Preliminary communication
DOI: /10.5513/JCEA01/27.3.5077
Journal of Central European Agriculture, 2026, 27(3), p.610-618

Preliminary evaluation of *Spirulina platensis* supplementation on reproductive traits composition in fluoride-affected rams from endemic regions

Anass BEN MOULA[1,2], Abdellatif RAHIM[3], Mounia SIBAOUEIH[1], Abdel Khalid ESSAMADI[3], Bouchra EL AMIRI[1,4] (✉)

[1] Animal Production Unit, Regional Center Agricultural Research of Settat, National Institute for Agricultural Research (INRA), Avenue Ennasr, P.O. Box 415 Rabat Principal, 10090 Rabat, Morocco

[2] Plant, Microbial, Marine, and Precision Agriculture Biotechnology research team; Department of Life Sciences, Polydisciplinary Faculty of Larache, Abdelmalek Essaadi University, 745 BP, 92004 Larache, Morocco

[3] Hassan First University of Settat, Faculty of Sciences and Techniques, Laboratory of Biochemistry, Neurosciences, Natural Resources and Environment, P.O. Box 577, 26000 Settat, Morocco

[4] African Sustainable Agriculture Research Institute (ASARI), Mohammed VI Polytechnic University (UM6P), Laayoune, Morocco

✉ Corresponding author: bouchra.elamiri@inra.ma

Received: October 26, 2025; accepted: December 8, 2025

ABSTRACT

This study examined the effects of nutritional *supplementation with Spirulina platensis* on scrotal circumference, semen characteristics, seminal plasma composition, and post-storage quality in rams reared in fluorosis-endemic regions, with an emphasis on fluoride-induced trace-element toxicity. Eighteen 5-month-old lambs of the Sardi breed were allocated to 3 groups: Group I (rams reared in a fluorosis-endemic area, Group II (rams reared in a fluorosis-free area), Group III (rams supplemented with 250 mg/kg BW/day of *Spirulina platensis*), and Group IV (rams supplemented with 500 mg/kg BW/day of *Spirulina platensis*). The trial was terminated at 18 months of age, corresponding to the dental development stage of full incisor eruption. The parameters measured during the last four months included the scrotal circumference, bi-weekly fresh and stored ejaculates' quality, and the chemical composition of the seminal plasma. Scrotal circumference was affected by fluorosis ($P<0.05$); nonetheless, there was no effect of Spirulina supplementation ($P>0.05$). However, significant ($P<0.05$) improvement was found in Group III and Group IV rams with regard to the quality of fresh semen (motility, viability, morphology) and the antioxidant level in comparison with Group I. The characteristics of liquid stored semen at 24 h showed continued benefits, with enhanced progressive motility, viability, and lowered sperm abnormalities ($P<0.05$) of samples from rams receiving *Spirulina platensis* supplements.

Keywords: fluorosis, stored semen, ram semen, seminal plasma, *Spirulina platensis*

INTRODUCTION

Animals that are exposed to high fluoride levels for an extended period of time are at serious risk for fluorosis (Choubisa, 2025), especially in places where natural fluoride concentrations are higher than what is advised (Srivastava and Flora, 2020); (Kashyap et al., 2021). Chronic fluoride toxicity can harm the reproductive system, among other organs, in both humans and animals (Katir Masnaoui et al., 2023; Li et al., 2023). Toxicological effects of fluoride exposure on sperm quality, including motility, viability, morphology, capacitation, antioxidant defense, and acrosome reaction, have been shown in laboratory animal studies to result in poor fertilization outcomes (Liang et al., 2020; Khan, 2022a).

For livestock productivity and genetic diversity, rams' reproductive performance and sperm quality are essential (El Amiri and Rahim, 2024). Furthermore, sperm production and quality are closely related to scrotal circumference, a crucial measure of testicular function (Pascal

et al., 2023a). In order to lessen the negative effects of fluorosis on sheep breeding programs, measures to increase sperm parameters and scrotal circumference are crucial.

Research has demonstrated that fluoride toxicity can be decreased in animals by supplementing them with minerals (Han et al., 2006), proteins, vitamins, and antioxidants (Nabavi et al., 2013). According to recent studies, natural sources like *Spirulina platensis* and *Tamarindus indica* fruit pulp have the potential to reduce fluoride toxicity (Banji et al., 2013; Amir-Ud-Din et al., 2022; Samal et al., 2016). However, a large portion of this research has only looked at fluorosis that has been created experimentally. *Spirulina platensis* improved oxidative stress markers and decreased dental fluorosis in lambs, according to a recent clinical trial (Rahim et al., 2023).

The antioxidant, anti-inflammatory, and immunomodulatory qualities of *Spirulina platensis* have drawn interest in animal nutrition for its nutritional and health benefits (Liang et al., 2020; El-Deeb et al., 2022; Ben Moula et al., 2024). Its impact on rams' ability to reproduce in regions where fluorosis is endemic is still unknown, though. Therefore, this study aimed to examine how supplementing with *Spirulina platensis* affected the scrotal circumference, semen properties, seminal plasma composition, and semen conservation in rams raised in El Fokra commune, which is part of Khouribga Province, Morocco. The objective of the study was to determine whether *Spirulina platensis* could decrease the reproductive problems associated with endemic fluorosis in sheep.

MATERIALS AND METHODS

Experimental design

The Hassan II Institute of Agronomy and Veterinary Medicine (Rabat, Morocco) and the Moroccan Ministry of Agriculture confirmed the ethical standards of all animal procedures in accordance with ARRIVE guidelines and European Union Directive 2010/63/EU. In order to eliminate duplication in verifying fluoride toxicity, this study builds on the findings of Rahim et al. (2023) by assessing reproductive parameters in the same cohort of rams that were previously validated for fluorosis status. To examine the effects of dietary supplementation in areas with and without endemic fluorosis, 18 males Sardi breed were split up into four different groups.

The groups were set up as follows:

- Group I (n = 5): Known as the endemic control group, these rams were raised in El Fokra, a region with endemic fluorosis in Khouribga Province, and fed only 400 g of barley daily without any supplements.
- Group II (n = 3): This non-endemic control group consists of rams from the fluorosis-free Settat region that are fed 400 g of barley daily without supplementation.
- Groups III and IV (n = 5 each): Purchased from El Fokra in Khouribga Province, a region where fluorosis is endemic, these rams were fed 400 g of barley daily along with *Spirulina platensis* at dosages of 250 mg/kg body weight (BW) for Group III and 500 mg/kg BW for Group IV.

In the previous study, fluorosis in Groups I, III, and IV was confirmed biochemically and clinically (Rahim et al., 2023). The groups had higher levels of oxidative stress markers (malondialdehyde, depleted glutathione) than Group I, plasma F^- levels above WHO thresholds (2.8-3.6 mg/L vs. 1.5 mg/L), and Dean's Fluorosis Index (DFI) scores ≥ 2 (mild to moderate fluorosis).

The main trial, which found that 500 mg/kg BW/day significantly reduced dental fluorosis and systemic oxidative stress, was replicated by the *Spirulina* dose protocol (Rahim et al., 2023). Doses were adjusted based on BW once every 15 days to ensure accuracy. While supplemented groups received barley fortified with *Spirulina*, rams grazed extensively on native pastures, indicating natural environmental trace element exposures. When the full set of incisors (dental maturity) erupted, the trial came to an end at 18 months of age.

Measurement of scrotal circumference

Throughout the final 4 months of the study, the scrotal circumference (SC) of each ram was assessed bi-weekly. This was done by holding the scrotal neck with one hand and using the fingers to gently push the testicles downward. A measuring tape was then looped around the scrotum and adjusted to the widest point between the two testicles. The measurement was recorded in centimeters.

Semen collection and evaluation

During the final 4 months of the experiment, semen samples were obtained from each ram every 15 days using a temperature-controlled artificial vagina set between 42 and 43°C. Immediately following collection, all ejaculates were incubated in a water bath at 37°C. The semen samples from each ram were then assessed for the following parameters: volume (ml), sperm concentration (109 spermatozoa/ml) and mass motility (rated on a scale from 0 for immotile to 5 for vigorous motility), as well as percentages of motility, viability, and abnormalities.

Semen conservation and assessment of its quality

Semen samples were diluted in skim milk to 800 × 10^6 spermatozoa/ml and stored at 15°C for 24 h. Total and progressive motilities were assessed using CASA (ISAS, version 1.0.17, Proiser, Valencia, Spain) according to (Yániz et al., 2008). Viability was determined via eosin-nigrosin staining, following Kulaksız et al. (2010). Abnormalities were identified using Diff-Quik staining, and plasma membrane integrity was assessed through the hypo-osmotic swelling test (HOST) following Revell (2003).

Seminal plasma assessments

Seminal plasma was separated by centrifugation (13,000 g, 10 min, 4 °C), pooled by ram groups, and stored at -20°C. Biochemical assessments were performed using a Shimadzu UV-1601 Spectrophotometer. Reduced glutathione levels were measured at 412 nm using Ellman's method (Ellman, 1959), with a standard curve prepared from reduced glutathione. Vitamin C levels were quantified following (Jagota and Dani, 1982) at 760 nm, using ascorbic acid as a standard. Lipid peroxidation was assessed as TBARS after incubation with $FeSO_4$, measured at 532 nm (Placer et al., 1966). Enzymatic activities of superoxide dismutase and catalase were determined according to Beyer and Fridovich (1987) and Ni et al. (2002), respectively, while protein concentrations were estimated via the method of Lowry et al. (1951), with bovine serum albumin as a standard.

Statistical analysis

Statistical analyzes were performed with JMP.SAS. VR 2011 software according to a standard least squares model. All the data were analyzed by a single-factor ANOVA (dietary treatment). When statistically significant differences were detected, the DUNETT test was used to compare the means and standard errors of the *Spirulina platensis* treatments compared to the positive control at $P<0.05$.

RESULTS

Scrotal circumference and semen characteristics

The scrotal circumference was significantly larger in the rams reared in a fluorosis-free area (Group II) compared to those reared in a fluorosis-endemic area (Group I), whether having *Spirulina* as a supplement or not ($P<0.05$) (Figure 1).

Regarding semen characteristics of the studied rams (Table 1), *Spirulina platensis* has a significant effect on volume ($P<0.01$), mass motility ($P<0.05$), individual motility ($P<0.01$), viability ($P<0.001$), and abnormalities ($P<0.001$). *Spirulina platensis*, on the other hand, did not affect spermatozoa concentration ($P>0.05$). More precisely, the rams of Groups II, III, and IV had higher sperm volume, mass motility, individual motility, and viability, as well as lower abnormalities than those of Group I (Table 1).

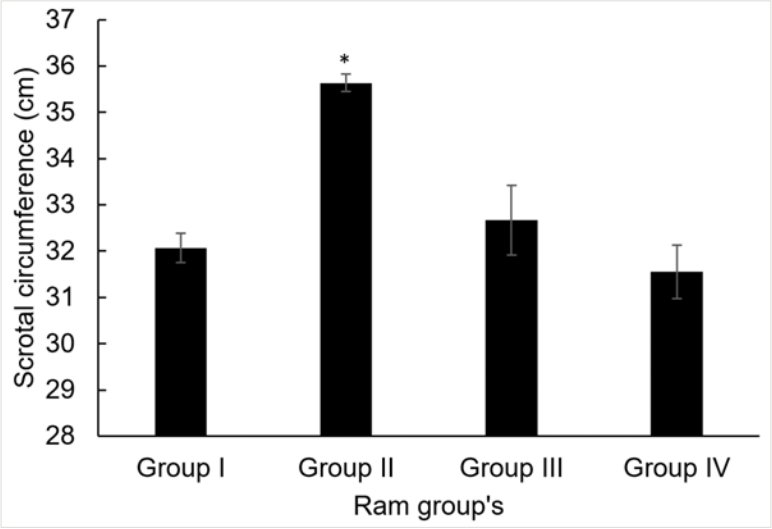


Figure 1. Effect of *Spirulina platensis* on scrotal circumference in the studied groups of rams

Group I: rams reared in fluorosis endemic area,
Group II: rams reared in fluorosis free area,
Group III: rams supplemented with 250 mg/kg BW/day of *Spirulina platensis*,
Group IV: rams supplemented with 500 mg/kg BW/day of *Spirulina platensis*.
*The lines with the index indicate a significant difference compared to Group II.

Table 1. Effect of *Spirulina platensis* on semen characteristics in the studied groups of rams

Ram group	Group I	Group II	Group III	Group IV
Volume (ml)	0.65 ± 0.15	1.47 ± 0.0*	1.27 ± 0.10 *	1.04 ± 0.12*
Mass motility	3.80 ± 0.2	4.23 ± 0.18*	4.4 ± 0.07*	3.96 ± 0.13*
Individual motility (%)	86.50 ± 1.5	97.67 ± 0.33*	94.2 ± 1.65*	91.37 ± 1.14*
Concentration (10^9 spz/ml)	3.7 ± 0.1	4.03 ± 0.47	4.19 ± 0.12	3.84 ± 0.10
Viability (%)	80.7 ± 0.7	98.33 ± 0.33*	97.27 ± 0.43*	95.6 ± 0.70*
Abnormality (%)	22.25 ± 1.25	7.53 ± 0.35*	8.77 ± 0.91*	8.63 ± 0.49*

Group I: rams reared in a fluorosis-endemic area;
Group II: rams reared in a fluorosis-free area;
Group III: rams supplemented with 250 mg/kg BW/day of *Spirulina platensis*;
Group IV: rams supplemented with 500 mg/kg BW/day of *Spirulina platensis*.
* The lines with the index indicate a significant difference compared to Group II.

Seminal plasma composition

Spirulina platensis has a significant effect on vitamin C, reduced glutathione, and lipid peroxidation levels, as well as on superoxide dismutase activity in seminal plasma (P<0.05). However, the catalase activity was not affected by the ram group (P<0.05). Precisely, the rams reared in fluorosis-free areas (Group II) and those reared in fluorosis-endemic areas supplemented with 250 (Group III) and 500 (Group IV) mg/kg BW/day of *Spirulina platensis* had lower levels of lipid peroxidation than those of Group I (P<0.05). On the other hand, vitamin C levels, reduced glutathione levels, and superoxide dismutase activity in the seminal plasma of Groups II, III, and IV were significantly higher than those of Group I (P<0.05) (Table 2).

Table 2. Effect of *Spirulina platensis* on the biochemical composition of seminal plasma in the studied groups of rams

Parameter	Group I	Group II	Group III	Group IV
Reduced glutathione (mmol/dl)	15.29 ± 0.08	19.27 ± 0.16*	18.01 ± 0.15*	19.84 ± 0.11*
Vitamin C (mg/ml)	72.47 ± 0.86	79.81 ± 0.45*	81.36 ± 0.56*	80.99 ± 0.46*
Lipid peroxidation (nmol/ml)	1.93 ± 0.01	1.68 ± 0.03*	1.69 ± 0.01*	1.70 ± 0.02*
Superoxide dismutase (IU/mg of protein)	21.69 ± 0.62	27.92 ± 0.15*	23.03 ± 0.16*	24.15 ± 1.27*
Catalase (IU/ mg of proteins)	231.39 ± 11.28	239.49 ± 14.22	240.89 ± 9.94	235.85 ± 10.26

Group I: rams reared in a fluorosis-endemic area;
Group II: rams reared in a fluorosis-free area;
Group III: rams supplemented with 250 mg/kg BW/day of *Spirulina platensis*;
Group IV: rams supplemented with 500 mg/kg BW/day of *Spirulina platensis*.
* The lines with the index indicate a significant difference compared to the Group II.

Semen conservation and assessment of its quality

The results of Table 3 showed that at 0 h of sperm storage, rams of Group II had significantly higher viability and lower percentage of abnormalities ($P<0.05$) than those of Group I. When compared to Group I, 250 mg/kg BW/day of *Spirulina platensis* (Group III) significantly enhanced progressive motility ($P<0.05$). Whereas 500 mg/kg BW/day of *Spirulina platensis* (Group IV) significantly improved progressive motility, viability and abnormalities ($P<0.05$). After 24 h sperm storage (Table 3), the rams of Group II recorded significantly higher progressive motility and a lower percentage of abnormalities compared to those of Group I ($P<0.05$). When compared to Group I, 250 mg/kg BW/day (Group III) and 500 mg/kg BW/day (Group IV) of *Spirulina platensis* significantly improved these two parameters ($P<0.05$). Plasma membrane integrity remained unaffected by the ram group regardless of storage time ($P>0.05$).

DISCUSSION

Endemic fluorosis affects over 20 countries, especially in tropical regions, due to natural fluoride sources like volcanic rocks and industrial activities (Singh et al., 2018; Rahim et al., 2022; Choubisa, 2025). In El Fokra (Khouribga, Morocco), hydrofluorosis (4.2-6.8 mg/L F^-) and trace element deficits (selenium: 0.05 ppm in soil) create a unique ecological stressor that impairs redox homeostasis in grazing animals (Maadid et al., 2017; Sibaoueih et al., 2022). The latest condition poses risks to sheep through water and plant consumption. This study revealed that rams from fluorosis-endemic areas exhibited altered scrotal circumference and semen characteristics, including reduced volume, motility, and viability, and increased abnormalities, which is consistent with findings in other species exposed to chronic fluoride toxicity (Liang et al., 2020; Khan, 2022). In fact, Chronic F^- exposure inhibits reproductive function in multiple ways (Talebi et al., 2025). Fluoride inhibits metalloenzymes involved in spermatogenesis, including Mg^{2+} dependent DNA polymerase β and Zn^{2+}dependent superoxide dismutase, by competitively binding divalent cations (Mg^{2+}, Ca^{2+}, Zn^{2+}) (Kumar et al., 2010; Rahim et al., 2023; Shams et al., 2025). Additionally, F^- overload depletes glutathione (GSH) and selenium-dependent glutathione peroxidase (GPx), permitting hydroxyl radical (•OH) accumulation that peroxidizes sperm membrane lipids, destabilizing mitochondrial cristae and axonemal dynein ATPases (Khan, 2022b). Furthermore, F^- downregulates androgen receptor expression in Sertoli cells, impairing testosterone-driven spermiogenesis and reducing seminiferous tubule diameter, reflected in diminished scrotal circumference (Pascal et al., 2023b; Owumi et al., 2025) (Figure 2).

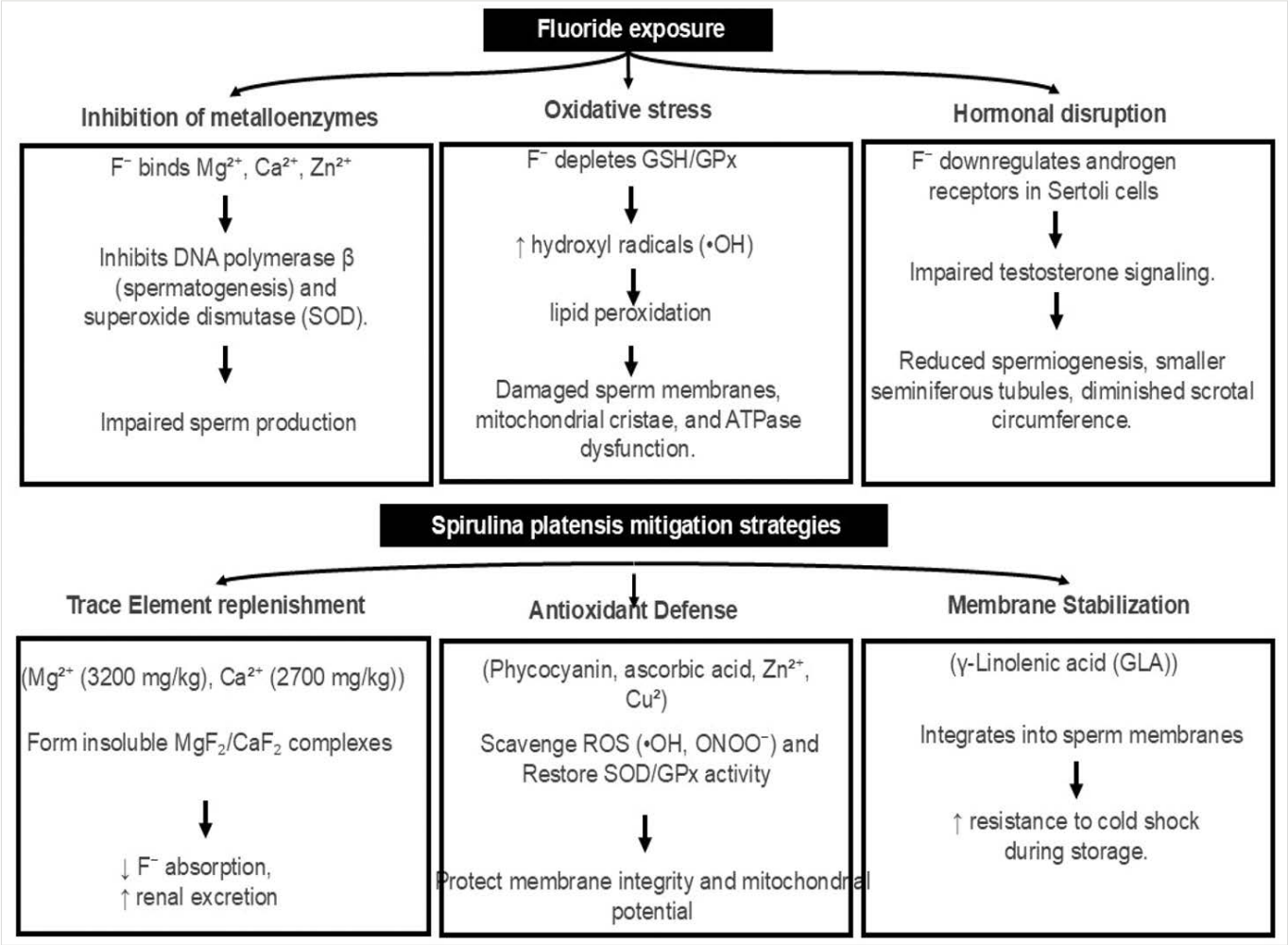


Figure 2. Effects of fluorosis on ram semen production and mitigation by *Spirulina platensis*

Semen preservation is crucial for sheep production, ensuring access to high-quality genetics for breeding (Ben Moula et al., 2024). However, preservation often decreases semen quality due to cold shock, sperm dilution, and oxidative stress, which generate reactive oxygen species that harm sperm membranes (Ben Moula et al., 2017; Ben Moula et al., 2023; El Amiri et al., 2023). Sperm motility, viability, and fertilization potential are all decreased by this oxidative damage (Rizkallah et al., 2022; Qamar et al., 2023). This leads to the hypothesis that rams from regions where fluorosis is endemic, which are already exposed to high fluoride levels, would undergo increased oxidative stress when storing their semen. In fact, compared to rams from the Settat area, rams from El Fokra showed higher sperm abnormalities and decreased progressive motility after storage, confirming our hypothesis.

Supplementing fluoride-affected rams with *Spirulina platensis* substantially improved the biochemical composition of seminal plasma and overall semen characteristics. *Spirulina* is known to contain high levels of vitamins, minerals, amino acids, pigments, and antioxidants that support reproductive function (Rahim et al., 2021). The S. platensis used in this study was particularly rich in total chlorophyll (12.85 ± 0.19 mg/g), carotenoids (1.69 ± 0.07 mg/g), c-phycocyanin (18.25 ± 1.81 mg/g), ascorbic acid (1150.3 ± 1.45 mg/kg), and several polar phenolic acids (Rahim et al., 2021). These potent antioxidant compounds likely mitigated the oxidative stress induced by fluoride exposure. Their combined action enhances endogenous antioxidant enzyme activity, scavenges reactive oxygen species, and reduces lipid peroxidation (Aissaoui et al., 2017; Gulcin, 2020), which may explain the improvements observed in seminal parameters.

Rahim et al. (2021) previously identified the mineral profile of *Spirulina platensis* used in this investigation. With magnesium at 3200 ± 2.89 mg/kg, calcium at 2700 ± 4.33 mg/kg, manganese at 24.08 ± 1.47 mg/kg, zinc at 17.61 ± 0.8 mg/kg, and copper at 0.9 ± 0.02 mg/kg, it has notable amounts of bivalent minerals. These minerals can form soluble complexes with fluoride to enhance its excretion through urine (Rahim et al., 2024). Supplementing with *Spirulina platensis* specifically reduced F^- toxicity (Figure 2) by utilizing antioxidant and trace element pathways in concert.

Mg^{2+} (3200 mg/kg) and Ca^{2+} (2700 mg/kg) in the *Spirulina* strain utilized here form insoluble complexes with F^- (MgF_2, CaF_2), decreasing intestinal absorption of F^- and increasing renal excretion (Aissaoui et al., 2017; Rahim et al., 2021). Zn^{2+} and Cu^{2+} simultaneously restored metalloenzyme activity, which is essential for neutralizing $O_2\bullet^-$ in seminal plasma. As demonstrated by decreased JC-1 monomeric fluorescence, phycocyanin and ascorbic acid directly scavenged •OH and $ONOO^-$, maintaining the integrity of the sperm membrane and the potential of the mitochondrial membrane (Aissaoui et al., 2017; Gulcin, 2020). Furthermore, *Spirulina*'s γ-linolenic acid (GLA) is integrated into sperm membranes, enhancing resistance to lipid peroxidation brought on by cold shock while being stored (Ben Moula et al., 2023). The enhanced post-storage semen resilience in supplemented serum highlights the dual function of *Spirulina* in chronic trace element rebalancing (Zn^{2+}/Se replenishment) and acute oxidative protection (phycocyanin-mediated ROS quenching). Interestingly, scrotal circumference did not change, which is consistent with F^-'s irreversible effects on collagen crosslinking in the testicular stroma, a structural insult that is less sensitive to antioxidant intervention (Pascal et al., 2023b).

CONCLUSION

In conclusion, this study's findings suggest that supplementing with *Spirulina platensis* may help reduce the reproductive dysfunctions caused by fluorosis in sheep. However, the results' power and generalizability are limited by the small sample size, so caution should be used when interpreting them. To validate these initial findings, optimise dosage schedules, and ascertain long-term efficacy, more research involving bigger, well-powered trials is required. By overcoming these methodological limitations, the evidence base will grow, enabling the development of practical solutions for enhancing the sustainability and reproductive health of sheep flocks affected by fluorosis.

ACKNOWLEDGMENTS

The authors would like to acknowledge the support through the RandD Initiative - Appel à projects APPHOS - sponsored by OCP (OCP Foundation, RandD OCP, Mohammed VI Polytechnic University, National Center of Scientific and Technical Research CNRST, Ministry of Higher Education, Scientific Research and Professional Training of Morocco MESRSFC) under the project SHS-ELM-01/2017.

This paper was supported by the RandD Initiative - Appel à projets APPHOS - sponsored by OCP (including OCP Foundation, RandD OCP, Mohammed VI Polytechnic University, National Center of Scientific and Technical Research CNRST, and the Ministry of Higher Education, Scientific Research and Professional Training of Morocco MESRSFC) under the project SHS-ELM-01/2017.

REFERENCES

Aissaoui, O., Amiali, M., Bouzid, N., Belkacemi, K., Bitam, A. (2017) Effect of *Spirulina platensis* ingestion on the abnormal biochemical and oxidative stress parameters in the pancreas and liver of alloxan-induced diabetic rats. Pharmaceutical Biology, 55 (1), 1304-1312. DOI: https://doi.org/10.1080/13880209.2017.1300820

Amir-Ud-Din, U., Ahmad, K. R., Kanwal, M. A., Inayat, I., Ahmad, S. N., Ali, R., Suleman, S., Sial, B., Younis, A., Abdullah, U., Siddique, S., Kanwal, N., Bilal, M. A. (2022) Ameliorations of medicinal fruit extracts on kidney against fluoride exposure in mice. Plant cell biotechnology and molecular biology, 15-22. DOI: https://doi.org/10.56557/pcbmb/2022/v23i15-167620

Banji, D., Banji, O. J. F., Pratusha, N. G., Annamalai, A. R. (2013). Investigation on the role of *Spirulina platensis* in ameliorating behavioural changes, thyroid dysfunction and oxidative stress in offspring of pregnant rats exposed to fluoride. Food Chemistry, 140 (1-2), 321-331. DOI: https://doi.org/10.1016/j.foodchem.2013.02.076

Ben Moula, A., Badi, A., El Fadili, M., El Khalil, K., Allai, L., El Hilali, A., El Amiri, B. (2017) Effect of season on scrotal circumference, semen characteristics, seminal plasma composition and spermatozoa motility during liquid storage in INRA180 rams. Animal Reproduction Science, 180, 17-22. DOI: https://doi.org/10.1016/j.anireprosci.2017.02.008

Ben Moula, A., Rahim, A., Chentouf, M., Raes, M., Kirschvink, N., El Amiri, B. (2023) Inclusion of *Spirulina platensis* and *Salvia verbenaca* extracts to boost semen quality and fertilization ability in sheep. Reproduction in Domestic Animals, 58 (5), 637-645. DOI: https://doi.org/10.1111/rda.14332

Ben Moula, A., Chentouf, M., Hmimsa, Y., El Amri, H., El Fatehi, S., El Amiri, B. (2024) Invited Review: Reproductive biotechnologies in Moroccan livestock: achievements, challenges, and future perspectives. Journal of Livestock Science and Technologies, 12 (2), 1-11. DOI: https://doi.org/10.22103/jlst.2024.23987.1560

Beyer, W. F., and Fridovich, I. (1987) Assaying for superoxide dismutase activity: Some large consequences of minor changes in conditions. Analytical Biochemistry, 161 (2), 559-566. DOI: https://doi.org/10.1016/0003-2697(87)90489-1

Choubisa, S. (2025) Focus on fluorosis in domestic animals in India. Journal of Veterinary Health Science, 6, 1-6.

El Amiri, B., Ben Moula, A., El Khelfaoui, N., Rahim, A., Gyoker, E., Horváth, A., Szenci, O. (2023) Comparative evaluation of Duragen® and skim milk extenders to enhance ram semen quality and fertility: A promising alternative in ovine artificial insemination. Reproduction in Domestic Animals, 58 (8), 1132-1138. DOI: https://doi.org/10.1111/rda.14414

El Amiri, B., Rahim, A. (2024) Exploring endogenous and exogenous factors for successful artificial insemination in sheep: A global overview. Veterinary Sciences, 11 (2). DOI: https://doi.org/10.3390/vetsci11020086

El-Deeb, M. M., Abdel-Gawad, M., Abdel-Hafez, M. A. M., Saba, F. E., Ibrahim, E. M. M. (2022) Effect of adding *Spirulina platensis* algae to small ruminant rations on productive, reproductive traits and some blood components. Acta Scientiarum. Animal Sciences, 45, e57546. DOI: https://doi.org/10.4025/actascianimsci.v45i1.57546

Ellman, G. L. (1959) Tissue sulfhydryl groups. Archives of Biochemistry and Biophysics, 82 (1), 70-77. DOI: https://doi.org/10.1016/0003-9861(59)90090-6

Gulcin, İ. (2020) Antioxidants and antioxidant methods: An updated overview. Archives of Toxicology, 94 (3), 651-715. DOI: https://doi.org/10.1007/s00204-020-02689-3

Han, B., Yoon, S.-S., Wu, P.-F., Han, H.-R., Liang, L.-C. (2006) Role of selenium in alteration of erythrocyte parameters in bovine fluorosis. Asian-Australasian Journal of Animal Sciences, 19 (6), 865-871.

Jagota, S. K., Dani, H. M. (1982) A new colorimetric technique for the estimation of vitamin C using Folin phenol reagent. Analytical Biochemistry, 127 (1), 178-182. DOI: https://doi.org/10.1016/0003-2697(82)90162-2

Kashyap, S. J., Sankannavar, R., Madhu, G. M. (2021) Fluoride sources, toxicity and fluorosis management techniques - A brief review. Journal of Hazardous Materials Letters, 2, 100033. DOI: https://doi.org/10.1016/j.hazl.2021.100033

Katir Masnaoui, L. A., Rahim, A., Bouchab, H., Amiri, B. E., Nasser, B., Khalid, E. A. (2023) Relationship between plasma fluoride levels, glutathione peroxidase activity, hemoglobin, and abortion in rural and urban pregnant women from settat (Morocco). Makara Journal of Health Research, 27 (2). DOI: https://doi.org/10.7454/msk.v27i2.1408

Khan, I. (2022a) Sperm quality parameters and oxidative stress: exploring correlation in fluoride-intoxicated rats. Journal of Human Reproductive Sciences, 15 (3), 219-227. DOI: https://doi.org/10.4103/jhrs.jhrs_65_22

Khan, I. (2022b) Sperm quality parameters and oxidative stress: Exploring correlation in fluoride-intoxicated rats. Journal of Human Reproductive Sciences, 15 (3), 219-227. DOI: https://doi.org/10.4103/jhrs.jhrs_65_22

Kulaksız, R., Çebi, Ç., Akçay, E., Daşkın, A. (2010) The protective effect of egg yolk from different avian species during the cryopreservation of Karayaka ram semen. Small Ruminant Research, 88 (1), 12-15. DOI: https://doi.org/10.1016/j.smallrumres.2009.11.014

Kumar, N., Sood, S., Arora, B., Singh, M., Beena, N.I. (2010) Effect of duration of fluoride exposure on the reproductive system in male rabbits. Journal of Human Reproductive Sciences, 3 (3), 148-152. DOI: https://doi.org/10.4103/0974-1208.74159

Li, Y., Zhang, J., Sun, L., Zhao, H., Jia, X., Zhang, Y., Li, Y. (2023) Fluoride-induced sperm damage and hur-mediated excessive apoptosis and autophagy in spermatocytes. Biological Trace Element Research, 201 (1), 295-305. DOI: https://doi.org/10.1007/s12011-022-03138-1

Liang, C., He, Y., Liu, Y., Gao, Y., Han, Y., Li, X., Zhao, Y., Wang, J., Zhang, J. (2020) Fluoride exposure alters the ultra-structure of sperm flagellum via reducing key protein expressions in testis. Chemosphere, 246, 125772. DOI: https://doi.org/10.1016/j.chemosphere.2019.125772

Liang, Y., Bao, Y., Gao, X., Deng, K., An, S., Wang, Z., Huang, X., Liu, D., Liu, Z., Wang, F., Fan, Y. (2020) Effects of *spirulina* supplementation on lipid metabolism disorder, oxidative stress caused by high-energy dietary in Hu sheep. Meat Science, 164, 108094. DOI: https://doi.org/10.1016/j.meatsci.2020.108094

Lowry, O., Rosebrough, N., Farr, A. L., Randall, R. (1951) Protein measurement with the Folin phenol reagent. Journal of Biological Chemistry, 193 (1), 265-275.

Maadid, H., El Mzouri, E. H., Mabrouk, A., Koulali, Y. (2017) Fluoride content in well waters for human and animal consumption with reported high incidence levels of endemic fluorosis in Beni Meskine (Morocco). Euro-Mediterranean Journal for Environmental Integration, 2 (1), 11.

Nabavi, S. M., Habtemariam, S., Nabavi, S. F., Sureda, A., Daglia, M., Moghaddam, A. H., Amani, M. A. (2013) Protective effect of gallic acid isolated from *Peltiphyllum peltatum* against sodium fluoride-induced oxidative stress in rat's kidney. Molecular and Cellular Biochemistry, 372 (1-2), 233-239. DOI: https://doi.org/10.1007/s11010-012-1464-y

Ni, J., Sasaki, Y., Tokuyama, S., Sogabe, A., Tahara, Y. (2002) Conversion of a typical catalase from Bacillus sp. TE124 to a catalase-peroxidase by directed evolution. Journal of Bioscience and Bioengineering, 93 (1), 31-36. DOI: https://doi.org/10.1016/S1389-1723(02)80050-0

Owumi, S., Oluwawibe, B. J., Agbarogi, H., Otunla, M. T., Owoeye, O., Arunsi, U. O. (2025) Integrated *In-silico* and *In-vivo* assessments of betaine's effect on the Hypothalamic-Pituitary-Testicular (HPT) axis in fluoride-treated rats. Biological Trace Element Research, 203 (9), 4671-4693. DOI: https://doi.org/10.1007/s12011-025-04519-y

Pascal, C., Nechifor, I., Florea, M. A., Pânzaru, C., Simeanu, D., Mierliță, D. (2023a) Diet influence on sperm quality, fertility, and reproductive behavior in karakul of botoșani rams. Agriculture, 13 (11). DOI: https://doi.org/10.3390/agriculture13112168

Pascal, C., Nechifor, I., Florea, M. A., Pânzaru, C., Simeanu, D., Mierliță, D. (2023b) Diet influence on sperm quality, fertility, and reproductive behavior in karakul of botoșani rams. Agriculture, 13 (11), 2168. DOI: https://doi.org/10.3390/agriculture13112168

Placer, Z. A., Cushman, L. L., and Johnson, B. C. (1966) Estimation of product of lipid peroxidation (malonyl dialdehyde) in biochemical systems. Analytical Biochemistry, 16 (2), 359-364. DOI: https://doi.org/10.1016/0003-2697(66)90167-9

Qamar, A. Y., Naveed, M. I., Raza, S., Fang, X., Roy, P. K., Bang, S., Tanga, B. M., Saadeldin, I. M., Lee, S., Cho, J. (2023) Role of antioxidants in fertility preservation of sperm—A narrative review. Animal Bioscience, 36 (3), 385-403. DOI: https://doi.org/10.5713/ab.22.0325

Rahim, A., Çakir, C., Ozturk, M., Şahin, B., Soulaimani, A., Sibaoueih, M., Nasser, B., Eddoha, R., Essamadi, A., El Amiri, B. (2021) Chemical characterization and nutritional value of *Spirulina platensis* cultivated in natural conditions of Chichaoua region (Morocco). South African Journal of Botany, 141, 235-242. DOI: https://doi.org/10.1016/j.sajb.2021.05.006

Rahim, A., Aydogmus-Öztürk, F., Cakir, C., Essamadi, A., El Amiri, B. (2022) Mitigating fluoride, lead, arsenic and cadmium toxicities in laboratory animals and ruminants through natural products. Records in Agricultural and Food Chemistry,2 (1). DOI: https://doi.org/10.25135/rfac.6.2202.2365

Rahim, A., Sibaoueih, M., Essamadi, A., El Amiri, B. (2023) An interventional clinical trial investigating the effects of *Spirulina platensis* on dental fluorosis and antioxidant system in lambs reared in endemic areas. Scientific Reports, 13 (1), 16858. DOI: https://doi.org/10.1038/s41598-023-44058-x

Revell, S. (2003) Assessment of the morphology of frozen thawed bull sperm in relation to its cryopreservation for artificial insemination. Veterinary Record, 152 (25), 765-767. DOI: https://doi.org/10.1136/vr.152.25.765

Rizkallah, N., Chambers, C. G., de Graaf, S. P., Rickard, J. P. (2022) Factors affecting the survival of ram spermatozoa during liquid storage and options for Improvement. Animals, 12 (3). DOI: https://doi.org/10.3390/ani12030244

Samal, P., Patra, R. C., Gupta, A. R., Mishra, S. K., Jena, D., Satapathy, D. (2016) Effect of *Tamarindus indica* leaf powder on plasma concentrations of copper, zinc, and iron in fluorotic cows. Veterinary World, 9 (10), 1121-1124. DOI: https://doi.org/10.14202/vetworld.2016.1121-1124

Shams, A., Kareem, A., Mahgoob, H., Zainualbdeen, K. (2025) A comprehensive review of the complex causes of fluorosis. Baghdad Journal of Biochemistry and Applied Biological Sciences, 6 (3), 117-126. DOI: https://doi.org/10.47419/bjbabs.v6i03.350

Sibaoueih, M., Rahim, A., Amiri, B. E. (2022). Les teneurs en fluorure des plantes influencent-elles leur qualité fourragère pour ruminant ? African and Mediterranean Agricultural Journal - Al Awamia, 136. DOI: https://doi.org/10.34874/IMIST.PRSM/afrimed-i136.34829

Singh, G., Kumari, B., Sinam, G., Kriti, Kumar, N., Mallick, S. (2018). Fluoride distribution and contamination in the water, soil and plants continuum and its remedial technologies, an Indian perspective- a review. Environmental Pollution, 239, 95-108. DOI: https://doi.org/10.1016/j.envpol.2018.04.002

Srivastava, S., Flora, S. J. S. (2020) Fluoride in drinking water and skeletal fluorosis: A review of the global impact. Current Environmental Health Reports, 7, 140-146.

Talebi, S. F., Seify, M., Bhandari, R. K., Shoorei, H., Oskuei, S. D. (2025) Fluoride-induced testicular and ovarian toxicity: Evidence from animal studies. Biological Research, 58 (1), 6. DOI: https://doi.org/10.1186/s40659-025-00586-6

Yániz, J. L., Santolaria, P., Marco-Aguado, M. A., López-Gatius, F. (2008). Use of image analysis to assess the plasma membrane integrity of ram spermatozoa in different diluents. Theriogenology, 70 (2), 192-198. DOI: https://doi.org/10.1016/j.theriogenology.2008.03.002