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Nano-Selenium Effect on Sexual Hormones and Enzymatic Activity in Relation to Sexual Puberty in NZW Rabbits

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ABSTRACT

The present study aims at investigating the impact of selenium nano form (Nano-Se) and sodium selenite (SSe) on sexual hormones and enzymatic activity of New Zealand White (NZW) rabbits at puberty. Seventy-two NZW rabbits (6 wk-old) were distributed into three equal groups of 24 animals (12 females and 12 males). The 1st group (control) was injected once a week with 4 ml distilled-water. The 2nd and the 3rd groups were injected with 4 ml of a solution containing 90 µg of Nano-Se and SSe /kg live body weight, respectively, once weekly for eight weeks.Testicles from male rabbits, and ovaries from female rabbits and serum from both males and females were obtained at the end of the experiment. Results showed that Nano-Se increased (P<0.01) ovarian and serum activities of lipase, amylase, and albumin concentration, and decreased (P<0.01) in alkaline phosphatase (ALP) activity. In contrast, Nano-Se decreased ovarian tissue activity of creatine phosphokinase (CPK) and serum alpha-fetoprotein (AFP) and increased (P<0.01) serum ALP in comparison with the control. Nano-Se increased (P<0.01) testicular amylase, lipase, AFP, serum CPK, and albumin. The opposite trend was observed in SSe treatment with a decrease (P<0.01) of serum ALP activity and albumin concentration. SSe increased (P<0.01) ovarian amylase, CPK, ALP activities, albumin, and serum ALP. However, SSe decreased (P<0.01) blood amylase, lipase (also in ovarian tissue), CPK, and AFP weighed to control. Estradiol-17β, progesterone, and testosterone levels increased (P<0.01) due to nano-Se treatment. . In Conclusion, nano-Se treatment significantly increased lipase, amylase and, CPK activities with a significant decrease in ALP and AFP activities, besides enhancement of male and female's sexual hormones, which indicates of pubertal NZW rabbits

INTRODUCTION

Rabbits, as farm animals potentially,suffer animal protein supply problems in developing countries [1]. Speeding up puberty and highly initial fecundity is a suitable basis for economic selection. Reaching puberty is affected by environmental factors (i.e., temperature & photoperiod), animals' age, breed, and heterosis [2]. Rabbits' ovarian follicle development occurs postnatal, with primordial follicle assembly presumably completed between 2 and 4 weeks of age [3]. Females reach puberty at around 10-14 weeks of age [4,5]. At the same time, the first manifestations of male sexual behavior appear at days 60 to 70 when the rabbit makes its first attempts at riding [6]. Sufficient nutrition has strong effects on developing reproductive and puberty traits in all animal species [7]. Young animals differ considerably when they reach puberty, as it is modulated by nutrition and management systems [8]. Selenium (Se) is indispensable to the antioxidant defence systems as a cornerstone of the selenoenzyme (glutathione peroxidase). Se is a part of selenocysteine proteins and works to prevent body tissues from oxidative damage [9]. During insufficient Se intake, the testes had the priority in Se supply over other organs, leading to assume the inclusion of Se in the biosynthesis

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of testosterone [10,11], since the testicular tissue contains high concentrations of Se, which is essential for the formation and normal development of spermatozoa [12,13]. In females, Se exists in various tissues, including ovaries (Behne, 1988)[14]. Investigations of Se bioaccumulation in ovarian tissue pointed to Se localization in the granulosa cell layer of large, healthy follicles [15]. Some enzymes and constituents are related to the growth of the ovarian follicles and maturation. Lipase, amylase, and CPK concentrations increase as the follicles mature, while ALP and AFP concentrations decrease with increasing follicular size [16]. Earlier, McNatty [17] reported that increased follicular P4 and androgens are associated with follicular atrophy, and the relation between increased P4 and ALP activity could be a useful metabolic indicator of follicular atresia [18]. So, this work aims at studying the impacts of selenium in nano form versus sodium selenite on enzymatic activities and sexual hormonal levels in serum and ovarian/testicular extracts of NZW rabbits for prediction the rabbits puberty.

MATERIALS and METHODS

The experimental work of the present study was conducted in the Rabbit's Research and Breeding Farm, the Nuclear Research Center, the Egyptian Atomic Energy Authority (EAEA). The EAEA Publishing Committee approved this work under No.191/2020. The authors followed the EU Directive 2010/63/EU guideline for rabbits' handling.

Experimental design:

Total seventy-two (36 male & 36 female) weaned NZW rabbits, with 6 wk-old and approximately equal body weight (665.5 ± 3.7 g for males and 642.3 ± 3.7 g for females) were used in the current work. Each sex was randomly divided into three groups (12/each); group one (control) was injected (i.m.) once a week with 4ml distilled water. Similarly, groups 2 and 3 were injected with 4ml of nano-Se and SSe solution, respectively. The solution was adjusted to provide 90 µg nano-Se or SSe (about 195.6 µg SSe used to get 90 µg Se) /kg live body weight. The experiment lasted for eight weeks.

Animal feeding:

Basal diet constituents and chemical analyses as presented in Table (1) were used according to NRC [19] requirments . Samples from the basal diet were pulverized by hammer mill with a 1-mm pore size mesh then analyzed in triplicate for their content of DM, ash, CP (N × 6.25), crude fiber (CF), ether extract (EE) as stated by AOAC [20]. Nitrogen-free extract (NFE) was calculated by the difference.

Table (1): The ingredients and the chemical compositions of the experimental ration

Ingredients	(%)
Clover hay	38.00
Wheat bran	24.00
Yellow corn	15.00
Soybean meal (44% CP)	16.00
Molasses	4.00
NaCl	1.00
Sodium bicarbonate	0.10
Di Calcium phosphate	1.60
Vitamins and Minerals premix*	0.30
Total	100
Chemical analysis	
Calculated analysis on dry matter basis**	
Crude protein (%)	17.20
Ether extract (%)	2.52
Crude fiber (%)	14.55
Digestible energy (Kcal/Kg diet)	2500
Calcium	1.00
Phosphorus	0.85
Starch	50.58
Lysine	0.84
Methionine	0.29

*Each 1Kg of vitamins and minerals premix contained: Vitamin A 10,000 IU, Vitamin D3,1,800 UI; Vitamin E, 15 mg; vitamin K3, 4.5 mg; Vitamin B1, 0.5 mg; Vitamin B2, 4 mg; Vitamin B12, 0.001 mg; Folic acid, 0.1 mg; Pantothenic acid, 7 mg; Nicotinic acid, 20 mg; I, 1 mg; Mn, 60 mg; Cu, 5.5 mg, Zn, 75 mg; Fe, 40 mg; Co, 0.3 mg; ;Se, 0.1mg Robenidine, 52.8 mg.** According to NRC (1977).

Nano-Se CAPSULE® was brought from Shanghai Stone Nano-Technology Port Co. Ltd., China. The product is based on liquid Nano-Se. The sizes of the elemental Se particles ranged from 60 to 80 nm; in orange powder-coated capsules, each one contains 0.225 g of powder, including Nano-Se (45 µg). Sodium selenite was brought from Signa-Aldrich®, Cat. No.214485. Nano-Se & SSe dosage, as claimed to live body weight, was dissolved in double distilled water using a magnetic stirrer overnight.

Animal management:

The farm design allows natural ventilation through wired windows beside controlled-sided exhaustion fans. The ambient temperature range during the experiment was from 20°C to 26°C, while relative humidity was from 45.0 to 78.0%. A recommended 12 hour light/dark period was provided for the animals. Rabbits were individually housed in galvanized wired battery cages (50×55×39cm); each cage had its feeder and automatic nipple drinker. Water and fodder were extended *ad libitum*. All rabbits were held under the same managerial, hygienic, and environmental conditions maintained and treated according to the approved

standards of handling animals by a human. The rabbits were vaccinated with clostridial enterotoxaemia bloat, bacterial and viral immunization (Veterinary Research and Vaccines, Research Institute, Cairo, Egypt).

Blood sampling and analysis:

At the 14th week of age (8 weeks from starting day), blood samples were drawn out from the marginal ear vein into clean tubes (without anticoagulant) in each group. The clotted blood samples were centrifuged at 4000 rpm for 10 min and the clear supernatants as serum was collected and stored at -70°C until conducting the subsequent biochemical and hormonal analyses. After that, eight males/females from each group were slaughtered by knife, the two testes and the two ovaries of each animal were directly removed and preserved at -70 C for analysis. Testes (dissected testicular tubes) and ovaries (without ovarian ligament) of each group were pooled and mixed well. Then, a weight of 250 mg mixed-tissue was added to 1 ml phosphate buffer saline (PBS) and minced at 12000/minute for a complete homogenization by IKEA homogenizer. The homogenate was centrifuged under cooling -3000 rpm- for 15 minutes, then the supernatant was transferred to clean tube and recentrifuged at 3000 rpm- for 15 minutes. The clear supernatant was gained and frozen at -20°C until parameter estimation [21]. Eight samples from the clear extract per group were arranged in duplicates for the subsequent analyses.

The following variables were analyzed in serum and tissue supernatants:

1-CPK and ALP were calculated using the reagent of Spectrum, Egyptian Company for Biotechnology (S.A.E), Cairo, Egypt, catalog No. (239001 and 214001, respectively).

2-Lipase, amylase, and albumin were estimated using the reagent of Arena Bioscience, Ismailia, Egypt, catalog No. (BS.1/ LIP02.020.0040, BS.1/ AMY02.025.0050, and BS.1/ AL02.100.0200, respectively).

3-AFP was determined by ELISA Kit provided by Ray Biotech, Inc., with catalog No. ELH- AFP.

4-Estradiol-17β (E2), progesterone (P4), and testosterone levels were analyzed using RIA coated-tube kits, samples were assayed in duplicate as described by DIA source ImmunoAssays® S.A. Belgium. The intra- and inter-assay CVs for E2, P4 and testosterone were 6.1% and 12.2%; 4.6% and 7.6%; 3.8% and 5.5%, respectively; with detection limits of 2.7pg/mL, 0.05ng/mL and 50pg/mL, respectively.

Statistical analysis:

Statistical data were analyzed using SAS GLM procedure [22]. The statistical model: $Yij = \mu + Ti + eij$. Where, Y = dependent variable, μ = overall mean, Ti = fixed effect of Treatment (1= Nano-Se, 2= SSe, 3= Control) and eij = random error, and the significant differences between means were verified by Duncan's Multiple Test [23].

RESULTS

Data of ovarian tissue enzymatic activities in NZW rabbits injected with Nano-Se and SSe are shown in Table (2). Nano-Se and SSe injection increased (P<0.001) ovarian enzymatic activities of amylase and lipase compared to control, with a 12% and 21% increase in amylase and lipase enzyme activity, respectiively due to Nano-Se as compared to the control. Likewise, CPK and ALP activities in ovarian tissue elevated (P<0.001) by 6% and 15%, respectively, as compared to the control due to SSe supplementation, an opposite trend was observed in the Nano-Se group with 20% decrement less than the control for ALP enzyme (Table 2).

Table (2): Ovarian tissue enzymatic activities (means ±SE) of NZW rabbits supplemented Nano-Se and SSe

	Amylase U/ L	Lipase U/ L	Total CPK U/ L	ALP U/ L
Nano-Se	100.0^{a}±1.62	68.0^{a}±0.29	323.0^{c}±0.71	15.0^{c}±0.24
SSe	98.0^{a}±0.36	59.0^{b}±0.37	350.0^{a}±0.75	22.0^{a}±0.31
Control.	89.2^{b}±0.51	56.0^{c}±0.37	329.0^{b}±0.71	19.2^{b}±0.58

a , b and c means within column in each parameter with different superscript are significantly different at P< <0.001.
Nano-se=Nano selenium, SSe= Sodium selenite, CPK=Creatin phosphokinase, ALP= Alkaline phosphatase

The influence of treatments on serum enzymatic activities in females NZW rabbits are shown in Table (3). Nano-Se administration caused a significant (P<0.001) increase in the activities of amylase, lipase, CPK, and albumin as compared to SSe and control groups, while it decreased (P<0.001) ALP and AFP activities by 26 and 52%, respectively less than the control (Table 3). On the contrary, amylase, lipase, CPK, and AFP activities decreased significantly (P<0.001) due to SSe injection when compared to the control group, with a marked decrease (11.2%) in CPK activity as compared to the control. Whereas SSe showed an increase (P<0.001) in ALP as compared to the Nano-Se and control groups (Table 3).

Table (3): Serum enzymatic activities (means ±SE) of females NZW rabbits supplemented Nano-Se and SSe

	Amylase U/L	Lipase U/L	Total CPK U/L	ALP U/L	AFP IU/mL	Alb. g/dL
Nano-Se	106.0^{a}±0.78	70.0^{a}±0.43	256.0^{a}±4.33	20.71^{c}±0.26	1.0^{b}±0.02	3.94^{a}±0.03
SSe	84.0^{c}±0.71	59.0^{c}±0.51	89.31^{c}±0.33	29.06^{a}±0.41	1.2^{b}±0.10	2.35^{b}±0.10
Control	88.0^{b}±0.36	62.1^{b}±1.02	100.6^{b}±1.03	28.01^{b}±0.74	2.1^{a}±0.09	2.19^{b}±0.07

a , b and c means within column in each parameter with different superscript are significantly different at P<0.001
Nano-se=Nano selenium, SSe= Sodium selenite, CPK=Creatin phosphokinase, ALP= Alkaline phosphatase, AFP=Alpha Fetoprotein. Alb= Albumine

The impacts of Nano-Se and SSe on testicular tissue enzymatic activities are presented in Table (4). Nano-Se exhibited a significant (P<0.001) increase in amylase and lipase activities as compared to the SSe and control groups. However, amylase, lipase, and AFP activities diminished (P<0.001) at puberty in the testicle tissue of SSe treatment in comparison with the other groups, as shown in Table (4).

Table (4): Testicular tissue enzymatic activities (means ±SE) of NZW rabbits supplemented Nano-Se and SSe

	Amylase U/L	Lipase U/L	AFP IU/mL
Nano-Se	98.3^{a}±0.65	72.6^{a}±0.42	1.8^{a}±0.15
SSe	85.0^{c}±0.64	59.0^{c}±0.57	1.1^{b}±0.17
Control	91.1^{b}±0.70	63.0^{b}±0.55	1.9^{a}±0.08

a,b and c means within column in each parameterwith different superscript are significantly different at P<0.001
Nano-se=Nano selenium, SSe= Sodium selenite, AFP= Alpha fetoprotein

The Nano-Se and SSe usage impacts on serum enzymatic activities of males NZW rabbits are listed in Table (5). Nano-Se caused a marked increase in CPK activity (80%; P<0.001) and albumin concentration (58%; P<0.001) as well as an increase(P<0.001) in ALP comparison to the control group. Whereas SSe had dramatic effects on serum enzymatic activity at puberty, it markedly increased ALP by 59%; P<0.001, decreased CPK activity by 30%; P<0.001 compared to the control, with no variation in Alb than control (Table 5).

Table (5): Serum enzymatic activities (means ±SE) of males NZW rabbits supplemented Nano-Se and SSe

	Total CPK U/L	ALP U/L	Alb. g/dL
Nano-Se	146.17^{a}±0.75	152.2^{b}±0.31	4.70^{a}±0.10
SSe	57.10^{c}±0.39	223.1^{a}±0.46	2.94^{b}±0.11
Control	81.34^{b}±0.47	139.9^{c}±0.51	2.98^{b}±0.17

a,b and c means within column in each parameter with different superscript are significantly different at P<0.001
Nano-se=Nano selenium, SSe= Sodium selenite, CPK=Creatin phosphokinase, ALP= Alkaline phosphatase, Alb= Albumine

Data of albumin concentrations in ovarian and testicular tissue of NZW rabbits supplemented with Nano-Se, and sodium selenite are illustrated in Table (6). Nano-Se and SSe induced a significant (P<0.0001) increase in albumin concentration in ovarian tissue as compared to the control. The highest mean values were for nano-Se,while nano-Se and SSe decreased (P<0.001) albumin concentration in testicular tissue as compared to the control group.

Table (6): Albumin concentrations (means ±SE) in ovarian and testicular tissues of NZW rabbits supplemented Nano-Se and SSe

	Ovarian tissue (in Female)	Testicular tissue (in Male)
	Alb. g/dL	Alb. g/dL
Nano-Se	6.05^{a}±0.13	1.64^{b}±0.03
SSe	5.44^{a}±0.24	1.59^{b}±0.01
Control	3.29^{b}±0.17	2.27^{a}±0.08

a,b and c means within column in each parameter with different superscript are significantly different at P<0.0001
Nano-se=Nano selenium, SSe= Sodium selenite, Alb= Albumine

Nano-Se injection increased (P<0.01) E2, P4, and testosterone concentrations weighed to the control group; on the other side, no changes (P<0.001) in these sexual hormones occurred due to SSe injection, except the increase of testosterone with 131%; P<0.001 more than the control group (Table 7).

Table (7): Serum sexual hormones (means ±SE) concentrations at puberty of females and males NZW rabbits supplemented Nano-Se and SSe

	E2 pg/mL	P4 ng / mL	Testo. ng/mL
Nano-Se	65.0^{a}±2.5	1.121^{a}±0.04	32.0^{a}±1.7
SSe	50.0^{b}±6.2	0.768^{b}±0.03	33.0^{a}±3.8
Control	41.8^{b}±3.0	0.718^{b}±0.03	13.8^{b}±1.0

a , b and c means within column in each parameter with different superscript are significantly different
Nano-se=Nano selenium, SSe= Sodium selenite, E2= Estradiaol-17β, P4= Progestrerone, Testo= Testosterone

DISCUSSION

Rabbit is an excellent laboratory animal that offers many advantages in the field of reproduction. It is easy to handle owing to its size. The current results showed a considerable decrease in ovarian extract and serum ALP activities due to nano-Se supplementation than SSe; a previous study stated a notable lowering in blood ALP enzyme caused by nano-Se treatment than the selenium and control groups. Likewise, SSe administration revealed an increase in ALP activity [24]. In contrast, others reported a diminished or unchanged blood ALP activity following Se and Vit-E supplementation [25]. A high relationship is stated between ovarian ALP activity and reproductive tissues [26]. The increased follicular ALP activity is associated with small and degenerated follicles (but large follicles have less ALP activity) due to the lysosomal enzyme effect upon phosphorylated receptor, which would lead to atresia [18]. It may appear because of small follicle contents from progesterone and dominant androgen, which assist ALP activity [27]. On the other side, the bioconversion of androgen to estrogen dominance may explain the decline of APL activity in the follicular milieu, coinciding with follicle development [16]. This leads to conclude that Nano-Se enhanced normal growth of follicles from atresia throughout decreasing ALP activity in ovarian tissue.

In concomitance with the current results, it is observed that high selenium concentration increases lipase activity whereas deficiency of Se lessens lipase activity resulting in degeneration of pancreatic cells in chicks [28,29]. Lipase function into the ovary is still unclear. Metabolically, the ovarian tissue is reached in fatty acids needed for the synthesis of lipids and cholesterol, the precursor of steroids [30], the binding of lipase to vascular endothelium is the first action in several mechanisms that facilitate the supply of fatty acids and cholesterol to ovarian parts including, growing follicle (including oocyte), and granulosa layer [30]. These support the highly significant lipase activity in ovarian, testicular tissues, and serum due to nano-Se supplementation more than the SSe and control groups. A clear trend toward increasing tissue lipase activity in chickens received Se than control [31], selenium acts through specific selenoproteins that modulate various enzyme probably included in lipid metabolism. Also, nano-selenium usage can improve feed digestibility and volatile fatty acids production [32]. The previous studies revealed high lipase activity in the ovary, adrenal cortex, and testes, correlated to steroid hormone production in these tissues [33]. Lipase is also engaged in triacylglycerol and cholesterol transport in the tissues where it is located by hydrolysis of phospholipids at the surface of HDL particles [34].

Amylases are enzymes involved in carbohydrate digestion that hydrolyze starches and glycogens into smaller polymers in many organisms [35,36]. In sheep, selenium supply increases amylase activity in blood, illustrating selenium turn in increasing amylase activity [37]. The activity of amylase in the ovarian tissue is highly correlated to follicular size; it becomes great during proestrus when follicular size is large, and vice versa is in the diestrus because the surrounding fluid is mainly gotten from the ovarian epithelium, the difference in amylase concentration that may be attributed to the activity of sexual steroids in ovary tissue [38]. The increase of amylase activities of testicular tissue and serum in the nano-Se as compared to the SSe and control groups is in contrast with that revealed no effects in amylase of testicular tissue due to selenium supplementation [39]. However, others reported that amylase increases in rats after Se supplementation [36].

In the present study, supplementing rabbits with nano-Se increased ovarian tissue extract and serum albumin as compared to the SSe and control groups. Using Se with/or without vitamin E causes significant increases in albumin; however, these increases are still within the standard range in growing rabbits [40]. The rise ($P<0.05$) of albumin content in follicular fluid more than blood plasma [41] suggests an active transport of albumin from the blood into follicles which are required for binding some blood constituent inside the follicle fluid that involved in the development and ripeness of follicles. These findings support our results. On the contrary, treatment with Se-algae caused a considerable reduction in albumin concentration of male growing New Zealand White rabbits [42].

SSe and Nano-Se supplementation increased CPK in the ovarian tissue enzymatic activities and serum enzymatic activities, respectively. Dietary sodium selenite elevated CPK activity besides the presence throughout follicular development and luteinization concentrated in ovarian thecal and luteal cells. On the contrary, feeding calves and lambs sodium selenite prevented plasma and/or serum rise of CPK enzyme[43].

The result of the AFP analysis indicated that selenium supplementation decreases AFP in serum and testicular enzyme activities, as it was previously mentioned that Se administration showed a better prevention efficacy in moderating the AFP plasma contents in rats [44,45].

Se induces corpora lutea cells proliferation of bovine through reducing the deleterious effects of peroxides [46]. Moreover, Se stimulates the viability and cell proliferation from small follicles and potentiates the FSH action in these cells [47]. The latest in-vitro study revealed significant production of E2 from the ovarian small and large follicles after 96 and 144 hrs of incubation with Se. This effect proposes that Se stimulates E2 output from the granulosa layer. Moreover, the motivative impact of FSH in small follicle cells is evidenced only with selenium's existence. P4 production from corpus luteum did not show any effect of Se with or without FSH presence [46].

Testosterone hormone is necessary for male germ cells' maturation, spermatogenesis, and sperm quality [48]. Supplementation of male rabbits with nano-Se or SSe significantly improved serum testosterone concentration [49,50], as found in the current data. Selenoenzyme, a molecular target for Se in male germ cell's [16], contributes to the proliferation, and spermatogenic cell differentiation, in addition to sperm maturation [51], which may explain the importance of Se to male testicular tissues. Nanoparticles have smaller particle sizes, greater surface area, higher mucosal permeability, improved intestinal absorption, and tissue deposits more than the common element forms [52]. These characteristics increase Nano-Se bioavailability in target tissues more than SSe as reported in the thyroid and liver of growing NZW rabbits [53].

CONCLUSION

Administration of Nano-Se increased amylase, lipase, and CPK, with the decrease of ALP enzymatic activities more than SSe; besides the elevation of estradiol-17β and testosterone, these indicate the regular growth of the ovarian follicles and testicular tissue of rabbits at puberty.

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Conflicts of Interest: None

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