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RESEARCH PAPER

Biosynthesized Selenium Nanoparticles Modulate Hormonal, Sperm Protein, and Antioxidant Parameters in Immature Male Rats (Rattus norvegicus)

Abdulsattar Mohammed Afrawee *, Aamir Al-Ghareebawi

Department of Physiology, Pharmacology, Biochemistry, College of Veterinary Medicine, University of Al-Shatrah, Iraq

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ABSTRACT

Shortening the time of the male reproductive system maturation aids in lowering the animal husbandry time. This study was designed to investigate whether biosynthesized selenium nanoparticles could modulate reproductive hormones, sperm-associated proteins, and antioxidant biomarkers in immature male rats and to evaluate their potential influence on reproductive development. The biosynthesized SeNPs were characterized by XRD, FTIR, Zeta Potential, and FE-SEM to confirm the utilized method produced nanoparticles. Forty immature male rats aged 1 month and weighing 100±5 gm was classified into 5 groups as follows: the control group received D.W. orally, G1 received 50 µg/kg B.W. orally, G2 received 100 µg/kg B.W. orally, G3 received 200 µg/kg B.W. orally, and G4 received 400 µg/kg B.W. orally. The treatment was daily for one month. Serum was obtained to estimate the concentrations of the studied parameters. Results of an experiment that deals with the characterization of biosynthesized Se-NPs showed an optimal method of synthesis. The concentrations of studied parameters revealed a significant increase in FSH, LH, Selenoprotein P, and acrosin in some treated groups as compared with the control. The levels of testosterone, glutathione reductase, glutathione peroxidase, and protamine exhibit significant inhibition in some treated groups as compared with the control group. Biosynthesized selenium nanoparticles significantly altered reproductive hormones, sperm protein, and antioxidant profiles in immature male rats.

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INTRODUCTION

The maturation and physiological regulation of the male reproductive system are under control of the hypothalamic, pituitary, and gonadal (HPG) axis, in which the hypothalamus produces hormone which regulates pituitary gonadotropins (LH and FSH) production, thereby modulating Leydig, and Sertoli cell function, androgen production, and male reproductive system fertility [1-3]. Testosterone is produced mainly by adult Leydig cells in the testicular interstitial tissue and is considered the principal androgen in males. It is essential for the maintenance of sperm production and for the development and

* *Corresponding Author Email: mailto:alhajey@gmail.com*

protection of the male reproductive system [4]. Current evidence suggests that selenium may indirectly help preserve testosterone production by protecting Leydig cell function from oxidative and inflammatory damage. Luteinizing hormone (LH) is secreted by the anterior pituitary in response to hypothalamic gonadotropin-releasing hormone (GnRH). In males, LH primarily acts on Leydig cells to stimulate testosterone synthesis, which is crucial for maintaining spermatogenesis and male fertility [5]. Follicular stimulating hormone (FSH) is synthesized and secreted by pituitary gonadotrophs under hypothalamic control through gonadotropin-releasing hormone (GnRH). In males, FSH is a major endocrine regulator of testicular development, pubertal maturation, and spermatogenesis [6,3,7]. Selenium (Se) is an essential trace element that is present as selenocysteine in a family of selenoproteins, which include glutathione peroxidases (GPXs), glutathione reductases (GRs), and Seleno protein P. These proteins collectively support antioxidant defense and thyroid hormone metabolism, both of which are essential for male reproductive function [8,9]. Oxidative stress results from an imbalance between the generation of reactive oxygen species (ROS) and antioxidant defense systems. Excessive ROS production disrupts redox homeostasis and promotes lipid peroxidation, protein oxidation, and DNA fragmentation. These changes impair sperm membrane integrity, motility, fertilizing capacity, and genomic stability, thereby contributing to male infertility [10,7]. Acrosin is a trypsin-like serine protease associated with the sperm acrosome and is stored predominantly as the inactive zymogen proacrosin, which becomes activated during acrosomal exocytosis. [11]. Functionally, acrosin appears to facilitate sperm passage through the zona pellucida by limited proteolysis and remodeling of the zona matrix rather than simple bulk degradation [12,13,11]. Protamines are small, highly basic (arginine-rich) nuclear proteins that play a central role in sperm chromatin remodeling during spermiogenesis. abnormal protamine ratios, impaired sperm chromatin packaging, increased DNA damage, and male infertility [14]. Nanotechnology refers to particles with a size of 1-100 nanometers [15,16]. Due to their superior biocompatibility and relatively low toxicity, selenium nanoparticles (SeNPs) have become a potential class of nanomaterials (compared with certain other nanomaterials), with tunable physicochemical properties. Recent reviews highlight their potential in antioxidant. anti-inflammatory [17]. antimicrobial [18], and anticancer applications [19], with growing interest in biomedical and translational uses [20]. At this scale, materials may display physicochemical and biological properties that differ greatly from their bulk equivalents, which underpins many of their biomedical uses [21,22]. Selenium nanoparticles (SeNPs) have emerged as a promising class of nanomaterials due to their excellent biocompatibility, comparatively low toxicity (compared with certain other nanomaterials). anti-inflammatory [17]. antimicrobial [18], and anticancer applications [19], with growing interest in biomedical and translational uses [20]. This study is among the first investigations evaluating biosynthesized starch-mediated selenium nanoparticles in immature male rats with simultaneous assessment of reproductive hormones, sperm-associated proteins (acrosin and protamine), and antioxidant biomarkers. This integrated approach provides new insights into the potential role of selenium nanoparticles in reproductive development. The present study aimed to evaluate the effects of biosynthesized selenium nanoparticles (SeNPs) on reproductive hormones (testosterone, luteinizing hormone, and follicle-stimulating hormone), sperm-associated proteins (acrosin and protamine), and antioxidant biomarkers (glutathione peroxidase, glutathione reductase, and selenoprotein P) in immature male rats. In addition, the study sought to investigate the biochemical and physiological responses associated with SeNP administration during the prepubertal stage.

MATERIALS AND METHODS

Ethical Approval

This study was prepared in accordance with Shatrah University's College of Veterinary Medicine's ethical guidelines (Approval No. SU-EC-2025-9).

Synthesis of Selenium nanoparticles

Selenium nanoparticles were manufactured by a green chemical reduction approach utilizing prepared 4% starch stabilization and ascorbic acid reduction [23]. First, the starch solution was gradually mixed with the prepared 0.346% sodium selenite solution while being continuously stirred to ensure that the selenium ions were

evenly distributed throughout the starch matrix. Following thorough mixing, the prepared 3.5% ascorbic acid solution was gradually added to the reaction mixture while stirring constantly until the PH of the solution dropped from 12 to (7 - 6,8). The solution was visually confirmed by a gradual change in the color of the solution from colorless to orange-red [24] indicating the formation of selenium nanoparticles. The reaction temperature was slightly raised to 50 ºC–60 ºC for 4.5 hours, Centrifugation was used to purify the produced selenium nanoparticles for 15 minutes at a speed of 6000 rpm to get rid of unreacted materials and extra stabilizing agents, the sediment was washed three times with distilled water and then one time with alcohol. After that, the product is dried at 40 degrees Celsius for a full day in an incubator. The biosynthesized SeNPs were characterized by XRD, FTIR, Zeta Potential, and FE-SEM to confirm the utilized method produced nanoparticles.

Preparation of dosage solution

To prepare the dosing solution, 0.4 mg of nano-selenium was dissolved in a liter of distilled water and placed in an ultrasonic device to dissolve the nano-selenium particles. The solution was then stored in an opaque bottle at a temperature of -4 ºC.

Animals of the study

Forty immature male white laboratory rats (Rattus norvegicus) weighing 100 ± 50 grams at one month of age were kept at the room temperature 25±2 °C, 12 hours of light per day from four lid lamps, a humidity level of between 40-50%, and daily access to food and water. The animals were divided into five groups with 8 male rats in each group. The five groups were given a daily oral single dose for 30 treatment days as follows: Group 1 (control): Administered orally 0.2 ml of D.W. Group 2: Administered orally 50 µg/kg BW of SeNPs daily. Group 3: Administered orally 100 µg/kg of SeNPs daily. Group 4: Administered orally 200 µg/kg BW of SeNPs daily. Group 5: Administered orally 400 µg/kg BW of SeNPs daily. After 30 days of oral administration of SeNPs, the 8 male rats of each group were sacrificed. Following chloroform anesthesia, by inhalation, the animal is secured, its abdomen is opened, and a 5 ml disposable syringe is used to collect blood from its heart. The collected blood centrifuged 3000 rpm for 10 minutes to obtain the serum which was used to estimate the concentration of studied hormones (testosterone, FSH, LH), Sperm protein (acrosin and protamine), and antioxidant (Glutathione peroxidase, Glutathione reductase and selenoprotein P) enzymes.

Collection of blood samples

After 30 days of oral SeNPs administration, blood samples were collected from anesthetized rats by cardiac puncture. Serum was separated by centrifugation at 3000 rpm for 15 minutes and stored at −20 °C for the estimation of testosterone, LH, FSH, sperm protamine, acrosin, selenoprotein, glutathione reductase, and glutathione peroxidase levels.

Hormonal and Oxidative Stress Biomarker Estimation by ELISA

Samples of rat blood serum hormonal concentration, antioxidant concentration, and sperm protein concentration were measured by using ELISA kits as follows:

The hormonal ELISA kits were bought from ELK Biotechnology, with the catalog numbers (ELK10883) and (ELK9279). And from Reed Biotech with the catalog numbers (RE 3119R), (RE3544R), and (RE2557R). And from ELK Biotechnology with the catalog numbers (ELK0073) and (ELK7733). The Human reader HS (Germany) ELISA reader was used to determine the concentration of enzymes and proteins in serum. The unit of each, according to the user manual, is:

Selenoprotein P = nanogram (ng), Glutathione peroxidase = picogram (pg), Glutathione reductase = picogram (pg).

The concentrations of testosterone, LH, FSH, sperm protamine, acrosin, selenoprotein, GR, and GPX in the examined samples were determined by interpolation from their respective standard calibration curves [25].

Statistical Analysis

Data were analyzed using one-way ANOVA. Mean comparisons were performed using Duncan's Multiple Range Test (DMRT) using the statistical software package [26].

RESULTS AND DISCUSSION

Green synthesis and characterization ofSelenium Nanoparticles SeNPs In visual observation (color changes)

In this study, selenium nanoparticles (SeNPs)

were synthesized using starch extract as a reducing agent. Over time, the color changed from a colorless aqueous solution to pale yellow, then to dark brown after 4.5 hours on the magnetic stirrer (Fig. 1).

Characterization of SeNPs

Fourier transform infrared spectroscopy (FT-IR) was used to establish the identity of different phytochemical constituents. The beak at 3434 cm^{-1} is typical of O–H stretching, which means that biomolecules act as reducing and capping agents. The peak at 1732 cm^{-1} refers to C=O stretching, and the peak at 1631 cm^{-1} refers to C=C. These all point to organic functional groups helping to stabilize the nanoparticles. But the real giveaway is the low-frequency bands at 539 and 468 cm^{-1}, which belong to Se–O or Se–Se vibrations. These strongly indicate the presence of SeNPs in the sample.

The FTIR spectroscopy showed successful biosynthesis of SeNPs as indicated by peaks in (Fig. 2).

The Zeta potential value refers to the charge which present on the surface of the particles. When it is higher than ±30, this indicates that the synthesized particles will be repellent and highly stable and will not aggregate together

Fig. 1. Color change of the solution.

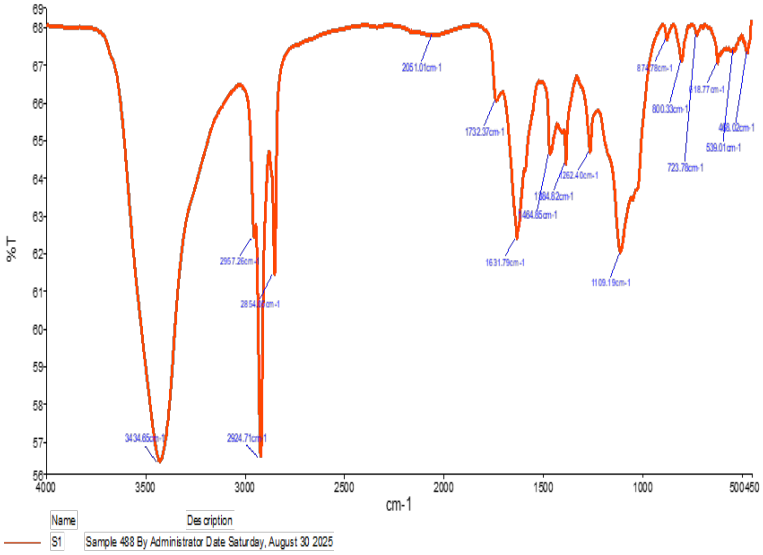

Fig. 2. FTIR Peaks of Se NPs.

in the suspension. In the current study, the zeta potential value is -58.3 mV (Fig. 3), that mean the synthesized SeNPs are highly stable in suspension for a long time. The effective synthesis of nano-selenium with good crystallinity and phase stability that matches the typical characteristics for elemental selenium is confirmed by the XRD examination.

The crystalline structure and phase purity of the synthesized Selenium Nanoparticles (Se-NPs) were characterized using X-ray diffraction (XRD). The diffraction pattern of the sample is presented in Fig. 4. The XRD pattern exhibits sharp and well-defined peaks, confirming the crystalline nature

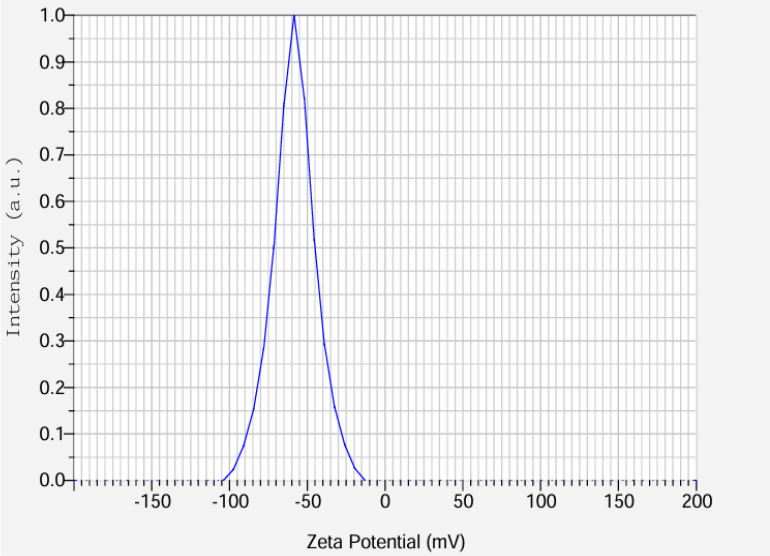

Fig. 3. Zeta potential (Mv) diagram of Se NPs.

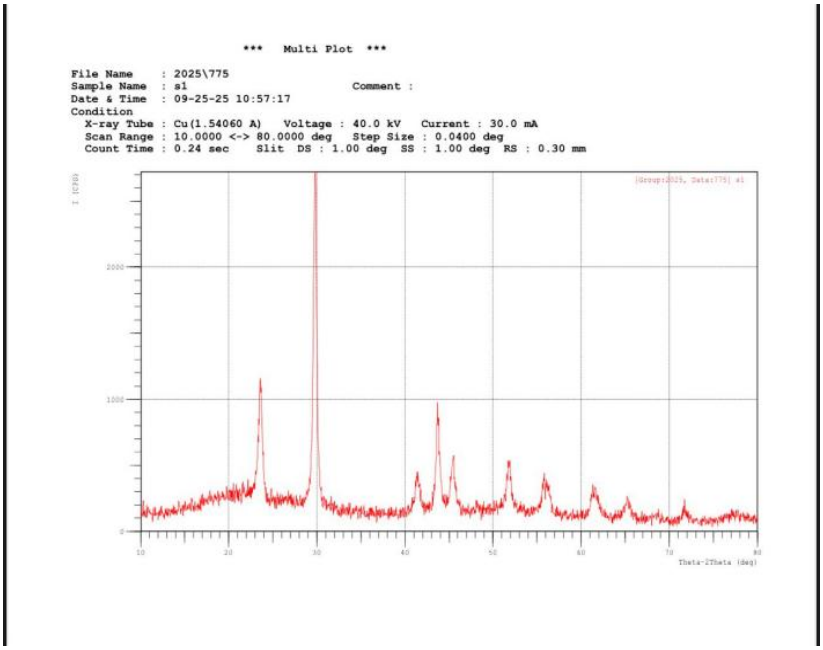

Fig. 4. XRD profile for Se NPs.

of the synthesized nano-selenium. The images in this study showed that the particles took an oval or semicircular shape and had diameters of 30-40 nanometers (30.72, 31.66, 31.94, 38.85), as in Fig. 5, indicating successful nanoparticle formation [27].

Effect of Se NPs on hormonal profile

Table 1 represents the concentrations of studied hormones of all groups, which reveals a significant (P≤0.01) decrease in Testosterone concentration in group G1 as compared with the control, G3, and G4 groups. At the same time, the G2 group did not show any significant(P>0.01) changes as compared to the G1 and G3 groups. Also, the G3 group showed non-significant (P>0.01) differences with the control, G2, and G4 groups. The luteinizing hormone concentration showed a significant (P≤0.01) increase in groups G1 and G2, as compared with the control and G4 groups; the concentration of LH in the G3 group appeared to show non-significant (P>0.01) differences with the control and G2 groups. The G4 group appeared non-significant (P>0.01) change to the control group and showed a significant (P≤0.01) decrease as compared with G1, G2, and G3 groups. The level of FSH in the current study showed a significant(P≤0.01) increase in G1 and G2 groups as compared to control, G3, and G4 groups. At the same time, the level of FSH in the G1 group appeared significantly (P≤0.01) increased when compared with the G2 group. The concentration of the studied hormone showed no significant (P>0.01) differences among the control, G3, and G4 groups.

Effect of Se- NPs on Antioxidant Enzyme and seleno protein level

At a significant level (P≤0.01), the concentration of Glutathione reductase decreased significantly (P≤0.01) in all groups in comparison with the control group. The G2 group showed a significant (P≤0.01) decrease compared with the G1group. Also, the G3 and G4 groups did not show significant (P>0.01) changes as compared to the G1 and G2 groups. The Glutathione peroxidase level study revealed a significant (P≤0.05) decrease in the G4 group when compared with the control group. But the G1, G2, and G3 groups showed no significant (P>0.05) differences when compared to the control and G4 groups. In addition to the above parameters, the concentration of selenoprotein studied showed a significant (P≤0.01) increase in the G4 group as compared with other groups except the G2 group, which showed non-significant (P≤0.01) changes when compared with the control and G4 group. The G1 and G3 groups decreased significantly (P≤0.01) as compared to the G2 and G4 groups, but not significantly (P>0.01) with the control group Table 2.

Table 3 illustrates the effect of selenium nanoparticle doses on serum acrosin and serum protamine levels in the different groups compared to the control group. It shows a significant increase in acrosine levels in the G3 and G4 groups compared to the control, G1, and G2 groups. No significant (P≤0.01) revealed among the control, G1, and G2 groups. As for protamine, it shows

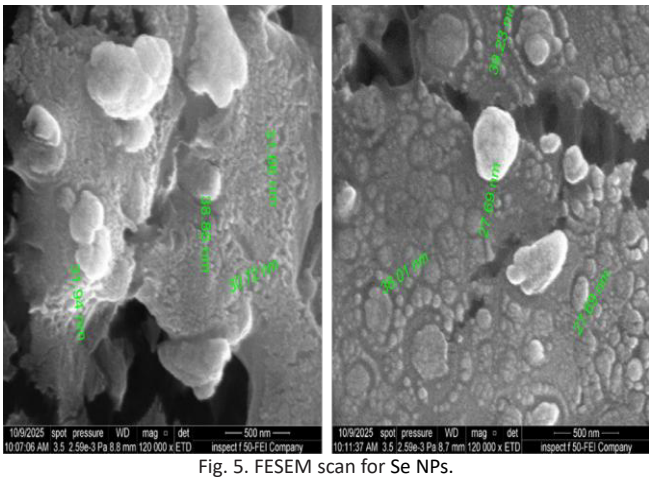

Fig. 5. FESEM scan for Se NPs.

a significant decrease in the G1, G2, G3, and G4 groups when compared to the control group, as well as a significant decrease in the G3 and G4 microgram groups in comparison to the G1 group. The G2 group showed non-significant (P≤0.01) changes with G1, G3, and G4 groups.

The FTIR, XRD, Zeta potential, and FESEM analyses confirmed the successful biosynthesis of selenium nanoparticles (SeNPs). The FTIR spectrum revealed characteristic absorption bands corresponding to functional groups involved in the reduction and stabilization of selenium nanoparticles. The XRD pattern showed distinct crystalline peaks, confirming the formation of elemental selenium nanoparticles, in agreement with the finding reported by Kazemi (2021) [28]. Furthermore, the Zeta potential value (-58.3 mV) indicated excellent colloidal stability and a low tendency for particle aggregation, which may enhance nanoparticle bioavailability and biological activity [29].

The FESEM images demonstrated that the synthesized nanoparticles possessed oval to semi spherical morphology with diameters ranging from 30 to 40 nm, confirming successful nanoparticle formation and supporting previous observations reported by Srivastava and Mukhopadhyay (2015) [27]. The present study demonstrated significant alterations in reproductive hormone concentrations following SeNPs administration. A significant reduction in testosterone levels was observed in treated groups. Although selenium is known to support testicular function through its antioxidant properties, the reduction in testosterone may indicate a dose-dependent physiological response. High concentrations of selenium nanoparticles may alter steroidogenic activity in Leydig cells, leading to reduced

Table 1. Effect of SeNPs dosage(µg/kg) on hormone levels in rats (Mean ± SE).

Groups	Testosterone ng/ml	LH mlU/ml	FSH mlU/ml
Control	14.059 ± 1.164 A	2.361 ± 0.392 DC	13.791 ± 1.022 C
G1	9.489 ± 0.224 C	6.055 ± 0.848 A	23.909 ± 1.232 A
G2	10.886 ± 0.224 BC	4.680 ± 0.542 AB	18.363 ± 1.297 B
G3	12.540 ± 0.531 AB	3.666 ± 0.423 BC	13.305 ± 0.733 C
G4	14.638 ± 1.011 A	2.010 ± 0.229 D	11.563 ± 1.161 C
Sign. Level	**	**	**

Means with different superscript letters differ significantly (P≤0.01) **, (P≤0.05) *

Table 2. Effect of Se-NPs dosage(µg/kg) on antioxidants levels in rats (Mean±SE).

Dosages	Glutathione reductase pg/ml	Glutathione peroxidase pg/ml	Selenoprotein ng/
Control	346.440 ± 11.351 A	1670.00 ± 86.023 A	1.600 ± 0.109 BC
G1	268.460 ± 33.471 B	1650.00 ± 128.452 AB	1.120 ± 0.245 C
G2	184.480 ± 8.057 C	1444.00 ± 83.88 AB	2.440 ± 0.519 AB
G3	205.500 ± 26.034 BC	1490.00 ± 85.732 AB	0.940 ± 0.188 C
G4	228.660 ± 19.027 BC	1340.00 ± 107.703 B	2.920 ± 0.389 A
Sign. Level	**	*	**

Means with different superscript letters differ significantly (P≤0.01) **, (P≤0.05) *).

testosterone synthesis despite the presence of adequate antioxidant support.

Similar findings were reported by Abdallah *et al*. (2023) [30], who observed decreased testosterone concentration following nano selenium administration. In contrast, significant changes in luteinizing hormone (LH) and follicular stimulating hormone (FSH) concentration were detected. These alterations may reflect modulation of the hypothalamic pituitary gonadal (HPG)axis. Selenium is an essential component of several selenoproteins that protect reproductive tissues against oxidative stress and maintain cellular homeostasis. Therefore, the observed increases in LH and FSH may represent a compensatory endocrine response aimed at maintaining reproductive function and supporting testicular activity. Similar observations have been reported by Gan *et al*. (2019) [31], Yuan *et al*. (2024) [32], and Dkhil *et al*, (2016) [33].

These findings support the concept that selenium does not necessarily stimulate testosterone production directly, but rather improves the physiological environment required for normal endocrine and reproductive function. This interpretation is consistent with the conclusions of Zecevic *et al*. (2025) [34], who suggested that selenium contributes to spermatogenesis and steroidogenesis primarily through its antioxidant and cytoprotective effects rather than through direct hormonal stimulation. The antioxidant biomarker results revealed a significant alteration in glutathione reductase (GR), glutathione peroxidase, and selenoprotein P concentrations. Selenium plays a fundamental role in the antioxidant defense system because it forms an integral component of several antioxidant enzymes and selenoproteins. The increase in selenoprotein P may indicate enhanced selenium transport and utilization within biological tissues. Conversely, the reduction observed in GR and GPx concentrations may reflect increased consumption of antioxidant defenses in response to metabolic changes induced by SeNPs exposure. Similar findings were reported by Nasirpour *et al*. (2017) [35], who attributed these alterations to enhanced antioxidant utilization during oxidative stress regulation. However, other studies reported increased antioxidant enzyme activities following selenium supplementation [36,37]. These discrepancies may be attributed to differences in selenium source nanoparticles, nanoparticle characteristics, dosage, duration of administration, animal species, and physiological condition. As suggested by Zhang *et al*. (2023) [38] and Yuan *et al*. (2024) [32], the biological effects of selenium nanoparticles are highly dependent on dose and experimental conditions regarding sperm associated protein; acrosin concentration increased significantly in some treated groups. Acrosin is a proteolytic enzyme that plays a critical role during fertilization by facilitating sperm penetration through the zona pellucida. The increase in acrosin concentration may indicate stimulation of biochemical pathways associated with sperm functional development and reproductive capacity. Similar findings were reported by Horkey *et al*. (2023) [39], who demonstrated beneficial effects of selenium nanoparticles on male reproductive performance. In contrast, protamine concentration decreased significantly in all treated groups. Protamine is an essential nuclear protein responsible for chromatin condensation and stabilization of sperm DNA during spermatogenesis; therefore, reduced protamine levels may indicate alteration in the chromatin remodeling process or disturbances in sperm nuclear protein synthesis. This finding suggests that although SeNPs may improve some reproductive biomarkers, excessive exposure may adversely affect specific molecular components. Similar observations were reported by Khalil *et al*. (2023) [40], who highlighted the importance of dose optimization when using SeNPs in

Table 3. Effect of Nano Selenium dosage(µg/kg) on sperm proteins levels in rats (Mean±SE)

Dosages	Acrosin ng/ml	Protamine ng/ml
Control	0.780 ± 0.058 C	6.920 ± 1.084 A
G1	1.360 ± 0.172 C	4.280 ± 0.265 B
G2	0.680 ± 0.115 C	3.160 ± 0.163 BC
G3	4.460 ± 0.911 B	1.660 ± 0.215 C
G4	6.940 ± 0.855 A	1.760 ± 0.307 C
Sign. Level	**	**

Means with different superscript letters differ significantly (P≤0.01) **, (P≤0.05) *).

reproductive studies.

CONCLUSION

Biosynthesized selenium nanoparticles altered reproductive hormonal, sperm protein, and antioxidant profiles in immature male rats. The results suggest that SeNPs influence reproductive and antioxidant functions in a dose-dependent manner. Further investigations are needed to clarify their mechanisms of action and evaluate their long-term reproductive safety.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

REFERENCES

1. Amato A, Tarantino I, Facciorusso A, Binda C, Crinò SF, Fugazza A, et al. Real-life multicentre study of lumen-apposing metal stent for EUS-guided drainage of pancreatic fluid collections. Gut. 2022;71(6):1050-1052.
2. Bhattacharya D, Aronsohn A, Price J, Lo Re V, Heald J, Demisashi G, et al. Hepatitis C Guidance 2023 Update: American Association for the Study of Liver Diseases–Infectious Diseases Society of America Recommendations for Testing, Managing, and Treating Hepatitis C Virus Infection. Clin Infect Dis. 2023.
3. Li L, Lin W, Wang Z, Huang R, Xia H, Li Z, et al. Hormone Regulation in Testicular Development and Function. Int J Mol Sci. 2024;25(11):5805.
4. Li Y, Li X, Cournoyer P, Choudhuri S, Guo L, Chen S. Comparing the cannabidiol-induced transcriptomic profiles in human and mouse Sertoli cells. Toxicology. 2025;512:154068.
5. Lei T, Yang Y, Yang W-X. Luteinizing Hormone Regulates Testosterone Production, Leydig Cell Proliferation, Differentiation, and Circadian Rhythm During Spermatogenesis. Int J Mol Sci. 2025;26(8):3548.
6. Oduwole OO, Huhtaniemi IT, Misrahi M. The Roles of Luteinizing Hormone, Follicle-Stimulating Hormone and Testosterone in Spermatogenesis and Folliculogenesis Revisited. Int J Mol Sci. 2021;22(23):12735.
7. Kaltsas A, Kyrgiafini M-A, Mamuris Z, Chrisofos M, Sofikitis N. Spermatogenesis Beyond DNA: Integrated RNA Control of the Epitranscriptome and Three-Dimensional Genome Architecture. Curr Issues Mol Biol. 2026;48(1):123.
8. Barchielli G, Capperucci A, Tanini D. The Role of Selenium in Pathologies: An Updated Review. Antioxidants. 2022;11(2):251.
9. Qi Z, Duan A, Ng K. Selenoproteins in Health. Molecules. 2023;29(1):136.
10. Bouhadana D, Godin Pagé M-H, Montjean D, Bélanger M-C, Benkhalifa M, Miron P, et al. The Role of Antioxidants in Male Fertility: A Comprehensive Review of Mechanisms and Clinical Applications. Antioxidants. 2025;14(8):1013.
11. Quan R, Wu X, Zhang J, Liu R, Han J, Liu D. Extraction, purification, biological effects and applications of acrosin: a review. Frontiers in Cellular and Infection Microbiology. 2025;15.
12. Kuske JX, Trainor BC. Mean Girls: Social Stress Models for Female Rodents. Current Topics in Behavioral Neurosciences: Springer International Publishing; 2021. p. 95-124. http://dx.doi.org/10.1007/7854_2021_247
13. Hua R, Xue R, Liu Y, Li Y, Sha X, Li K, et al. ACROSIN deficiency causes total fertilization failure in humans by preventing the sperm from penetrating the zona pellucida. Hum Reprod. 2023;38(6):1213-1223.
14. Arévalo L, Merges GE, Schneider S, Oben FE, Neumann IS, Schorle H. Loss of the cleaved-protamine 2 domain leads to incomplete histone-to-protamine exchange and infertility in mice. PLoS Genet. 2022;18(6):e1010272.
15. Kumarasamy RV, Natarajan PM, Umapathy VR, Roy JR, Mironescu M, Palanisamy CP. Clinical applications and therapeutic potentials of advanced nanoparticles: a comprehensive review on completed human clinical trials. Frontiers in Nanotechnology. 2024;6.
16. Murray AM, Swingle KL, Mitchell MJ. Engineering Nanoparticles for Gynecologic Cancer Therapy. ACS Nano. 2025;19(34):30758-30785.
17. Khurana A, Allawadhi P, Singh V, Khurana I, Yadav P, Sathua KB, et al. Antimicrobial and anti-viral effects of selenium nanoparticles and selenoprotein based strategies: COVID-19 and beyond. J Drug Deliv Sci Technol. 2023;86:104663.
18. Pan Z, Huang J, Hu T, Zhang Y, Zhang L, Zhang J, et al. Protective Effects of Selenium Nanoparticles against Bisphenol A-Induced Toxicity in Porcine Intestinal Epithelial Cells. Int J Mol Sci. 2023;24(8):7242.
19. Wadhwani S, Gorain M, Banerjee P, Shedbalkar U, Singh R, Kundu G, et al. Green synthesis of selenium nanoparticles using Acinetobacte sp. SW30: optimization, characterization and its anticancer activity in breast cancer cells. International Journal of Nanomedicine. 2017;Volume 12:6841-6855.
20. Zhang Y, Qin X, Wan Z, Deng C, Deng T. Selenium Nanoparticles: Biomedical Application Advances and New Stomatology Pathways. International Journal of Nanomedicine. 2025;Volume 20:16027-16053.
21. Egeberg M. 10. The European Commission. European Union Politics: Oxford University Press; 2022. p. 141-154.
22. Szczyglewska P, Feliczak-Guzik A, Nowak I. Nanotechnology–General Aspects: A Chemical Reduction Approach to the Synthesis of Nanoparticles. Molecules. 2023;28(13):4932.
23. Vishakha V, Abdel-Mohsen AM, Jancar J. Green Synthesis and the Stabilization of Selenium Nanoparticles Using Carboxymethyl Starch. NANOCON Conference Proeedings: TANGER Ltd.; 2020. p. 433-439.
24. Al-ghareebaw AM, Al-Okaily BN, Ibrahim OMS, Mohammed AD. Role of Olive leaves Zinc Oxide Nanoparticles in Alleviating the Molecular and Histological Changes of Kidney in Female Goats-Induced by Gentamicin (Part III). The Iraqi Journal of Veterinary Medicine. 2020;44((E0)):14-20.
25. Ahirwar R, Bhattacharya A, Kumar S. Unveiling the underpinnings of various non-conventional ELISA variants: a review article. Expert Rev Mol Diagn. 2022;22(7):761-774.
26. Pao CV, Taiping H. Numerical Methods for Coupled Systems of Quasilinear Elliptic Equations with Nonlinear Boundary Conditions. Journal of Applied Analysis and Computation. 2016;6(2):543-581.
27. Srivastava N, Mukhopadhyay M. Biosynthesis and Structural Characterization of Selenium Nanoparticles Using Gliocladium roseum. J Cluster Sci. 2015;26(5):1473-1482.
28. Kazemi M, Akbari A, Sabouri Z, Soleimanpour S, Zarrinfar H, Khatami M, et al. Green synthesis of colloidal selenium

nanoparticles in starch solutions and investigation of their photocatalytic, antimicrobial, and cytotoxicity effects. Bioprocess and Biosystems Engineering. 2021;44(6):1215-1225.
29. Chandramohan S, Sundar K, Muthukumaran A. Monodispersed spherical shaped selenium nanoparticles (SeNPs) synthesized by Bacillus subtilis and its toxicity evaluation in zebrafish embryos. Materials Research Express. 2018;5(2):025020.
30. Abdallah ABE, El-Ghannam MA, Hasan AA, Mohammad LG, Mesalam NM, Alsayed RM. Selenium Nanoparticles Modulate Steroidogenesis-Related Genes and Improve Ovarian Functions via Regulating Androgen Receptors Expression in Polycystic Ovary Syndrome Rat Model. Biol Trace Elem Res. 2023;201(12):5721-5733.
31. Gan X, Zhang X, E Q, Zhang Q, Ye Y, Cai Y, et al. Nano-selenium attenuates nickel-induced testosterone synthesis disturbance through inhibition of MAPK pathways in Sprague-Dawley rats. Environ Toxicol. 2019;34(8):968-978.
32. Yuan S, Zhang Y, Dong P-Y, Chen Yan Y-M, Liu J, Zhang B-Q, et al. A comprehensive review on potential role of selenium, selenoproteins and selenium nanoparticles in male fertility. Heliyon. 2024;10(15):e34975.
33. Dkhil M, Zrieq R, Al-Quraishy S, Abdel Moneim A. Selenium Nanoparticles Attenuate Oxidative Stress and Testicular Damage in Streptozotocin-Induced Diabetic Rats. Molecules. 2016;21(11):1517.
34. Zečević N, Šarac I, Perović M, Zeković M, Stojsavljević A. Impact of selenium and selenoproteins on idiopathic male infertility: a comprehensive review. Frontiers in Nutrition. 2026;12.
35. Boostani A, Sadeghi AA, Mousavi SN, Chamani M, Kashan N. Effects of organic, inorganic, and nano-Se on growth performance, antioxidant capacity, cellular and humoral immune responses in broiler chickens exposed to oxidative stress. Livestock Science. 2015;178:330-336.
36. Shi L, Yue W, Zhang C, Ren Y, Zhu X, Wang Q, et al. Effects of maternal and dietary selenium (Se-enriched yeast) on oxidative status in testis and apoptosis of germ cells during spermatogenesis of their offspring in goats. Anim Reprod Sci. 2010;119(3-4):212-218.
37. Zhou X, Wang Y, Gu Q, Li W. Effects of different dietary selenium sources (selenium nanoparticle and selenomethionine) on growth performance, muscle composition and glutathione peroxidase enzyme activity of crucian carp (Carassius auratus gibelio). Aquaculture. 2009;291(1-2):78-81.
38. Zhang F, Li X, Wei Y. Selenium and Selenoproteins in Health. Biomolecules. 2023;13(5):799.
39. Horky P, Urbankova L, Bano I, Kopec T, Nevrkla P, Pribilova M, et al. Selenium Nanoparticles as Potential Antioxidants to Improve Semen Quality in Boars. Animals. 2023;13(15):2460.
40. Khalil I, Yehye WA, Etxeberria AE, Alhadi AA, Dezfooli SM, Julkapli NBM, et al. Nanoantioxidants: Recent Trends in Antioxidant Delivery Applications. Antioxidants. 2019;9(1):24.