Toxicology International, *32(3) : 449-457; 2025. DOI: 10.18311/ti/2025/v32i3/45059*

Online ISSN : 0976-5131; Print ISSN : 0971-6580

Toxicology International

Journal Homepage: https://informaticsjournals.co.in/index.php/toxi



Protective Role of Ginger Extract Against Bisphenol A - Induced Sperm Damage in Goats

Thu Anh Huynh[1], Chon Minh Nguyen[2], Men Thanh Tran[3] and Khuong Thi Thanh Tran[1*]

[1]Stem Cell Laboratory, Institute of Food and Biotechnology, Can Tho University, Can Tho City – 900000, Vietnam; tttkhuong@ctu.edu.vn

[2]Applied Biochemistry Laboratory, Institute of Food and Biotechnology, Can Tho University, Can Tho City – 90000, Vietnam

[3]Department of Biology, College of Natural Sciences, Can Tho University, Can Tho City – 900000, Vietnam

Abstract

Common environmental contaminant found in plastics and consumer goods, bisphenol A (BPA), seriously compromises male reproductive health by causing oxidative stress and reducing sperm functioning. This study aimed to determine the optimal dose for improving sperm quality and to evaluate the protective effect of ginger extract against BPA-induced sperm damage in goats. For 15 min, two crossbred male goats' semen was exposed to BPA at dosages of 0, 10, 20, and 30 µM. With overall motility, progressive motility, viability, and membrane integrity declining to 35.57 ± 3.23%, 7.45 ± 1.20%, 60.67 ± 3.50%, and 61.17 ± 3.43%, the results showed that 30 µM BPA significantly reduced sperm quality. Following their 30 µM BPA exposure, samples were incubated for 5 min at 0.0, 0.1, 0.2, and 0.3 mg/mL concentrations using ginger extract. Particularly with regard to general motility (50.60 ± 3.28%) and progressive motility (36.12 ± 1.54%), the 0.2 mg/mL concentration significantly improved all evaluated sperm parameters. Especially at a dose of 0.2 mg/mL, the data show that ginger extract greatly reduces BPA-induced oxidative damage and restores sperm function. This emphasises its possibility as a natural antioxidant approach to protect male fertility under environmental toxicant contact.

Major/Key Findings: (i) Bisphenol A (BPA) is harmful to goat sperm, (ii) Ginger extract has the ability to improve goat sperm quality, (iii) Ginger is a natural antioxidant approach to protect male fertility.

Keywords: Bisphenol A, Ginger Extract, Goat, Sperm Quality, Spermatotoxicity

1. Introduction

Bisphenol A (BPA) is a synthetic xenoestrogen extensively used in the manufacture of polycarbonate plastics and epoxy resins, found in everyday items such as food packaging, water bottles, dental sealants, and thermal paper[1]. Due to its widespread presence, BPA exposure is nearly ubiquitous in both humans and animals, primarily through dietary intake, skin absorption, or inhalation[2]. As a recognised endocrine-disrupting chemical, BPA exerts deleterious effects on male reproductive health by interfering with estrogen and androgen receptor pathways, leading to impaired spermatogenesis, hormonal imbalance, and testicular dysfunction[3,4].

One of the major mechanisms underlying BPA-induced male infertility is oxidative stress. BPA elevates intracellular Reactive Oxygen Species (ROS) levels and inhibits the activity of key antioxidant enzymes

**Author for correspondence*

Article Received on: 22.07.2024 Revised on: 21.05.2025 Accepted on: 28.05.2025

such as Superoxide Dismutase (SOD), Catalase (CAT), and Glutathione Peroxidase (GPx), resulting in lipid peroxidation, mitochondrial dysfunction, and DNA fragmentation in spermatozoa[5,6]. These biochemical disruptions manifest as decreased sperm motility, viability, morphology, and fertilisation capacity[7]. The harmful effects of BPA have been confirmed in various animal models, but protective strategies for mitigating BPA-induced sperm damage remain limited.

To counteract BPA-induced oxidative damage, the application of natural or synthetic antioxidants has emerged as a promising strategy in reproductive biotechnology. Among natural compounds, Ginger Extract (GE)—derived from Zingiber officinale—has attracted significant attention due to its rich composition of antioxidant phytochemicals such as 6-gingerol, shogaols, polyphenols, flavonoids, and ascorbic acid[8,9]. These compounds scavenge free radicals, enhance endogenous antioxidant activity, and support the structural and functional integrity of reproductive cells. Numerous studies in rodents and humans have reported that GE supplementation improves semen parameters, including sperm motility, viability, and testosterone levels, particularly under toxic or oxidative stress conditions[10-12]. However, these findings are not directly translatable to livestock. There is a lack of evidence concerning the optimal GE concentration, treatment duration, and mechanism of action in livestock species, especially goats, whose sperm are known to be highly sensitive to oxidative insults. Goats have gained recognition as a suitable model for reproductive toxicology due to their well-characterised semen physiology, economic relevance, and susceptibility to environmental stressors[13,14]. Although a few antioxidant studies have been conducted in ruminants[15], the dose-response relationship and protective efficacy of Ginger Extract (GE) under BPA-induced oxidative damage in goat sperm remain uninvestigated. This constitutes a critical knowledge gap that this study aims to address. We hypothesise that ginger extract can mitigate BPA-induced oxidative damage and improve sperm motility, viability, and membrane integrity in a concentration-dependent manner, with a specific dose providing maximal benefit. This study aimed to determine the optimal concentration of GE for restoring goat sperm quality after BPA exposure using a controlled *in vitro* model. The novelty of this research lies in its stepwise design to simulate sperm damage and recovery under toxic conditions, thereby offering insights into the potential application of plant-derived antioxidants in semen preservation and environmental reproductive toxicology.

2. Materials and Methods

2.1 Animals

In this study, two male crossbred goats, Bach Thao (♀) × Boer (♂), 2 years old and weighing an average of 45 kg, were used. The goats were fed a standard diet and freely provided access to drinking water three times each day, ensuring they had a sufficient supply to avoid thirst. The animal experimental farm of Can Tho University's Stem Cell Lab provides a barn area that was built to be spacious, cool, and comfortable. The goats were fully vaccinated against illnesses and had their health monitored on a regular basis. The study received ethical permission for the housing, care, and semen collection of animals in accordance with the rules set forth by Can Tho University's Animal Ethics Committee (CTU-AEC24013).

2.2 Ginger Extract

Ginger harvested from gardens in Can Tho was washed to remove dirt and debris, and damaged parts were discarded. It was then thinly sliced and dried at 50°C for 72 h. The dried samples were ground into powder. The powdered samples were kept in airtight packaging at 4°C for long-term use. Ginger powder was soaked for 72 h with 96% ethanol in the dark, sealed conditions at room temperature. The soaked samples were filtered once, removing the filter residue6. The solution was then evaporated under minus pressure (-70 mbar, 50-55°C). Ethanol was recovered through the rotary evaporator system. The extract from the evaporation was dried in a Forced Convection Basic Drying cabinet at a temperature of 45°C, and then, it was stored at 4°C for subsequent experiments.

2.3 Experimental Design

An artificial vagina was used for collecting semen from the goats (2 ejaculations/week). The semen was then assessed for parameters such as colour, volume, pH, viability, motility, and membrane integrity.

Experiment 1: The semen samples were diluted with Tris Citrate Glucose (TCG) solution supplemented with BPA at concentrations of 0 µM, 10 µM, 20 µM, and 30 µM. After 15 min, sperm quality was evaluated.

Experiment 2: The samples with low sperm quality in experiment 1 were centrifuged at 1000 rpm for 5 min to remove the medium and BPA. The samples were then treated with ginger extract at levels of 0 mg/mL, 0.1 mg/mL, 0.2 mg/mL, and 0.3 mg/mL. After 5 min, the sperm quality was evaluated.

2.4 Assessment of Sperm Concentration

Sperm concentration was assessed by counting the number of sperm on a counting chamber with a magnification of 40× under a microscope (Nikon) according to World Health Organisation (WHO) guidelines[16].

2.5 Assessment of Sperm Motility

Sperm samples were viewed under a microscope (Nikon) at 40× magnification, sperm motility was assessed and classified as follows: progressive motility, non-progressive motility, and immotility[17].

2.6 Assessment of Sperm Viability

The Eosin-Nigrosin staining was used for testing the sperm viability[18]. Sperm samples were viewed under a microscope (Nikon) at 40× magnification. Live sperms were unstained, while dead sperms were stained (Figure 1).

2.7 Assessment of Sperm Membrane Integrity

The Hypo-Osmotic Swelling Test (HOS Test) was used for assessing the integrity of the sperm membrane, and an Eclipse Si microscope (Nikon) with 40× magnification for observation was used[19]. Sperm with intact membranes show a tail curling response, while sperm with damaged membranes show no reaction (Figure 2).

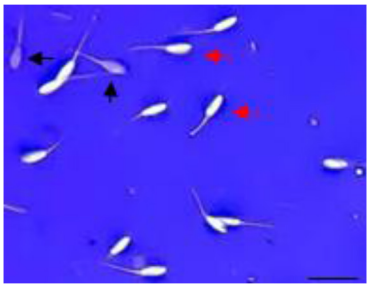


Figure 1. Goat sperm stained with Eosin-Nigrosin. Live sperm do not take up the dye (red arrow), while dead sperm take up the dye (black arrow). Scale bar = 50µm.

2.8 Statistical Analysis

Excel (2016) and the R 4.3.1 software were used for analysing the data. After confirming normality and variance homogeneity, the data were analysed using a Linear Mixed Model ANOVA, with mean comparisons between treatments using the Tukey method in the R.4.3.1 program. In Experiment 1, the random effects were goats and ejaculators, while the fixed effect was BPA concentrations. In Experiment 2, the random variables were goats and ejaculators, while the fixed effect was ginger extract concentrations. Results were presented as mean ± standard error. Statistical significance was set at $p<0.05$, reflecting high confidence in the findings.

3. Results and Discussion

3.1 Fresh Sperm Quality

The quality of goat fresh sperm is shown in Table 1. The overall characteristics, pH, colour, and volume of the goat sperm samples were all normal and characteristic of goat sperm. The results of assessing fresh sperm quality of this study were similar to the results of studies by Khuong *et al.*[20] and Duy *et al.*[21]. Based on the initial quality assessment results, the goat sperm samples were of sufficient quality to proceed with the experiments.

3.2 The Effect of Different BPA at Concentrations of 0 µM, 10 µM, 20 µM, and 30 µM on Sperm Quality

After 15 min of treatment with BPA, sperm quality parameters were assessed. The results in Figures 3-5 demonstrated that sperm quality was significantly

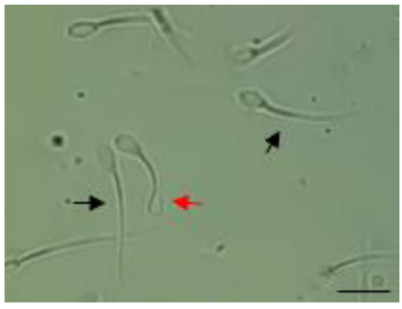


Figure 2. Goat sperm was tested for membrane integrity. Sperm with intact plasma membranes show tail coiling (red arrow), while sperm with damaged plasma membranes do not show any response (black arrow). Scale bar = 50 µm.

($p<0.05$) affected by BPA concentration. Sperm quality fell significantly ($p<0.05$) in treatments post-activation with 10 µM, 20 µM and 30 µM BPA in comparison with the control. The differences in viability and membrane integrity between 10 µM and 20 µM concentrations of BPA were not statistically significant ($p>0.05$). At 30 µM BPA concentration, the sperm quality was recorded as the lowest and significantly different ($p<0.05$) from other concentrations. Specifically, the progressive motility rate was 22.45%, the overall motility rate was 35.55%, the viability rate was 40.67%, and the membrane integrity rate

Table 1. Fresh sperm quality

Assessment criteria	Results
Volume (mL)	0.73 ± 0.03
pH	7.01 ± 0.07
Sperm progressive motility (%)	68.65 ± 0.62
Sperm overall motility (%)	85.29 ± 0.46
Sperm concentration ($\times 10^9$ cells/mL)	2.88 ± 0.04
Sperm membrane integrity (%)	77.63 ± 0.68
Sperm viability (%)	88.81 ± 0.45

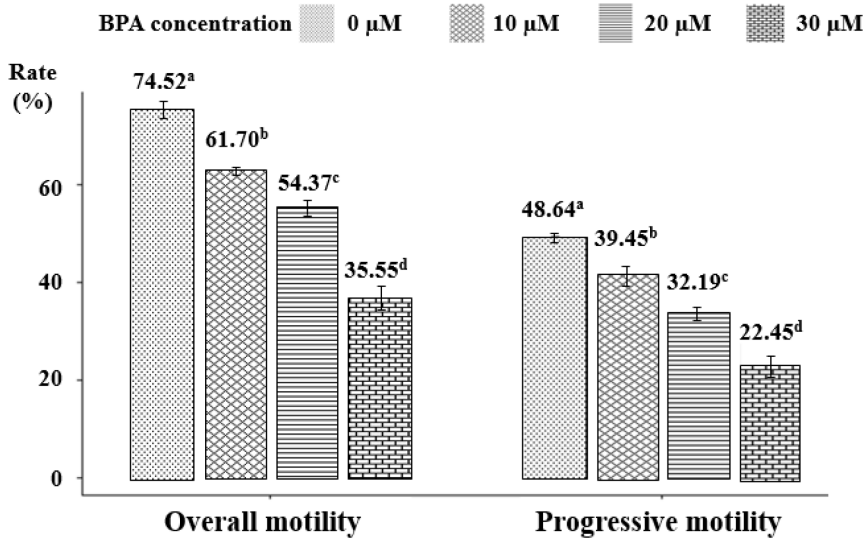


Figure 3. The sperm motility after BPA treatment. [a, b, c, d] Values in each assessment criterion that have distinct superscripts are different.

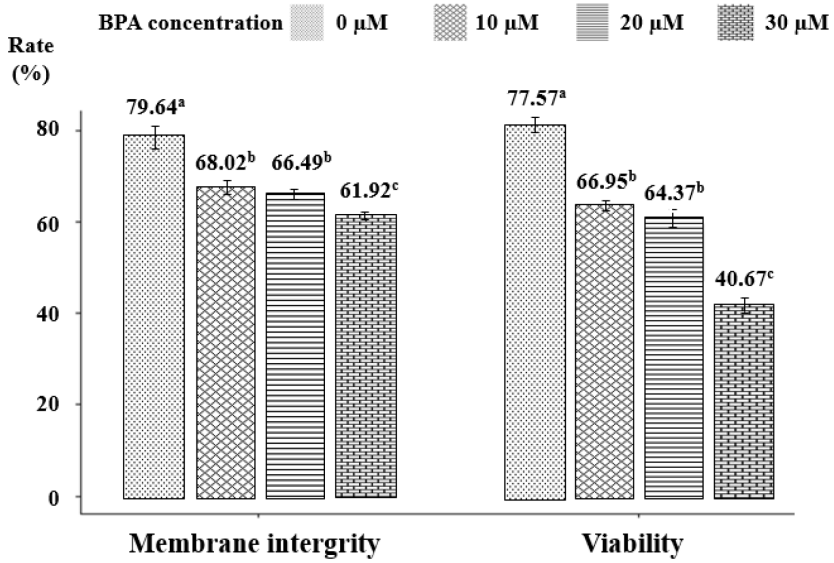


Figure 4. The sperm viability and membrane integrity after BPA treatment. [a, b, c, d] Values in each assessment criterion that have distinct superscripts are different.

was 61.92% were affected. However, the sperm quality at 30 µM BPA concentration still met WHO standards[16].

3.3 The Effect of Different Ginger Extract Concentrations (0 mg/mL; 0.1 mg/mL; 0.2 mg/mL; 0.3 mg/mL) on BPA Treated-Sperm Quality

After 15 min exposure to 30µM BPA, the sperm samples were treated with ginger extract at four levels of 0 mg/mL, 0.1 mg/mL, 0.2 mg/mL, and 0.3 mg/mL for 5 min. Then, the sperm quality was assessed. The data showed that the sperm quality was improved due to the positive effects of ginger extract (Figures 6-8). The highest sperm overall motility rate was recorded in experimental treatment with 0.2 mg/mL ginger extract (50.60%) and was significantly different from 0.3 mg/mL and 0 mg/mL ginger extract. However, the differences in overall motility between 0.1 mg/mL and 0.2 mg/mL concentrations of ginger extract were not statistically significant. The experimental treatment with 0.2 mg/mL ginger extract recorded the highest sperm progressive motility rate (36.12%), and the difference was statistically significant compared to other treatments. There was no statistically significant difference in viability and membrane integrity rate between treatments treated with 0 mg/mL, 0.1 mg/mL and 0.2 mg/mL ginger extract. However, the highest viability and membrane integrity rate were recorded in

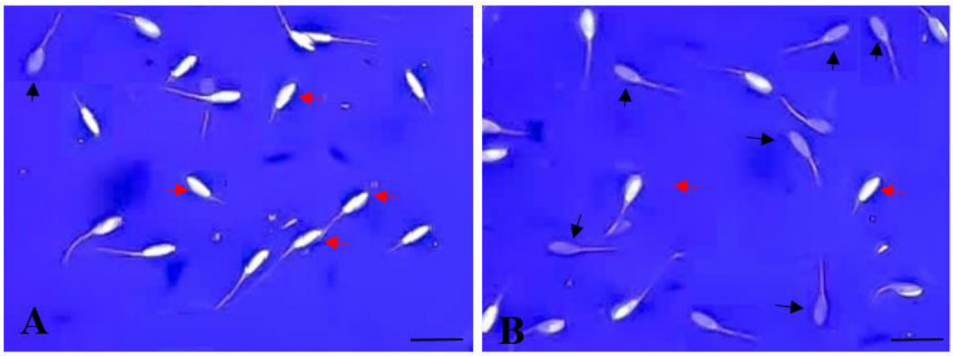


Figure 5. **(A)** The sperm viability before and **(B)** after 30 µM BPA treatment. Live sperm do not take up the dye (red arrow), while dead sperm take up the dye (black arrow). Scale bar = 50µm.

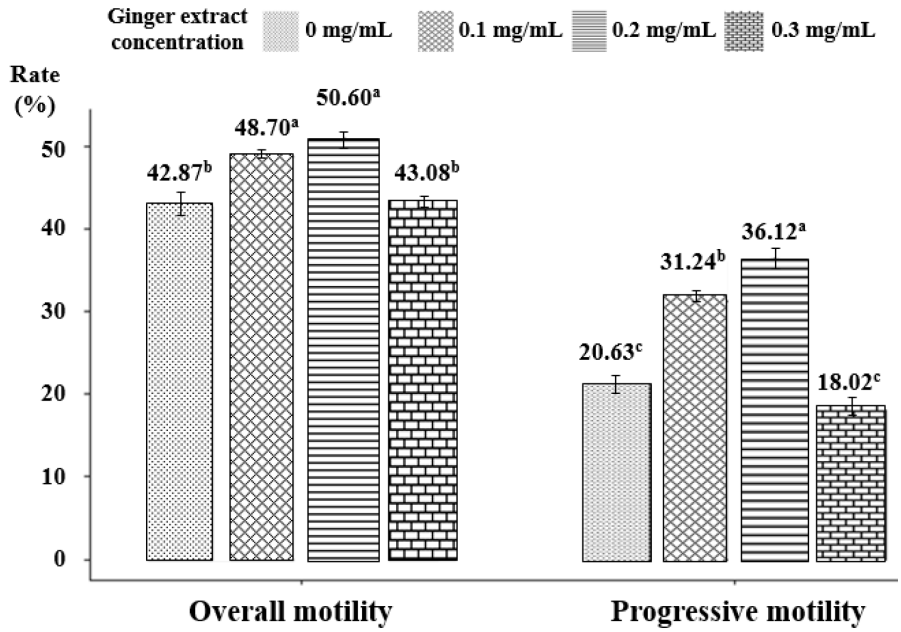


Figure 6. The sperm motility after ginger extract treatment. [a, b, c, d] Values in each assessment criterion that have distinct superscripts are different.

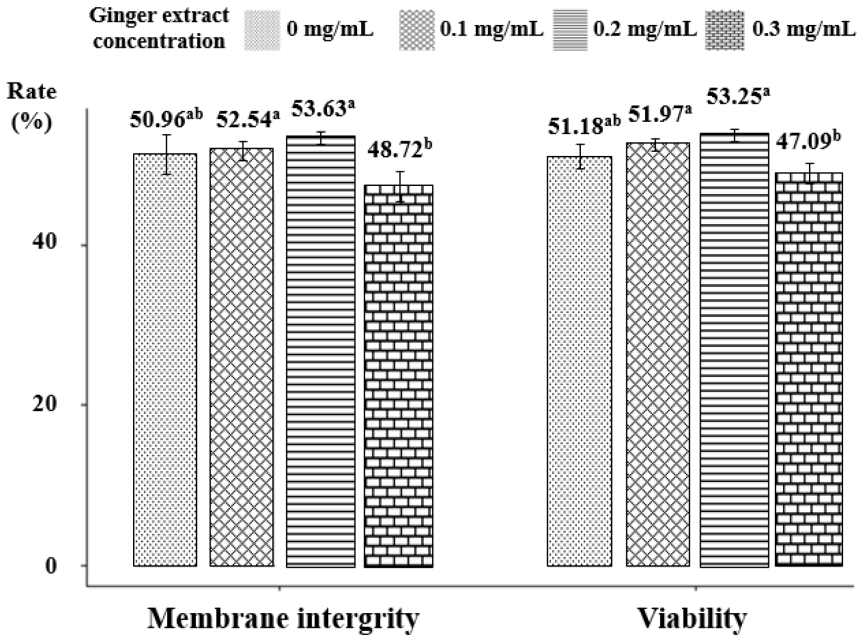


Figure 7. The sperm viability and membrane integrity after ginger extract treatment. [a, b, c, d] Values in each assessment criterion that have distinct superscripts are different.

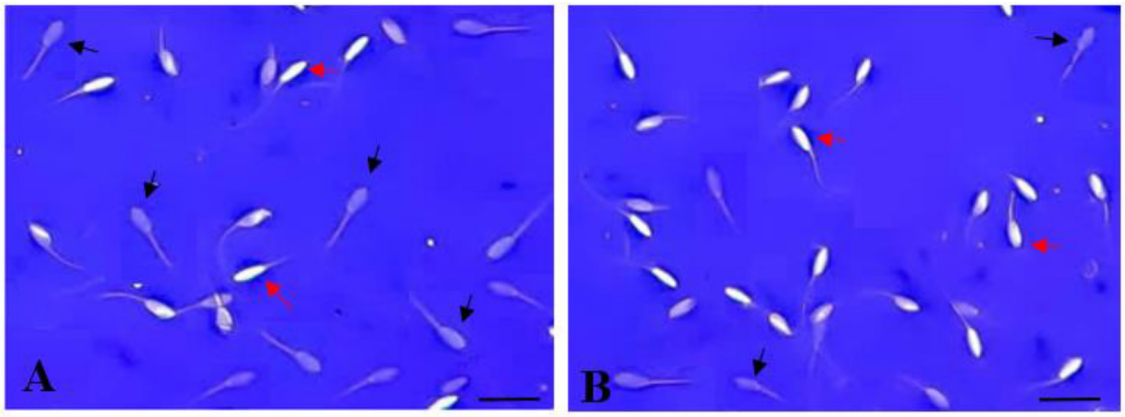


Figure 8. The sperm viability before **(A)** and after 0.2 mg/mL Ginger extract treatment **(B)**. Live sperm do not take up the dye (red arrow), while dead sperm take up the dye (black arrow). Scale bar = 50µm.

the experimental treatment with 0.2 mg/mL, and the difference was statistically significant compared to the experimental treatment with 0.3 mg/mL ($p<0.05$). The 0.2 mg/mL ginger extract showed the best ability to improve sperm quality after sperm was treated with 30 µM BPA.

3.4 Discussion

This study investigated the protective effects of ginger extract on goat sperm quality following exposure to Bisphenol A, a known endocrine-disrupting chemical that induces oxidative stress and reproductive dysfunction. Our results demonstrate that BPA significantly impairs sperm motility, viability, and membrane integrity, whereas supplementation with ginger extract, particularly at 0.2 mg/mL, can partially restore these parameters *in vitro*.

Among the parameters assessed, progressive motility emerged as the most sensitive indicator of BPA-induced toxicity and ginger-mediated recovery. BPA exposure at 30 µM reduced progressive motility to 7.45%, a level far below the WHO[16] fertility threshold of 32%. This pronounced decline reflects severe mitochondrial dysfunction and oxidative damage, consistent with previous reports highlighting BPA's capacity to inhibit ATP synthesis and

disrupt axonemal function[3,22]. Treatment with GE at 0.2 mg/mL improved progressive motility to levels exceeding the fertility threshold, while also enhancing overall motility, membrane integrity, and viability. These results confirm our hypothesis that GE exerts a dose-dependent protective effect against BPA-induced sperm damage.

The extent of BPA-induced sperm dysfunction in our study aligns with earlier findings in fish and mammalian models[7,23]. Likewise, the protective role of ginger extract corroborates rodent studies where GE enhanced sperm motility and viability under toxic or stress-induced conditions[8,24]. Our data also agree with Hosseini *et al.*[25], who observed improved membrane integrity and reduced oxidative damage and DNA fragmentation in sperm treated with ginger extract. The observed decline in sperm quality following BPA exposure is largely attributed to elevated ROS and inhibition of endogenous antioxidants such as SOD, CAT, and GPx[6]. This imbalance promotes lipid peroxidation and DNA fragmentation, compromising sperm function and viability.

Conversely, ginger's protective effects are likely mediated through its phytochemical constituents, [6]-gingerol, shogaols, zingerone, and flavonoids, which scavenge ROS, stabilise mitochondrial membranes, and restore redox homeostasis[8,9]. GE has also been shown to enhance enzymatic antioxidant activity, reduce malondialdehyde levels, and maintain membrane fluidity, thereby preserving sperm functional capacity under stress[26]. Interestingly, the highest tested concentration of GE (0.3 mg/mL) did not yield further improvement and slightly reduced certain parameters, suggesting a biphasic antioxidant response. Similar dose-dependent effects have been observed in other antioxidant therapies, where excessive supplementation disrupts physiological ROS signalling required for sperm function[27].

These findings provide valuable insights for optimising semen extenders in artificial insemination and sperm preservation protocols, especially in regions where environmental contaminants like BPA are prevalent. The application of ginger extract as a natural, low-cost antioxidant supplement offers practical benefits for maintaining fertility in livestock under oxidative conditions. Moreover, the study supports broader adoption of plant-derived bioactive compounds in veterinary andrology and highlights the potential for integrating ginger extract into cryopreservation strategies for long-term semen storage.

While this study offers promising results, it is limited by its short *in vitro* exposure time and the lack of molecular or genomic assessments. Parameters such as DNA fragmentation, mitochondrial membrane potential, acrosome integrity, and gene expression profiles of antioxidant enzymes were not evaluated. Furthermore, the results are species-specific and may not be directly extrapolated to other livestock or *in vivo* systems. To enhance the translational relevance of these results, future research should investigate the long-term effects of ginger extract on fertilisation outcomes and embryo development, examine gene expression changes associated with oxidative stress and sperm motility, assess the *in vivo* efficacy of ginger extract in goats and other ruminants, and evaluate its incorporation into semen freezing and thawing protocols in comparison with conventional antioxidants.

4. Conclusion

This study demonstrates that bisphenol A exposure significantly decreases goat sperm quality, particularly regarding motility, viability, and membrane integrity. Ginger extract at 0.2 mg/mL exhibited optimal protective effects, enhancing all critical parameters of sperm function and mitigating BPA-induced oxidative damage. These findings corroborate ginger's intrinsic antioxidant effects in improving sperm quality under toxic stress. Further investigation is required to elucidate the underlying molecular mechanisms of ginger extract's protective role and evaluate its impact on reproductive outcomes, including conception rates and offspring quality.

5. Acknowledgements

We thank the staff and facilities at the Institute of Food and Biotechnology, Can Tho University, for their crucial support in conducting this study. Funding for this study was provided by the Ministry of Education and Training, Vietnam, Code: B2024-TCT- 04.

6. Author contribution

Conceptualisation, H.A.T. and N.M.C.; methodology, T.T.M. and T.T.T.K.; software, H.A.T. and T.T.M.; validation, T.T.T.K. and N.M.C.; formal analysis, H.A.T. and T.T.T.K.; investigation, N.M.C. and T.T.M.; resources, T.T.M. and T.T.T.K.; data curation, H.A.T. and N.M.C.; writing—original

draft preparation, T.T.T.K. and N.M.C.; writing—review and editing, N.M.C. and T.T.M.; visualisation, T.T.M. and H.A.T.; supervision, N.M.C. and T.T.T.K. All authors have read and agreed to the published version of the manuscript.

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