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Dietary Organic Selenium Supplementation Improved Some Histo-Morphological Features and Stimulated G1 Cell Cycle Progression in Testis of Pre-Pubertal Male Goat

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Key words
Selenium, Testicular development, Testosterone, Proliferation, Cyclin, Goat

ABSTRACT

Selenium (Se) nutrition improves testicular growth in young ruminants. We speculated that Se supplementation would modulate proliferative gene expression and improve morphological characteristics in testes, which eventually result in pre-pubertal testicular growth. To test this hypothesis, twenty young male goats, approximately 3 months old and 11 kg body weight, were divided into control (CN, n=10) and Se-Yeast (SY, n=10) groups and offered same plane of nutrition without (CN) or with (SY) Se supplementation at the dose rate of 0.3 mg.kg^{-1} diet for 10 weeks. Results revealed that the intact weight and organ index of testes significantly increased ($P < 0.05$) by approximately 16 % and 8.8 %, respectively in SY goats compared with control. Histo-morphological evaluation showed that germinal epithelium height (21.80 ± 0.53 µm in SY vs 18.84 ± 0.12 µm in CN) was significantly higher ($P < 0.05$) by 15.7 % in SY compared with CN. Simultaneously, the area and diameter of seminiferous tubule (ST) increased ($P < 0.05$) by 8.6 % and 10.6 %, respectively, in testis of Se-fed (SY) goats compared with control. However, the ST lumen diameter showed no significant difference ($P > 0.05$) between the groups. Serum testosterone level significantly increased ($P < 0.05$) by approximately 30 % in SY than in CN. Concurrent with changes in testicular organ index (TOI) and histomorphometry, the expression levels of *cyclin D1* and *cyclin-dependant kinase-4 (CDK-4)* increased ($P < 0.05$) by 0.71-fold and 0.38-fold, respectively, in testicular tissues of SY compared with CN. However, *CDK-6* expression was not different ($P > 0.05$) between the groups. The findings of the present study demonstrate that dietary Se supplementation improved organ index and some histological features, increased serum testosterone concentrations accompanied with early G1 phase cell cycle progression in testis of goat.

INTRODUCTION

Selenium (Se) is a vital micronutrient, which is very well-known for its role in antioxidant defence mechanism and reproduction (Samo *et al*., 2020). Its anti-oxidative defensive role is based on a seleno-enzymes family, the glutathione peroxidases (GSH-Px) which ensure the cellular membranes and organelles protection by detoxifying the highly hazardous free radicals such as super-oxides, peroxides and hydroxyl ions formed during oxidative metabolism (Bano *et al*., 2023; Shah *et al*., 2022). Plants usually uptake inorganic Se i.e., selenate and selenite and store them as organic Se i.e., seleno-methionine and seleno-cysteine that is utilized as feed source for animals, the Se availability thus varies with soil-plant-animal interaction (Yang *et al*., 2022). It is therefore important in the regions of Se-deficient soils that supplemental Se form be added in the food to ensure optimal Se intake by the animal. Se-yeast, a synthetic organic Se, is preferably

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used over inorganic Se supplements because of its greater tissue retention and biological role in ruminants (Samo *et al*., 2018; Yang *et al*., 2022).

Growth and development of male reproductive organs especially the testicular development is critical for efficient reproductive performance of an animal (Ashraf *et al*., 2022). Se is crucial for spermatogenic and steroidogenic differentiation and Sertoli cell maturation and multiplication in the testis (Ramírez-Acosta *et al*., 2022). Seleno-enzymes, GSH-Px1-4 provide protection against oxidants thus help in sperm maturation, additionally, seleno-protein-P and GSH-Px4 (phospholipid hydro-peroxide GSH-Px or PHGSH-Px) regulate functions in germ cells and spermatogenesis (Ramírez-Acosta *et al*., 2022; Jamali *et al*., 2019). Moreover GSH-Px4 is termed as male fertility factor and the mitochondrial (mGSH-Px4) and sperm nuclear (snGSH-Px4) act as structural components of mature sperm (Maciejewski *et al*., 2022; Ramírez-Acosta *et al*., 2022). Therefore, Se deficiency in pre-pubertal nutrition of young ruminants leads to retard testicular development, impaired spermatogenesis along with qualitative and morphological sperm abnormalities (Xu *et al*., 2023). Testicular development is linked with early attainment of puberty. Puberty or the sexual maturity is a complex biological process in which an animal undergoes the gradual physical, physiological, and behavioural changes to attain the reproductive competence (Ashraf *et al*., 2022). Early attainment of puberty is one of the important strategies practiced by livestock farmers to maximize the reproductive potential of an animal (Mojapelo and Lehloenya, 2019). Pre-pubertal Se supplementation to two months old Saanen male goat kids enhanced testicular development and shortened onset of puberty from 6 months to approximately 5 months of age (Mojapelo and Lehloenya, 2019). In addition, Se nutrition increased gonado-somatic index (GSI) and improved histo-architecture such as seminiferous tubules (ST) number and luminal epithelium size in ST in testes accompanied with elevated serum testosterone levels in young goat kids (Bano *et al*., 2019). Moreover, the influence of Se on testicular development starts during foetal development and it is reported that dam fed Se in breeding season and during pregnancy period affects the expression of genes related to steroidogenesis and spermatogenesis in testes of neonatal calves and kids. These data suggest that Se nutrition in early life of young male animals stimulate neuroendocrine hormone axis, enhance body growth with simultaneous testicular development and growth which eventually culminate into early onset of puberty.

Testicular growth is a sophisticated biochemical process in which cellular homeostasis is maintained by proliferation, differentiation, maturation, and apoptosis of both germ and somatic cells (Ashraf *et al*., 2022). Se-induced histological improvement consequently increases parenchymal tissue mass in testes, which may be attributed to proliferative effects of Se (Xu *et al*., 2023). Cellular proliferation consists of cell cycle in which a cell undergoes four distinct sequential phases viz., G1, S, G2 and M, strictly regulated by proteins including cyclins, cyclin dependent kinases (CDKs) and CDK inhibiters (Ding *et al*., 2020). We speculated that Se-induced alteration in parenchymal tissue might have been associated with modulation in cell cycle proteins expression. The present study was, therefore, designed to investigate the effects of dietary Se supplementation on testicular histo-morphology, serum testosterone and early G1 related cell cycle gene expression.

MATERIALS AND METHODS

Animal assortment and feeding management

Twenty young male goats, approximately 3 to 3.5 months of age and 11-13 kg body weight (BW) were purchased and brought at livestock experimental station, SAU Tandojam. After 2-weeks adaptation, the animals were randomly divided into control (CN, n=10) and Se-Yeast (SY, n=10) groups and offered same plane of nutrition without (CN) or with Se supplementation (SY). Hence were kept animals' diets controlled during the experiment. Se was added from organic source i.e., Se-yeast at the dose rate of 0.3 mg Se kg^{-1} diet. The source of Se was (Selemax™, Lencois, Paulista, São Paulo, Biorigin®, Brazil) and mixed in the diet. The diet consisting of concentrate to roughage ratio (35:65, Table I), was fed twice a day, while water was always available before the animals. The background Se level in control diet was 0.037 mg Se kg^{-1} diet, quantified through inductively coupled plasma-mass spectrometry (ICP-OES Optima 2100-DV, Perkin Elmer) as illustrated by Samo *et al*. (2020). The trial lasted for 10 weeks.

Table I. Ingredients and nutrient level in diet fed to experimental animals.

Ingredients (% of DM)		Nutrient level	
Berseem	65	DM (%)	85.41
Corn	25.6	Crude protein (% of DM)	16.74
Soybean meal	7.4	Crude fat (% of DM)	3.81
Lime stone	0.5	Crude fiber (% of DM)	6.85
Calcium phosphate dibasic	0.8	Crude ash (% of DM)	8.11
Salt	0.4	ME (MJ/kg of DM)	10.88
Mineral Premix[1]	0.4		

DM, dry matter; ME, metabolizable energy. [1]Per kg of premix = Vitamin A 6 000U; Vitamin D2 500U; Vitamin E 80 mg; Cu 6.25 mg; Fe 62.5 mg; Zn 62.5 mg; Mn 50 mg; I 0.125 mg; Co 0.125 mg; Mo 0.125 mg.

Blood sample collection and hormonal assay

On the day of trial completion, the blood sample (5 mL) were collected from the jugular vein of each animal into a sterile vacutainer. Serum was obtained by centrifuging at 3000 g for 10 min and stored at -20 °C for hormonal analysis. Testosterone concentration was measured using an ELISA assay kit (Blue Gene, Shanghai, China) following manufacturer directions.

Slaughter and tissue sample collection

Following live BW record, the goats were slaughtered via Halal Method. The testicles along with epididymis were hygienically detached. Immediately, the epididymis was gently separated from each testis and weighed, and then two testicular tissues sampling was performed. First, a few tissue pieces (approximately 0.5 cm^2) from left testis were taken and fixed in 4 % paraformaldehyde (PFA) solution for histological evaluation. Another tissue sample from right testis was taken in Eppendorf tube and stored frozen for RNA extraction and PCR analysis.

Organ index and histology of testis

The testicular-organ index (TOI, g/kg) was determined as a ratio of testicular weight to live final BW (Bano *et al*., 2019). For histology, the overnight PFA-fixed testicular tissue was rinsed (distilled water), dehydrated (graded alcohol), cleared (xylene) and then embedded in paraffin. Thin sections (4-6 µm thick) were cut, mounted onto glass slides, and stained by the standard hematoxylin and eosin (H and E) procedure. At least, five slides from each group were observed under microscope and microphotographs were taken using digital camera (Tuscen CMOS Camera: IS500, resolution: 5.0 megapixels). The protocol adapted was followed to perform histomorphometry. The cross-sectional area of seminiferous tubule (ST), diameters of ST and ST lumen, and the germinal epithelium height were measured using computerized image analysis programme (Image-Pro Plus, IPP, Ver. 6, Media Cybernetic Inc). The histological assemblies of the testes were noticed by using light microscope using 10x magnifications. Six replicate measurements for each of the 10 goats per dietary treatment were taken and averaged for statistical analysis. The SFT diameter, SFT area, germinal epithelium height and SFT lumen diameter was measured by using microscope model: image J software at (Bar scale= 50µm). The snapshots of each sample were taken for better estimation of the results.

Total RNA isolation and real-time qRT-PCR

An acid guanidinium thiocyanate-phenol-chloroform (GTC) technique was used to extract total RNA from a sample of testicular tissue (Malhi *et al*., 2013). The concentration of RNA was determined using the nano drop spectrometer at 260 and 280 nm. All of the samples showed good RNA purity, as evidenced by the absorbance ratios between 1.72 and 1.84. Real-time PCR was performed in a 20 ul total volume using a mixture of forward and reverse primers (Table II), along with 1xiQ SYBR Green supermix (Bio-Rad Laboratories, Inc., Hercules, CA), a certain volume of sterile water and one cDNA template. A first cycle of 30 s at 95 °C was employed to denaturize the cDNA. After that, 40 PCR cycles were conducted, which included primer annealing and extension at 55 °C for 30 sec and denaturation at 95 °C for 10 sec. The amplification efficiencies of all primers were determined using standard dilution series before PCRs were conducted for experimental materials. Every sample was examined three times, and a melt analysis was done following the PCR analysis. GAPDH was used to normalize gene expression (ΔCt = Ct target – Ct GAPDH). The method $2^{-\Delta\Delta Ct}$ was utilized to calculate the relative expression values, following the instructions provided by (Malhi *et al*., 2013).

Statistical analysis

Data were analysed by Student's t test using statistical software SPSS25.0 (Stat Soft, Tulsa, OK, USA). Results were displayed as Means ± SEM and the differences were considered significant at $P < 0.05$.

Table II. Primers used in quantitative real-time PCR analysis.

Gene	Primer sequence 5 to 3	Accession number	Size (bp)
CCND1	GGTCCTGGTGAACAAACTC TTGCGGATGATCTGCTT	EU525165.1	114
CDK4	TGAGCATCCCAGTGTTGT CCTTGTCCAGATACTTCCT	NM-001127269.1	122
CDK6	AGAGTGATTGCAGCTTTATGTCCA TGCCCAGGTTGCTCACTTC	GAAI01006376.1	158
GAPDH	TTGTCTCCTGCGACTTCA CCACCACCCTGTTACTGTT	HM043737.1	135

CCND1, cyclin D1; CDK4, cyclin-dependent kinase 4; CDK6, cyclin-dependent kinase 6; GAPDH, Glyceraldehyde 3-phosphate dehydrogenase; bp, base pairs. The first primer listed for each gene is the forward primer and the second primer is reverse primer.

RESULTS

Organ index and histo-morphology of testis

The effect of diet without and with selenium (Se) supplementation on final live body weight (BW), and intact weight and organ index of testis in goat is presented in Table III. Final BW (17.22 ± 0.36 kg in SY vs 16.09 ± 0.31 kg in CN) and intact testicular weight (10.81 ± 0.40 g in SY vs 9.32 ± 0.31 g in CN) significantly increased ($P < 0.05$) in SY compared with CN. Simultaneously, the testicular organ index (TOI) significantly increased ($P < 0.05$) by approximately 8.8 % in SY compared with CN.

Table III. Effect of dietary selenium yeast supplementation on final body weight, and intact weight and organ index of testis in goat.

Items	Groups		P-value
	CN	SY	
Final BW (kg)	16.09 ± 0.31	17.22 ± 0.36*	0.0382
Testicular weight (g)	9.32 ± 0.31	10.81 ± 0.40*	0.0129
TOI (g/kg)	0.57 ± 0.01	0.62 ± 0.01*	0.0104

BW, body weight; TOI, Testicular-organ Index. Goats were fed diet without (CN) or with (SY) selenium at the rate of 0.3 mg kg^{-1} diet. Values (mean ± SE) differ at $P < 0.05$ between two groups.

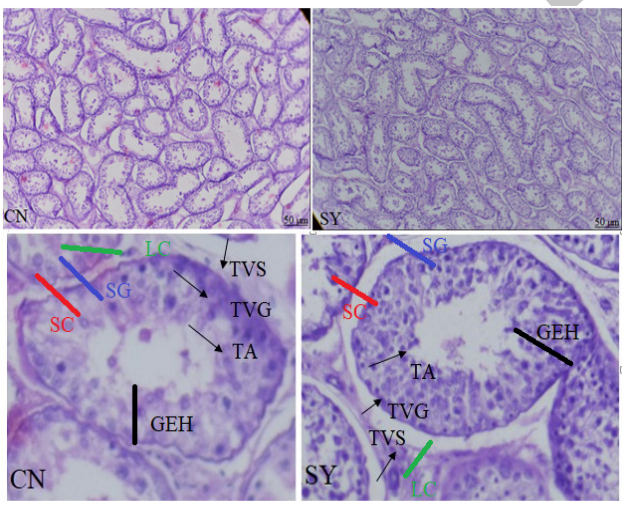

Fig. 1. Microphotographs showing histological structures of goat testis. Germinal epithelium height (black line), Sertoli Cells (SC, red line), Spermatogonia Sells (SG, blue line) and leydig cell (LC, green line). Additionally, inner most layer tunica vasculosa (green line, TVS), inner layer tunica vaginalis (red line, TVG) and middle layer dense tunica albuginea (blue line, TA). Goats were fed diet without (CN) or with (SY) selenium at the rate of 0.3 mg kg^{-1} diet. (Bar scale = 50µm).

Figure 1 shows the histological features of goat testis fed Se-supplemented (SY) or non-supplemented diets (CN). In general, no abnormality or degeneration was observed in testes of treated group, suggesting that the background of dietary Se levels were sufficient to avoid testicular tissue damage. Histologically, the testis was found to occupy three coverings in its germinal epithelium that comprises various epithelial layer where the different cells are located, the tunica vasculosa (innermost layer) contains leydig cells that is involve in producing testosterone hormone, the second and third epithelial layers are dense tunica albuginea (middle layer) and the tunica vaginalis (inner layer) that contain Sertoli cells showing slightly light bright cells however, spermatogonia cells showing highly bright during pre-pubertal testicular growth as shown in Figure 1. The inner capsulated portion was employed by the seminiferous tubules (ST) containing of different shapes like elongated, straight, comma and elliptical. Denser ST and higher Sertoli cells count per slide were observed in testicular parenchyma of SY compared with CN goats. Histomorphometric analysis (Table IV) showed that germinal epithelium height (21.80 ± 0.53 µm in SY vs 18.84 ± 0.12 µm in CN) was significantly higher ($P < 0.05$) by 15.7 % in SY compared with CN. Simultaneously, ST area (32560 ± 604.1 in SY v 29990 ± 363.7 in CN, $P < 0.05$) and ST diameter (76.33 ± 1.8 in SY v 69.01 in CN, $P < 0.05$) increased by 8.6 % and 10.6 %, respectively, in testis of Se-fed (SY) goats compared with control. However, the ST lumen diameter (28.13 ± 0.71 µm in SY vs 33.54 ± 0.34 µm in CN) showed no significant difference ($P > 0.05$) between the groups (Table IV).

Table IV. Effect of dietary organic selenium supplementation on testicular hostomorphometry in goat.

Items	Groups		P-value
	CN	SY	
GEH (µm)	18.84±0.12	21.80±0.53*	0.004
ST diameter (µm)	69.01±1.38	76.33±1.81*	0.012
ST lumen diameter (µm)	33.54±0.34	28.13±0.71	0.19
ST area ($µm^2$)	29990±363.7	32560±604.1*	0.007

GEH, Germinal epithelial height; ST, Seminiferous tubules; Goats were fed diet without (CN) or with (SY) selenium at the rate of 0.3 mg kg^{-1} diet. Values (mean ± SE) differ at $P < 0.05$ between two groups.

Serum testosterone level

The effect of diet without and with Se addition on serum testosterone level in goat is depicted in Figure 2. The serum testosterone level significantly increased by approximately 30 % in goats fed Se supplemented diet

(SY) compared with those fed non-supplemented diet (CN).

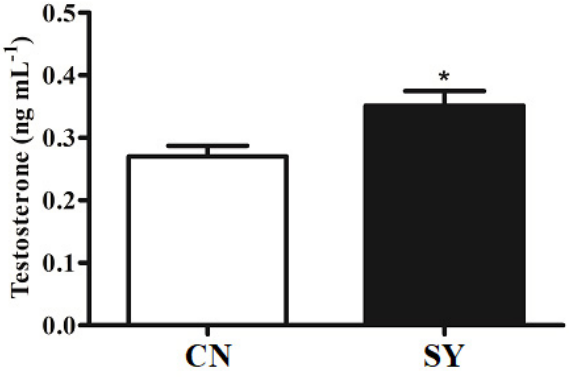

Fig. 2. Effect of dietary organic selenium supplementation on serum testosterone concentration in goat. Goats were fed diet without (CN) or with (SY) selenium at the rate of 0.3 mg kg^{-1} diet for 10 weeks.

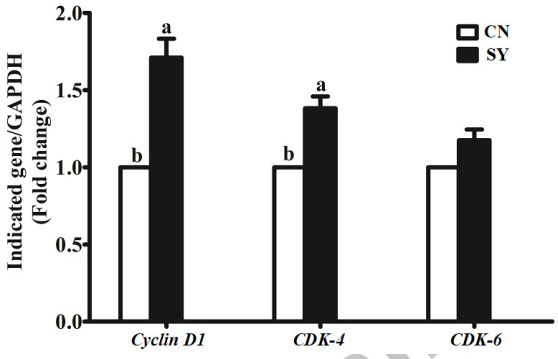

Fig. 3. Effect of dietary organic selenium supplementation on cyclin D1, cyclin-dependant kinase 4 (CDK4), and CDK6 mRNA expression in testicular tissue of goat. Goats were fed diet without (CN) or with (SY) selenium at the rate of 0.3 mg kg^{-1} diet for 10 weeks. Gene expression level was calculated with real-time PCR in comparison with GAPDH rRNA. In both groups different lower-case letters (a-b) are statistically significantly different. Values are mean ± S.E on the bars exhibit the differences between groups with $P < 0.05$.

Proliferative genes expressions in testicular tissue

Concurrent with changes in TOI and histomorphometry, dietary organic Se produced significant effect on the expression of cyclin and cyclin-dependant kinases (CDK) in the testicular tissue (Fig. 3). The expression levels of cyclin D1 and CDK-4 were increased ($P < 0.05$) by 0.71-fold and 0.38-fold, respectively, in testicular tissues of SY compared with CN goats. However, the CDK-6 expression was not different ($P > 0.05$) between the groups.

DISCUSSION

Selenium (Se) addition in the pre-pubertal diet increases the reproductive lifespan of ruminants by reducing the time to attain puberty or sexual maturity. The progressive effects of Se on male puberty involve the stimulation of the neuroendocrine hormone axis, enhanced body growth, and simultaneous testicular development and growth. This study aimed to understand the underlying molecular effects of Se on pre-pubertal testicular growth.

Our results demonstrate that dietary Se supplementation increased final live body weight (BW), intact testicular weight, and testicular organ index (TOI). Consistent with our findings, previous studies reported that feeding Se-supplemented diets in early life increased BW and testicular weight in goats and boars (Bano *et al*., 2019). Increased BW and TOI are important reproductive determinants indicating sexual maturity in animals (Mojapelo and Lehloenya, 2019). Specifically, pre-pubertal Se supplementation to two-month-old Saanen male goat kids enhanced testicular development and shortened the onset of puberty from six months to approximately five months of age (Mojapelo and Lehloenya, 2019). Similarly, boars fed Se-supplemented diets from a pre-pubertal age exhibited greater onset of spermatogenesis and higher proportions of seminiferous tubules (ST) leading to sperm formation compared to non-supplemented boars.

Histological evaluation in this study revealed that Se improved several ST features in the testes. The ST area, ST diameter, and germinal epithelium height increased in Se-fed goats. Additionally, we observed higher Sertoli cells and spermatogonia cell counts per slide in the testicular parenchyma of Se-fed goats. These findings are consistent with previous studies where Se supplementation increased epithelial height and ST diameter in young bucks and boars (Bano *et al*., 2019). Similarly, it was observed an insignificantly higher number of Sertoli cells in the testis of boars in response to Se feeding at 6.2 months of age; however, this response led to a significant increase in Sertoli cells population by 18 months of age. This suggests that Se influences the early development of Sertoli cells, increasing their number in adulthood, where they are crucial for spermatogonia support and spermatid development. Se supplementation also improved ST area, ST diameter, and germinal epithelium thickness in testicular tissues of native Turkish ganders (Bas *et al*., 2023).

Concurrent with improved testicular parameters, serum testosterone concentration increased by 30% in kids fed Se-supplemented diets. Although the kids in both groups remained pre-pubertal until slaughter, the higher

testosterone levels in Se-fed goats suggest accelerated progression towards puberty. Mojapelo and Lehloenya (2019) reported that Se feeding to pre-pubertal male goats resulted in an increase in blood testosterone levels at various pre-pubertal time points, indicating Se-induced testicular development and enhancement of puberty onset under the regulation of the hypothalamo-pituitary-gonadal axis.

The improved TOI observed in this study is likely due to enhanced histo-architecture, such as larger ST area, higher germinal epithelial height, and increased Sertoli cells population, indicating hypertrophic (increased cell size) and hyperplastic (increased cell number) effects. These effects may be attributed to the proliferative impact of Se on both germ and somatic cells in the testicular parenchyma (Wang *et al*., 2023; Shahid *et al*., 2020). In vivo studies have shown that Se supplementation improved histological features and increased epithelial tissue mass in the colon and rumen of young goats (Samo *et al*., 2020; Shahid *et al*., 2020). Dietary Se increased muscle quality in goats (Memon *et al*., 2024) and induced muscle hypertrophy in grass carp (Wang *et al*., 2023). These data demonstrate the hypertrophic or hyperplastic effects of Se on tissues, reflecting Se's role in proliferation.

Cellular proliferation is controlled by the cell cycle, in which a cell grows, replicates its DNA, and divides into two daughter cells. The cell cycle is tightly regulated by specific proteins such as cyclins, cyclin-dependent kinases (CDKs), and CDK inhibitors (Yan *et al*., 2024). Mammalian cells undergo four distinct sequential phases: G1, S, G2, and M, with each phase requiring specific cyclins and their *CDKs* to cross checkpoints. Cyclin D1 and CDK4/6 form a complex that helps cells cross the G1 checkpoint (Soomro *et al*., 2018), promoting the transition from G0/G1 to S phase (Yan *et al*., 2024). In the present study, dietary Se up regulated the mRNA expressions of cyclin D1 and CDK4, suggesting that Se increased the cell proliferation rate by enhancing early G1 cell cycle progression in testicular tissue. Adequate dietary Se is required for normal reproductive health and testicular development in animals. Diets deficient or excessive in Se can induce testicular damage through oxidative stress-mediated modulation of proliferative and apoptotic genes in rats and roosters (Xu *et al*., 2023; Yan *et al*., 2024). Dietary Se induced muscle hypertrophy and enhanced myoblast proliferation through cyclin modulation in juvenile grass carp (Wang *et al*., 2023). Rats fed Se-supplemented diets showed upregulation of proliferative genes, including cyclin *D1* and cyclin *E*, in normal esophageal tissues (Younesian *et al*., 2023).

Comparing our findings with existing literature, it is evident that Se plays a crucial role in reproductive development across various species. Our results are in alignment with studies on goats, boars, and ganders, highlighting the consistency of Se's effects on enhancing testicular growth and accelerating puberty onset. The histological improvements observed in our study, such as increased ST area and germinal epithelium height, corroborate findings in other species, emphasizing Se's role in cellular proliferation and tissue development. The upregulation of cyclin *D1* and *CDK4* expressions in response to Se supplementation further supports the molecular mechanisms underlying Se-induced cell cycle progression, as reported in studies on grass carp and rats.

CONCLUSION

This study provides comprehensive evidence that dietary Se supplementation enhances pre-pubertal testicular growth through improved histological features, increased testosterone levels, and up regulation of cell cycle regulatory genes. These findings align with and extend existing research on the beneficial effects of Se on reproductive development. Further studies are warranted to elucidate the precise molecular mechanisms and optimize Se supplementation strategies for enhancing reproductive health in ruminants.

DECLARATIONS

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IRB approval

The study proposal received approval from Board of Advanced Studies and Research (BASR) under reference number (DAS/2464) in the year 2023.

Ethical approval

The procedures performed in this research such as handling of animals, sampling, and analytical methods, received prior approval in 152nd meeting of Board of advanced studies and research (No. DAS/2464/of 2023), Sindh Agriculture University (SAU) Tandojam.

Statement of conflict of interest

The authors have declared no conflict of interest.

REFERENCES

Ashraf, M., Zeeshan, M., Ahmad, N., Javed, K., Riaz, A. and Riaz, H., 2022. Changes in the testicular histomorphometry and their association with genes expression pattern of testes from birth to puberty in Beetal goat kids. *Small Rumin. Res.*, **210**: 106666. https://doi.org/10.1016/j.smallrumres.2022.106666

Bano, I., Malhi, M.C., Khatri, P., Soomro, S.A., Sajjad, H., Leghari, A., Awais, M., Kandhro, S., Lakho, S.A. and Soomro, M., 2019. Effect of dietary selenium yeast supplementation on morphology and antioxidant status in testes of young goat. *Pakistan J. Zool.*, **51**: 979-988. https://doi.org/10.17582/journal.pjz/2019.51.3.979.988

Bano, I., Malhi, M. and Talpur, H.S., 2023. Effects of dietary selenium yeast supplementation on oxidative biomarkers of the brain and blood in goats. *Biol. Trace Elemen. Res.,* **202**: 3999-4006. https://doi.org/10.1007/s12011-023-03966-9

Baş, H., Taşkesen, H.O., Boz, M.A., Sarıca, M., Erensoy, K., Dotas, V. and Symeon, G., 2023. The effects of varying combinations of dietary selenium, vitamin E, and zinc supplements on antioxidant enzyme activity, and developmental and histological traits in testicular tissues of 1-year-old native Turkish ganders. *Sustainability*, **15**: 12245. https://doi.org/10.3390/su151612245

Ding, L., Cao, J., Lin, W., Chen, H., Xiong, X., Ao, H. and Cui, Q., 2020. The roles of cyclin-dependent kinases in cell-cycle progression and therapeutic strategies in human breast cancer. *Int. J. mol. Sci.*, **21**: 1960. https://doi.org/10.3390/ijms21061960

Jamali, N.U., Kaka, A.K., Khatri, P.K., Malhi, M.M., Naeem, M.N., Memon, A.A. and Kalhoro, D.H., 2019. *Effect of* in vitro *selenium addition to the semen extender on the spermatozoa characteristics before and after freezing in Kundhi buffalo bull and* in vivo *fertility rate*. https://doi.org/10.17582/journal.pjz/2019.51.1.317.323

Maciejewski, R., Radzikowska-Büchner, E., Flieger, W., Kulczycka, K., Baj, J., Forma, A. and Flieger, J., 2022. An overview of essential microelements and common metallic nanoparticles and their effects on male fertility. *Int. J. environ. Res. Publ. Hlth*., **19**: 11066. https://doi.org/10.3390/ijerph191711066

Malhi, M., Gui, H., Yao, L., Aschenbach, J.R., Gäbel, G. and Shen, Z., 2013. Increased papillae growth and enhanced short-chain fatty acid absorption in the rumen of goats are associated with transient increases in cyclin D1 expression after ruminal butyrate infusion. *J. Dairy Sci*., **96**: 7603-7616. https://doi.org/10.3168/jds.2013-6700

Memon, M.A., Malhi, M., Kachiwal, A.B. and Barham, G.S., 2024. Impact of dietary supranutritional selenium on improving the meat quality of goats: *Futur. Biotech*, pp. 48-52. https://doi.org/10.54393/fbt.v4i01.91

Mojapelo, M.M. and Lehloenya, K.C., 2019. Effect of selenium supplementation on attainment of puberty in Saanen male goat kids. *Theriogenology*, **138**: 9-15. https://doi.org/10.1016/j.theriogenology.2019.06.044

Ramírez-Acosta, S., Selma-Royo, M., Collado, M.C., Navarro-Roldán, F., Abril, N. and García-Barrera, T., 2022. Selenium supplementation influences mice testicular selenoproteins driven by gut microbiota. *Sci. Rep.,* **12**: 4218. https://doi.org/10.1038/s41598-022-08121-3

Samo, S.P., Malhi, M., Kachiwal, A.B., Gadahi, J.A., Parveen, F., Kalhoro, N.H. and Lei, Y., 2020. Supra-nutritional selenium level minimizes high concentrate diet-induced epithelial injury by alleviating oxidative stress and apoptosis in colon of goat. *BMC Vet. Res.*, **16**: 1-10. https://doi.org/10.1186/s12917-020-02653-4

Samo, S.P., Malhi, M., Gadahi, J., Lei, Y., Kaciwal, A.B. and Soomro, S.A., 2018. Effect of organic selenium supplementation in diet on gastrointestinal tract performance and meat quality of goat. *Pakistan J. Zool.,* **50**: 995-1003. https://doi.org/10.17582/journal.pjz/2018.50.3.995.1003

Shah, T., Malhi, M., Kachiwal, A.B., Bhutto, B., Shah, Q.A., Lei, Y. and Gui, H., 2022. Ameliorative effects of supra-nutritional selenium on TLR-4-NF-kB-TNF-α-mediated hepatic oxidative injury and inflammation in goats fed high concentrate diet. *Fd. Sci. Nutr.,* **10**: 3842-3854. https://doi.org/10.1002/fsn3.2980

Shahid, A.B., Malhi, M., Soomro, S.A., Shah, M.G., Kalhoro, N.H., Kaka, A. and Sanjrani, M.N., 2020. Influence of dietary selenium yeast supplementation on fermentation pattern, papillae morphology and antioxidant status in rumen of goat. *Pakistan J. Zool.,* **52**: 565-571. https://doi.org/10.17582/journal.pjz/20190205120240

Soomro, J., Lu, Z., Ui, H.B., Zhang, B. and Shen, Z., 2018. Synchronous and time-dependent expression of cyclin, cyclin- dependent kinases, and apoptotic genes in the rumen epithelia of butyrate- infused goats. *Front. Physiol.*, **9**: 496. https://doi.

org/10.3389/fphys.2018.00496

Wang, Y., Jiang, W.D., Wu, P., Liu, Y., Jin, X.W., Jiang, J. and Zhou, X.Q., 2023. Dietary selenium promoted muscle growth (myofiber hypertrophy rather than hyperplasia) by reducing endoplasmic reticulum stress in juvenile grass carp (*Ctenopharyngodon idella*). *Aquacult. Res.*, **575**: 739796. https://doi.org/10.1016/j.aquaculture.2023.739796

Xu, Z.J., Liu, M., Niu, Q.J., Huang, Y.X., Zhao, L., Lei, X.G. and Sun, L.H., 2023. Both selenium deficiency and excess impair male reproductive system via inducing oxidative stress-activated PI3K/AKT-mediated apoptosis and cell proliferation signalling in testis of mice. *Free Radic. Biol. Med.*, pp. 15-22. https://doi.org/10.1016/j.freeradbiomed.2023.01.024

Yan, Y.Q., Liu, M., Xu, Z.J., Xu, Z.J., Huang, Y.X., Li, X.M. and Sun, L.H., 2024. Optimum doses and forms of selenium maintaining reproductive health via regulating homeostasis of gut microbiota and testicular redox, inflammation, cell proliferation, and apoptosis in roosters. *J. Nutr.*, **154**: 369-380. https://doi.org/10.1016/j.tjnut.2023.12.021

Yang, H., Yang, X., Ning, Z., Kwon, S.Y., Li, M.L., Tack, F.M. and Yin, R., 2022. The beneficial and hazardous effects of selenium on the health of the soil-plant-human system: An overview. *J. Hazard. Mater.*, **422**: 126876. https://doi.org/10.1016/j.jhazmat.2021.126876

Younesian, O., Arabi, S.M. and Jafari, S.M., 2023. Long-term excessive selenium supplementation affects gene expression in esophageal tissue of rats. *Biol. Trace Elem. Res.*, **201**: 3387–3394. https://doi.org/10.1007/s12011-022-03413-1