Scientific Works. Series C. Veterinary Medicine. Vol. LXIX (2), 2023
ISSN 2065-1295; ISSN 2343-9394 (CD-ROM); ISSN 2067-3663 (Online); ISSN-L 2065-1295

EVOLUTION OF THE REPRODUCTIVE SYSTEM MORPHOLOGY IN ROOSTERS FED ON VITAMIN A AND VITAMIN E SUPPLEMENTED DIETS

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Abstract

The paper presents the effects of long-term dietary supplementation with vitamin A and vitamin E on the testicle and epididymis morphology in roosters. The roosters were fed on diets enriched in vitamin A (600 IU/kg of diet) and/or vitamin E (60 IU/kg of diet) from 40 to 57 weeks of age. Histology and morphometry studies were performed on the testicle and on epididymis duct of the experimentally fed rooster. A better preservation of the seminiferous epithelium, refinement of seminiferous pericanalicular connective tissue, small islands of Leydig cells as well as the relative maintenance of the richness of the seminiferous pericanalicular blood vasculature are noted for vitamin supplemented groups versus control. Both vitamins diminished the ageing effects on the thickness and structure of the epididymis epithelium. Both vitamins prolonged the maintenance of Sertoli cell density ($P < 0.01$ versus control). The lumen epididymis fluid contains smaller amount of detached cytoplasmic fragments, cilia, and nuclei versus control. Vitamin A mainly protects the spermatogenesis line, while vitamin E mainly protects Sertoli and Leydig cells. No mutual inhibition or potentiating effects of the two vitamins were revealed.

Key words*: epididymis, rooster, testicle, vitamin A, vitamin E.*

INTRODUCTION

The use of antioxidant vitamins in poultry practice to improve the reproductive performance of roosters is well known. Among the most frequently used vitamins in this regard are the vitamin A and the vitamin E. Many scientific works are focused on the effects of these vitamins on some characteristics on semen biological properties such as ejaculate volume, semen density, semen motility, semen viability as well as on morphological characteristics of spermatozoa (Danikowski et al., 2002; Biswas et al., 2009; Baba & Asrol, 2017; Bălăceanu et al., 2019). Some scientific works are focused on the influence of antioxidant vitamins on the macro- and microscopic structure of the reproductive system of roosters. Thus, Sukmawati et al. (2019) studied the effect of vitamin E on testicle histology (in rats) and reported there is an ameliorative effect of vitamin E on testicle histology structure such as tubules diameter, epithelial thickness, Sertoli cell number, and antigen binding protein levels of rats. Saddein et al. (2019) reported the protective effect of vitamin E on the seminiferous epithelium in guinea pig males in experimental mancozeb intoxication. Yokota et al. (2018) reported a decrease in testicular volume in mice fed excess vitamin A (1000 IU/kg diet from 3 to 10 weeks of age), an effect qualified by the authors as toxic. Bosakowski et al. (1988) found no changes in testicular volume in rats fed diets containing twice the amount of vitamin A administered by Yokota et al. (2018). Triques et al. (2019) investigated the long-term effect (from hatching to 66 weeks of age) of other antioxidants (vitamin C, canthaxanthin and lycopene) on testis morphometry properties in roosters of different breeds and they found that supplementation with an antioxidant blend composed of canthaxanthin, vitamin C, and lycopene in roosters led to higher testicles (as length, thickness, width, and weight). Thus, the data regarding the effects of long-term treatments with vitamin A or E on testicular and epididymal morphology in roosters are rare. The purpose of this work was to determine the

effects of long-term vitamin A and vitamin E dietary supplementation on testicular morphometry, on the structure of the seminiferous epithelium and on the structure and epididymis morphometry in hybrid Cornish roosters.

MATERIALS AND METHODS

The experiment was carried out on 40-week-old Cornish hybrid roosters. A number of 64 animals were involved, divided in four groups (16 animals each one): a control group, an A group, an E group and an A+E group. The animals were housed and maintained in compliance with the technological norms of industrial breeding. All groups were fed on a commercial diet based on maize 35.6%, wheat 27.4%, soy extruded 21.3% and containing 15.43% crude protein, 3.89% calcium, 0.39% phosphorus, and 2,880 kcal/kg ME (as calculated values). Furthermore, group A diet was supplemented with 180 μg vitamin A (as retinal)/kg diet. Group E diet was supplemented with 270 mg vitamin E (as vDL-α-tocopherol acetate vitamer/kg diet. Group A+E diet was supplemented by both, A and E vitamins (same quantities). The basal commercial diet already contained 360 μg vitamin A/kg and 4.5 mg vitamin E/kg. The experimental feeding begun with the birds at 40 weeks and lasted 17 weeks. Five 40-week-old roosters were euthanized and then, five 57-week-old roosters from each group were euthanized at the end of the experimental feeding period. Testicle and epididymis tissues were immediately sampled and were histologically processed and stained with hematoxylin eosin (H-E), Malory and Malachite green according to the methods described by Cornilă & Manolescu (1996). The histological preparations were examined using an optical microscope at different magnifi-cations. Image captures were used for testicular and epididymal morphometric analysis (semini-ferous duct and epididymal duct diameter, and seminiferous and epididymal epithelium thickness) using the "ImageJ" program.

All procedures in this study were conducted in accordance with Directive 2010/63/EU on the protection of animals used for scientific purposes by Member States of the European Union. The experiment was approved by the ethic committee of the USAMV of Bucharest. Testicular and epididymal morphometry data were statistically processed and expressed as mean ± standard error of the mean. Differences between groups were analyzed based on the Student's *t* test, being considered significant for $P < 0.05$.

RESULTS AND DISCUSSIONS

Regarding the seminiferous tubular morpho-metry, in our experiences, it is found that the seminiferous tubules undergo a process of decreasing diameter and decreasing density, by age (Table 1). The decrease in seminiferous tubule diameter was made both by decreasing the thickness of the epithelium and by shrinking the tubular lumen.

Table 1. Morphometry of seminiferous ducts in rooster groups fed on vitamin A or vitamin E - enriched diets from 40 to 57 weeks of age versus control

	40-week-old roosters	57-week-old roosters			
		Control	Vitamin A	Vitamin E	Vitamin A+E
Duct diameter (mm)	332.3±98.5	254±36.3	294±22.4	279±66.6	288±12.0
Duct epithelium thickness (mm)	111.0$^{\#}$±12.3	65.5$^{\#;a;b}$±4.4	89.0^{a}±11.3	74.4±10.0	86.6±6.6
Density of seminiferous ducts[1]	233.3±45.4	190.7±33.0	213.3±16.6	209.9±33.0	211.0±8.5
Density of Sertoli cells[2]	22.2±1.9	11.0$^{a;b;c}$±2.2	14.4^{a}±3.2	16.2^{b}±3.6	13.3±6.5

[1]mean values on 10 microscopic fields (ob.10 x oc.10) taken randomly;
[2]mean values on 10 microscopic fields (ob. 100 x oc. 10) taken randomly;
Note: - values represent the average of the two testicles of the roosters;
- values represent the mean ± the standard error of the mean;
- n = 5 for 40-week-old roosters and n = 5 for 57-week-old roosters;
- values on the same line with the same superscripts differ significantly ($P < 0.05$).

Part of the diameter decrease was compensated by the increase in the thickness of the intertubular connective tissue, so there is no direct proportionality between the evolution of the diameter, density of the seminiferous ducts and the testicular volume. The fact oriented us

towards the analysis mainly the effect on the thickness of the wall of the seminiferous tubules. The results reveal a protective effect of both, vitamin A and E diet supplementation, on the thickness of the seminiferous tubules, maintaining its size. The protective effect was greater in the case of vitamin A. Thus, a physiological decrease, of 45% of the thickness was found for the epithelium of the seminiferous tubules from 40 to 57 weeks, in control roosters. The mean tubule thickness values are significantly higher in groups A compared to the control, revealing a protective effect of the vitamin A on spermatogenesis line. Vitamin E also showed a protective effect, but of a weaker amplitude. In this regard, according to Sarabia Frogoso et al. (2013), 44% of testicular weight in broiler hybrids can be lost between 36 and 55 weeks of age, and this is usually accompanied by a decrease in the diameter of the seminiferous tubules, a decrease that agrees with the results found in Cornish hybrid roosters of the present experiment.

Both vitamins A and E also led to the maintenance of Sertoli cell density (significant differences versus control, $P < 0.01$ in all supplemented groups). This fact explains the physiological effects of vitamin A predominantly on spermatogenesis line cells (finally reflected in semen density and spermatozoa count, in particular) and the predominant effect of vitamin E on the biological properties of sperm (motility, in particular). Our results are confirmed by other researchers who found that vitamin E can maintains the thickness of the seminiferous tubule epithelium, increasing the number of epithelial cells, with positive effects on testis weight and testosterone concentration in the rat (Kumar et al., 2004) and rabbit (Yousef, 2010).

The histological structure of the seminiferous epithelium of roosters fed on vitamin A-enriched diet is closer as structure, dimensions and component elements to that of the seminiferous epithelium of 40-week-old roosters compared to the control testicular seminiferous epithelium, denoting a protective effect of the vitamin A against the erosion process supported by seminiferous tubules, induced by the physiology ageing process.

Aspects of testicular tissue histology in roosters fed the vitamin E-supplemented diet are notable for maintaining a high density of seminiferous Sertoli cells and interstitial Leydig cells (Figure 1).

The results obtained in our research are in agreement with previous studies (Yokota et al., 2018) which demonstrated that vitamin A deficiency in rats induces a progressive loss of spermatogenic germ cells, ultimately leading to the appearance of seminiferous tubules containing only Sertoli cells and premeiotic germ cells, straight spermatogonia (Huang et al., 1988 cited by Yokota et al., 2018) and administration of vitamin A to vitamin A deficient rats resumes spermatogenesis, as a regulator of gene expression (Yokota et al., 2018).

Manson and Mauer (1974) revealed a remarkable regenerative response in hamsters experimentally deficient in vitamin E: when the degeneration had reached quite advanced stages, the restoration of the germinal epithelium in most tubules was good as a result of vitamin E administration, but a variable number of seminiferous tubules showed only limited repair.

Similarly, in rats, Bensoussan et al. (1998) showed that reintroducing dietary vitamin E to deficient rats restored a normal appearance to the structure of the testis and epididymis, indicating that the effects on these tissues are reversible.

Taken together, these data indicate that vitamin E plays an important role in maintaining the viability of the spermatid population, allowing epididymal epithelial cells to acquire their appearance.

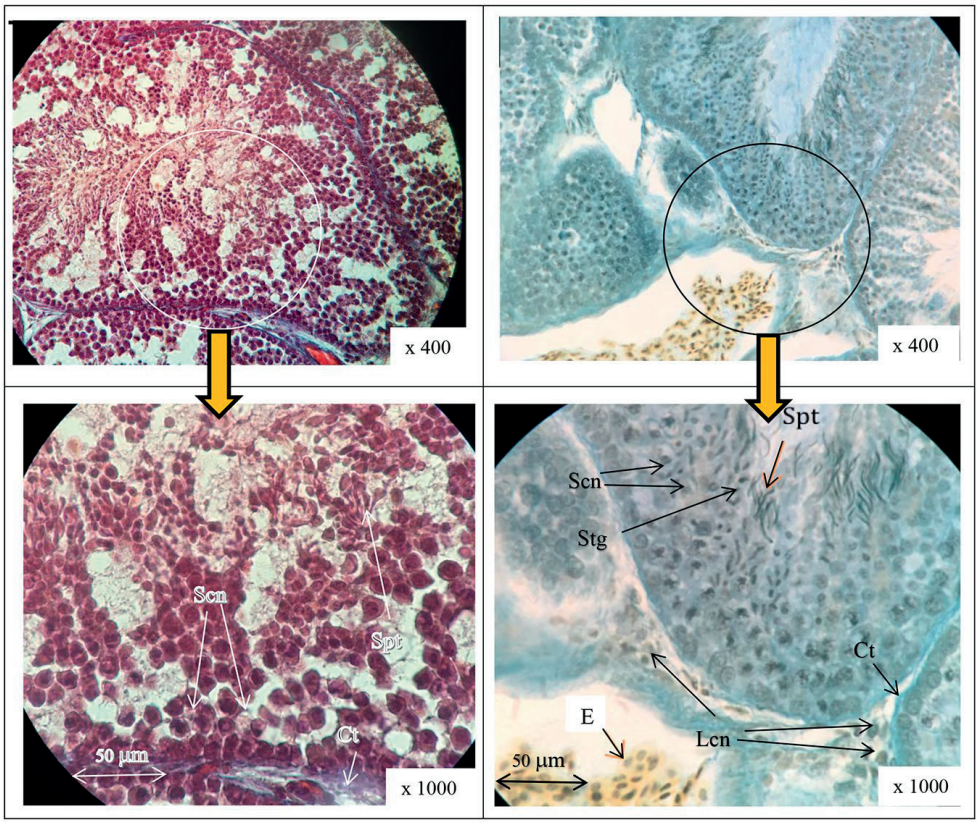

Figure 1. *Left:* testis of a 57-week-old control rooster. Disorganization of the seminiferous structure is noted, with the appearance of numerous vacuoles. Above, thick sheets of connective tissue can be seen, surrounding the seminiferous tubules. At the bottom, numerous spermatides (Spd) can be seen, still retaining a high mass of residual cytoplasm. Scn = Sertoli cell nuclei; Spr - spermatids; Ct -connective tissue. Mallory staining
Right: testis of 57-week-old rooster fed the vitamin A-enriched diet for 17 weeks. The preservation of the full development of the seminiferous epithelium, the fine lamellae of seminiferous pericanalicular connective tissue, small islands of Leydig cells as well as the relative maintenance of the richness of the seminiferous pericanalicular blood vasculature are noted. Green Malachite staining.
E - erythrocytes (with pink-yellow colored cytoplasm); Spt - spermatozoa; Stg - spermatogonia; Lcn - Leydig cell nuclei; Scn - Sertoli cell nuclei; Ct- connective tissue

During the aging process, from 40 to 57 weeks, there was a 36% decrease in the thickness of the epididymis epithelium in the control (Figure 2). In contrast, in groups supplemented with vitamin A, this decrease in epididymis epithelial thickness was less (only 18% in group A), suggesting a higher protective effect of vitamin A on this structure in the long-term vitamin treatment, the differences being significant compared to the control group ($P < 0.01$) and 33% in group E.

A similar situation was identified in the case of the diameter of the epididymis duct, the created space being replaced by connective tissue.

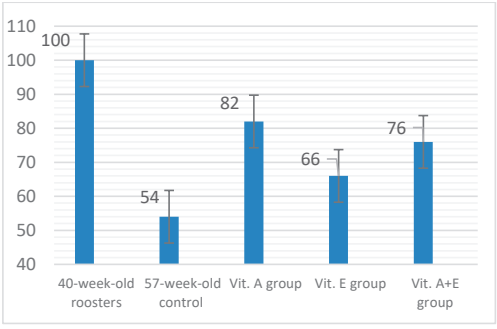

Figure 2. Long-term evolution of the vitamin A or E enriched diets on the thickness of the epididymis epithelium in roosters (% from values at 40 weeks of age)

With regard to the histological structure and the content of the epididymis and deferent duct lumen (Figure 3), in 57-week-old control roosters, spermatogonia, spermatocytes and fragments of basophilic or eosinophilic cytoplasmic mass originating from Setoli cells or epididymal epithelial cells appear among the spermatozoa (Figure 3, A).

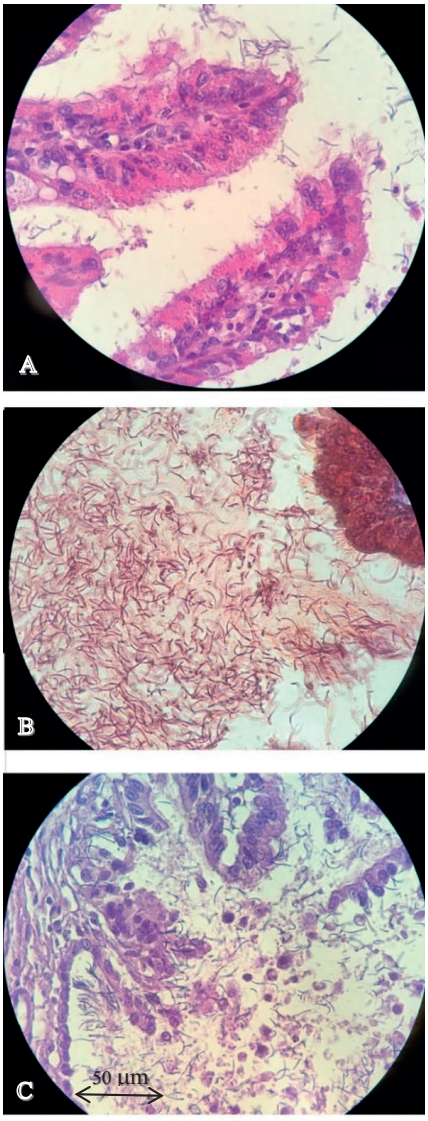

Figure 3. Comparative structural aspects of the epididymis epithelium and epididymis luminal content in 57-week-old roosters, following 17 weeks of experimental feeding with vitamin A (midle) or E (down) enriched diets versus a control (up) (see detailed explanations in the text). H-E (A and C) and Malory (B) staining (x1000)

Much reduced amount of eosinophilic cytoplasmic mass and spermatogonia were found in 57-week-old roosters fed for 17 weeks on diets enriched in vitamin A. (Stereo)epididymal cilia are well developed (Figure 3, B). In 57-week-old roosters fed for 17 weeks with diets enriched in vitamin E, the epididymal epithelium is eroded, reduced in some places to a single row of cells (Figure 3, C). In the lumen, numerous large spermatogonia, spermatocytes and fragments of cytoplasmic mass from Sertoli cells or from epididymis epithelium cells are identified. Data from the literature regarding the protective effects of antioxidant vitamins on the epididymal epithelium confirm our results: protective effects of vitamins (ascorbic acid) on the epididymis structure (affected by the experimental toxic action) were reported by Chitra et al. (2003) in the rat.

Similarly, Krishnamoorthy et al. (2007) demonstrated the protective effect of vitamin E on epididymal spermatozoa, explained by improving the biochemistry of epididymal secretions, also in rats. Again, Bensoussan et al. (1998) provide, as shown, a more edifying example of the protective role of vitamin E on the epididymis in rats after five weeks of feeding them a diet deficient in vitamin E compared to animals fed on a commercial diet. All the effects of the researched vitamins must be interpreted in the context of their antioxidant effect on the enzymes involved in redox processes in tissues (Escorcia et al., 2020).

CONCLUSIONS

Long-term feeding (17 weeks) on diets enriched in vitamin A or E of hybrid Cornish roosters allowed the identification of specific protective effects of the two vitamins on the structure of the seminiferous epithelium and the epididymal one. Vitamin A mainly protects the spermatogenesis line, while vitamin E mainly protects Sertoli cells and Leydig cells. Both vitamins have a protective effect on the epididymal epithelium against the physiology ageing process, especially vitamin A. No mutual inhibition or mutual enhancement of the two studied vitamins were identified. The effects of the vitamin A and vitamin E diet supplements on the testicular morphology will be reflected in the

improvement of the biological properties of the semen, allowing the extension of the economic exploitation of Cornish hybrid roosters over a longer period of time than that foreseen in the commercial technologies.

REFERENCES

Baba A.R., & Asrol, M.A. (2017). Effect of vitamin E supplementation on semen quantity and quality of Local Kampong roosters. *Mal. J. Anim. Sci*. 20(1): 37-43.

Bălăceanu, R.A., Sima, L., & Dojană, N. (2019). The effect of different level of vitamin E diet supplementation on the sperm in adult cocks raised in industrial system. *Scientific Papers Veterinary Medicine Timisoara*, ISSN 1221-5295.

Bensoussan K., C R Morales C.R., & Hermo L. (1998). Vitamin E deficiency causes incomplete spermatogenesis and affects the structural differentiation of epithelial cells of the epididymis in the rat. *J. Androl*. 19(3):266-88.

Biswas A., Mohan J., & Sastry K.V.H. (2009). Effect of higher dietary vitamin E concentrations on physical and biochemical characteristics of semen in Kadaknath cockerels, *British Poultry Science*, 50:6, 733-738. DOI: 10.1080/00071660903264369

Bosakowski, T., Levi, A.A., & Edgcomb, J.H. (1988). Studies onthe testicular effects of vitamin A palmitate in the Sprague dawley rat. *Food Chem. Toxicol*., 26, 767-773.

Chitra,, K.C., Ramachandra Rao K., & Mathur, P.P. (2003). Effect of bisphenol A and co-administration of bisphenol A and vitamin C on epididymis of adult rats: a histological and biochemical study. *Asian J Androl.* 5(3):203-8.

Cornilă, N., & Manolescu, N. (1996). *Structura şi ultrastructura organelor la animalele domestice*. Editura Ceres, Bucureşti.

Danikowski, S., Halle, I., & Flachowsky, G. (2002). Influence of high levels of vitamin E on semen parameters of cocks. *Journal of Animal Physiology and Animal Nutrition.* 86: 11-12, 376-382.

Escorcia, L.M., Sánchez-Godoy, F., Ramos-Vidales D., Medina-Campos, O.N., & Pedraza-Chaverri, J. (2020). Effect of the Age and Body Weight of the Broiler Breeders Male on the Presentation of Oxidative Stress and Its Correlation with the Quality of Testicular Parenchyma and Physiological Antioxidant. *Vet. Sci*. 7, 69.

Krishnamoorthy, G., Venkataraman, P., Arunkumar, A., Vignesh, R.C., Aruldhas, M. M., & Arunakaran, J. (2007). Ameliorative effect of vitamins (alpha-tocopherol and ascorbic acid) on PCB (Aroclor 1254) induced oxidative stress in rat epididymal sperm. *Reprod Toxicol.* 23(2):239-45. doi: 10.1016/j.reprotox.2006.12.004. Epub 2006 Dec 22.

Kumar, R., Prakash, S., Kushwah, A., & Vijayan, V. (2004). Effects of vitamin C and E induced oxidative stress, androgen binding protein and lactate in rat Sertoli cells. *Reprod Toxicol*. 19:201–8; https://doi.org/10.1016/j.reprotox.2004.08.001.

Manson, K.E., & Mauer, S.I. (1974). Reversible Testis Injury in the Vitamin E-deficient Hamster. *J. Nutr*. 105(4):484-90. doi: 10.1093/jn/105.4.48.

Saddein E., Haghpanah, T., Nematollahi-Mahani S., Seyedi, F., & Ezzatabadipour, M., (2019). Preventative Effects of Vitamin E on Testicular Damage and Sperm Parameters in the First-Generation Mice Pups due to Pre- and Postnatal Mancozeb Exposure. *Journal of Toxicology*. https://doi.org/10.1155/2019/4763684.

Sarabia Fragoso, J., Pizarro Díaz, M., Abad Moreno, J.C., Casanovas Infesta, P., Rodriguez-Bertos, A., & Barger, K., (2013). Relationships between fertility and some parameters in male broiler breeders (body and testicular weight, histology and immunohistochemistry of testes, spermatogenesis and hormonal levels). *Reprod. Domest. Anim*., 48, pp. 345-352

Sukmawati, Y., Arisanty, D., Tofrizal, A., & Amir A. (2019). Vitamin E ameliorates testicular histological features and androgen binding protein levels in testicle of rats induced by allethrin. *Journal of Advanced Veterinary and Animal Research*. 6: 4, 486–491.

Triques, G.E., De Cristo, A.B., Canevese, M., Santos Marques, P.F., Burin, A.M., & Fernandez, J.I.M. (2022). Effect of antioxidant supplementation in diets of roosters during the post-peak phase on reproduction and production characteristics of offspring. *Zootechnia*. C*ienc. anim. bras*. 20, 1-13, e-43072.

Yokota, S., Shirahata, T., & Yusa, J. (2018). Long-term dietary intake of excessive vitamin A impairs spermatogenesis in mice. *J. Toxicol. Sci*., 44:4, 257-271.

Yousef, M.I. (2010). Vitamin E modulates reproductive toxicity of pyrethroid lambda-cyhalothrin in male rabbits. *Food Chem. Toxicol. Elsevier*. 48(5):1152–9; https://doi.org/10.1016/j.fct.2010.02.002.

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