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