

EFFECT OF TAURINE ADMINISTRATION ON THE STRUCTURE OF RABBIT TESTES

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ABSTRACT

The aim of this work was to analyze the effect of taurine (TAU) on the microscopic structure of rabbit testes, focusing on individual parameters of the seminiferous tubules. We used 5 months old sexually mature male rabbits, which were divided into four experimental groups. Rabbits from the experimental groups E1, E2, and E3 received taurine dissolved in drinking water (doses 321.675 mg/rabbit/day, 643.35 mg/rabbit/day and 965.025 mg/rabbit/day) for four weeks. To compare the results of our experiment a control group of rabbits (KK) was created without any TAU administration. We analyzed the following morphometric parameters: the diameter of the seminiferous tubules (μm), the height of the epithelium and the diameter of the lumen of the seminiferous tubules (μm), the area (μm^2) and the perimeter (μm) of the seminiferous tubules. Based on our results, we have found that the oral administration of TAU caused a significant increase ($P < 0.05$) of the diameter (E1, E2, E3), height of epithelium (E3), lumen thickness (E1), area (E3) and perimeter (E3) of the seminiferous tubules when compared to the control group (KK). Based on these findings we can conclude that oral administration of taurine at the highest dose (965.025 mg/rabbit/day, group E3) caused the most significant changes in the structure of the rabbit seminiferous tubules. We consider that administration of taurine induced increasing values of the studied parameters in the seminiferous tubules, which indicates a positive effect of taurine on spermatogenesis.

Keywords: taurine, testes, morphometry, rabbit

INTRODUCTION

Taurine (2-aminoethansulfonic acid, TAU) is one of the most abundant free β -amino acid that is present in all tissues of most animal species (Ahmed, 2015). Taurine is not integrated in a protein or metabolic pathway (Yang *et al.*, 2010). Taurine can be synthesized endogenously mainly in the liver from cysteine or methionine, released into circulation (Aly and Khafagy, 2014) but it can be also biosynthesized by other tissues, such as the central nervous system, kidney, retina and mammary gland (Yang *et al.*, 2010), or it can be assimilated from exogenous dietary sources (Ahmed, 2015). Taurine shows several physiological functions, such as osmoregulation, membrane stabilization, calcium modulation, antioxidation, radioprotection, xenobiotic conjugation, energy storage (Yang *et al.*, 2010), neuromodulation, detoxication, cytoprotective effects and anti-inflammatory actions (De Luca *et al.*, 2015). Deficiency of this amino acid leads to a lower production of bile, a reduced fat absorption and liver function (Lambert *et al.*, 2008). Its cellular concentration is primarily controlled by taurine transporters and biosynthetic enzymes such as cysteine dioxygenase and cysteine sulfinate decarboxylase (Li *et al.*, 2006).

Taurine has been identified as an important free β -amino acid in the male reproductive system (Aly and Khafagy, 2014). This amino acid has been found in the male reproductive system especially in peritubular myoid cells (Li *et al.*, 2006), Leydig cells, vascular endothelial cells, and some other interstitial cells of testis (Yang *et al.*, 2010). Taurine has various roles in the male reproductive system and sperm cells. It is known for its ability to stabilize cell membranes and regulate cellular osmosis (Ahmed, 2015), and can traverse the sperm plasma membrane (Kumar and Atreja, 2012) but it also acts as a sperm motility factor and as a capacitating agent (Aly and Khafagy, 2014). Taurine also can inhibit lipid peroxidation (LPO) due to its antioxidant properties (Ahmed, 2015) and protect the cells against the accumulation of reactive oxygen species (ROS) (Chhillar *et al.*, 2012). Taurine is also known as the most abundant free amino acid of the sperm cells and seminal fluid (Li *et al.*, 2006; Yang *et al.*, 2010).

Despite its importance, the effect of taurine on the male reproduction is still unclear. The aim of this study was to examine the effects of taurine on selected morphometric parameters of rabbit testes.

MATERIAL AND METHODS

The experiment included twenty-four 5-month old Hybrid Hyla rabbits that have been bred at NPPC-National Agricultural and Food Centre (Lužianky, Slovak Republic), with an average weight of 3.40 kg to 5.27 kg. Water was provided *ad libitum*. The rabbits were divided into three experimental groups and one control group that received water without the addition of TAU. Water containing TAU was connected to the feed pump system and administered to rabbits in the experimental groups. Taurine was added in the following concentrations (for 1 rabbit per day) - the experimental group 1 was administered taurine at a dose 321.675 mg (n=6), group 2 the taurine was administered at a dose of 643.5 mg (n=6), group 3 was administered at a dose of taurine 965.025 mg (n=6), control group received water without the addition of TAU (n=6). The exact concentrations of taurine taken in by rabbits in water in each group are only estimated since it was impossible to measure exact volume of water accepted by individual rabbit each day. The experiment took 4 weeks. After killing the testes were fixed in 10% formaldehyde until their processing at the University of Veterinary Medicine and Pharmacy in Košice, Department of Anatomy, Histology and Physiology (Slovak Republic). The samples were dehydrated in ethanol and embedded in paraffin wax. The whole sections were cut into 4-5 micron thick sections, which were stained with hematoxylin and eosin. Each sample was evaluated at the Slovak Agricultural University at the Department of Physiology (Slovak Republic) by a light microscope (Olympus CX-41) and the images were taken at the same place by digital camera (OLYMPUS U-CMAD3). The slides were observed at a magnification of 100x, several pictures were taken to be used for further evaluation. The photos were evaluated using the computer software Quick Photo Micro 3.1. (PROMICRA, Czech Republic). All the necessary measurements have been made according to themorphometric criteria (Massanyi *et al.*, 2003; Weibell

et al., 1966; Toman et al., 2002). We analyzed the following morphometric parameters: the diameter of the seminiferous tubules (μm), the height of the epithelium and the diameter of the lumen of the seminiferous tubules (μm), the area (μm^2) and the perimeter (μm) of the seminiferous tubules. The values of the measured structures are expressed as the arithmetic mean \pm standard deviation. The results were statistically evaluated using the SPSS 17.0 software. To determine statistical significance ($P<0.05$) the variance analysis (One - Way ANOVA), Tukey and Games - Howell tests were used.

RESULTS

In this study we analyzed the morphometric parameters of rabbit testes following oral administration of TAU at different doses (groups E1, E2, E3) and compared them against the control group without any addition of taurine (KK). The results of the analysis are shown in Table 1 and Figures 1, 2, 3, 4 and 5.

Table 1 Basic mathematical and statistical indicators (mean \pm SD) of testes from the control and experimental groups

Measured structures/Groups	E1	E2	E3	KK	TEST	
					T	GH
Diameter of ST (μm)	123.77 \pm 9.40	121.46 \pm 13.68	133.81 \pm 14.57	112.21 \pm 11.47	E1*, E2*, E3*	-
Height of the epithelium of ST (μm)	36.24 \pm 3.54	35.04 \pm 6.52	40.88 \pm 6.77	35.02 \pm 5.12	-	E3*
Diameter of the lumen of ST (μm)	45.66 \pm 6.70	41.70 \pm 6.72	41.73 \pm 8.79	38.30 \pm 5.70	E1*	-
Area of ST (μm^2)	17 062.43 \pm 1 908.74	16 028.17 \pm 2 597.70	20 391.53 \pm 4 246.39	15 375.99 \pm 3 728.64	-	E3*
Perimeter of ST (μm)	516.31 \pm 28.09	488.60 \pm 40.92	544.98 \pm 63.88	483.73 \pm 58.65	-	E3*

Legend: * $P<0.05$; ST - seminiferous tubule; T - Tukey test; GH - Games-Howell test;

Figure 1 shows the values of the diameter of the seminiferous tubules from each rabbit experimental group. We found that administration of TAU in individual doses (E1 - 321.675 mg/rabbit/day, E2 - received 643.35 mg/rabbit/day and E3 - 965.025 mg/rabbit/day) resulted a significant increase ($P<0.05$) of the diameter of the seminiferous tubules in all experimental groups when compared to control (KK) group without addition of TAU.

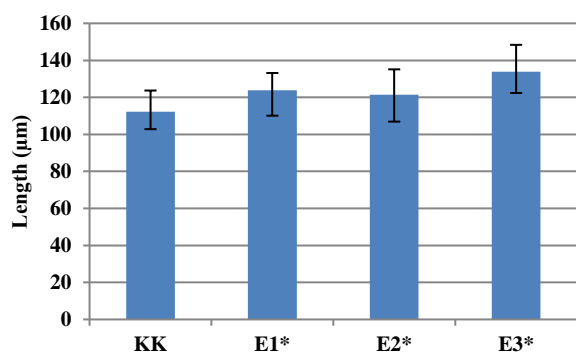


Figure 1 The values of the diameter of the seminiferous tubules
Legend: Groups of rabbit with doses of TAU: KK - 0.0 mg/rabbit/day; E1 - 321.675 mg/rabbit/day; E2 - received 643.35 mg /rabbit/day; E3 - 965.025 mg/rabbit/day;

Figure 2 shows the values for the height of the seminiferous epithelium for each experimental group of the rabbits. From the results we found, that the administration of taurine in the highest dose (965.025 mg/rabbit/day) resulted a significant increase ($P<0.05$) of the height of the seminiferous epithelium compared to the control group of rabbits. With respect to the other administered TAU concentrations, there were no significant differences.

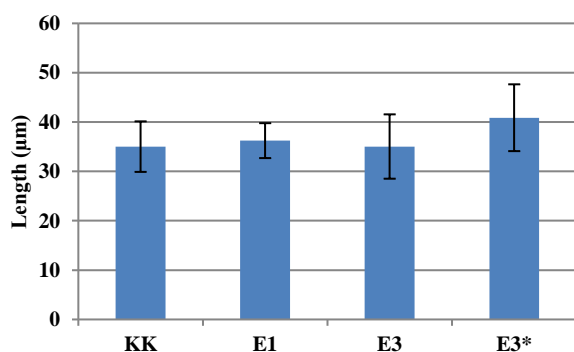


Figure 2 The values of the height of the seminiferous epithelium
Legend: Groups of rabbit with doses of TAU: KK - 0.0 mg/rabbit/day; E1 - 321.675 mg/rabbit/day; E2 - received 643.35 mg /rabbit/day; E3 - 965.025 mg/rabbit/day;

Figure 3 shows the results of the diameter of the lumen of seminiferous tubules. We can conclude that the lowest dose of TAU (321.675 mg/rabbit/day) caused

a statistically significant increase ($P<0.05$) in this parameter when compared to the control group. However, it is important to add that taurine at higher doses did not cause any significant changes.

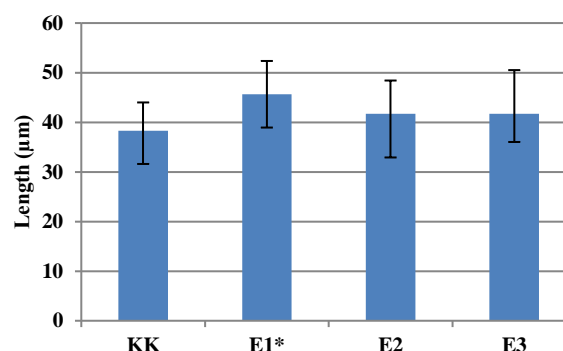


Figure 3 The values of the diameter of the lumen of seminiferous tubules
Legend: Groups of rabbit with doses of TAU: KK - 0.0 mg/rabbit/day; E1 - 321.675 mg/rabbit/day; E2 - received 643.35 mg /rabbit/day; E3 - 965.025 mg/rabbit/day;

As seen in figure 4, we observed the mean area of the seminiferous tubules. By comparing the experimental groups with the control group, we found that there was a significant increase ($P<0.05$) of the mean area size in rabbits with the highest administered dose of TAU (965.025 mg/rabbit/day), but the rest of the experimental groups did not show any significant differences in the size of the area of the seminiferous tubules.

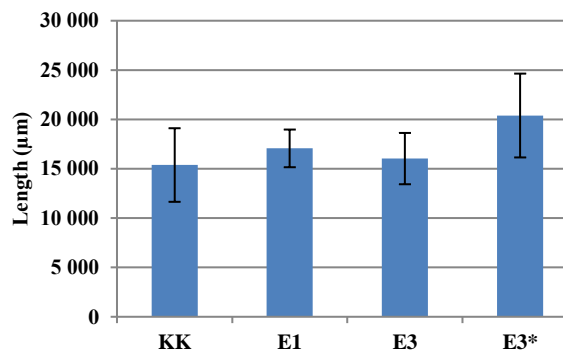


Figure 4 The values of the area of the seminiferous tubules
Legend: Groups of rabbit with doses of TAU: KK - 0.0 mg/rabbit/day; E1 - 321.675 mg/rabbit/day; E2 - received 643.35 mg/rabbit/day; E3 - 965.025 mg/rabbit/day;

Figure 5 depicts the mean values of the perimeter of the seminiferous tubules. We found that the highest dose of TAU (965.025 mg/rabbit/day) caused a significant increase ($P<0.05$) of this parameter when compared to the control group. It is also

important to add that the effect of TAU on the measured testicular structure is dose-dependent as we did not notice any statistically significant changes in the other experimental groups.

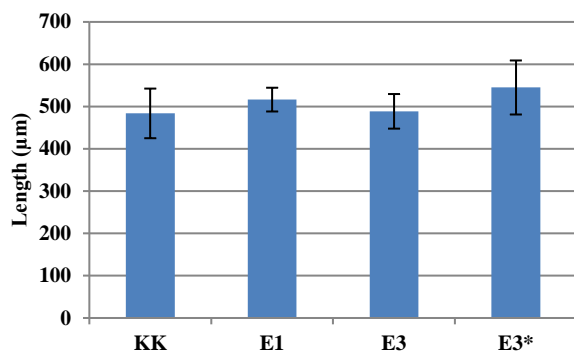


Figure 5 The values of the perimeter of the seminiferous tubules.

Legend: Groups of rabbit with doses of TAU: KK - 0.0 mg/rabbit/day; E1 - 321.675 mg/rabbit/day; E2 - received 643.35 mg /rabbit/day; E3 - 965.025 mg/rabbit/day;

DISCUSSION

In this experiment, we determined the impact of the increasing levels of intake of taurine in water on the structure of rabbit testes. After the evaluation, we may conclude that the effect of taurine to the structure of testes is dose-dependent. The results of our work suggest that oral administration of TAU causes a statistically significant increase of the diameter of the seminiferous tubules, the height of the seminiferous epithelium, the diameter of the lumen of seminiferous tubules, the area seminiferous tubules and the perimeter of the seminiferous tubules. We may state say that the most significant changes were noticed following the supplementation of the highest dose of TAU (965.025 mg/rabbit/day).

Taurine plays important roles in male reproduction, particularly in aged male animals. Taurine as a supplement was able to stimulate the secretion of male reproductive hormones (luteinizing hormone and testosterone), to increase the levels of testicular enzymes, fortify the testicular antioxidant system and improve the sperm quality (Yang et al., 2010).

Abdel-Moneim (2013) emphasized on the protective effect of TAU against histomorphological and ultrastructural changes in the testes of mice caused by an acute *in vivo* exposure to aluminium chloride (AlCl₃). A single intraperitoneal dose of AlCl₃ caused sloughing, tubular atrophy, germ-cell degeneration, Leydig cell hyperplasia, destruction of inter-Sertoli cell tight junctions, apoptotic cell death of spermatogonia and primary spermatocytes, various abnormalities in spermatid morphology and mitochondrial damage in Leydig cells. Taurine administration attenuated these changes and significantly reduced the number of affected tubules when compared to poisoned mice.

Taurine is known as an attractive candidate for attenuating various drug- and toxin-induced pathophysiological conditions through its antioxidant actions. Aly and Khafagy (2013) showed that endosulfan decreased the rat testes weight, inhibited steroidogenesis and spermatogenesis, induced oxidative stress and apoptosis. Inversely, TAU counteracted endosulfan-induced oxidative stress and apoptosis in rat testis (Aly and Khafagy, 2013).

Our results show that application of TAU induced an increase of the assessed parameters of the seminiferous tubules, which probably indicates its positive effect on the spermatogenesis. These results correspond with Yang et al. (2015), who suggested that TAU administration markedly increased the sperm count, motility and viability, decreased sperm abnormalities, decreased testicular apoptosis and suppressed the deterioration of testicular functions.

Taurine is also important during the regulation of the male reproductive system and spermatogenesis (Ahmed, 2015). Taurine and hypotaurine have been found in seminal plasma and spermatozoa of numerous species and are known to have beneficial effects on spermatozoa characteristics in mammals. Previous reports studying the effect of TAU on rabbit spermatozoa motility *in vitro*. The total progressive motility and general motility were evaluated immediately following TAU administration, after 2 hours and 24 hours of incubation. The results confirm that taurine supplementation increases motility and progressive motility of rabbit spermatozoa. With the length of incubation and increase of taurine concentration the parameters of motility were stimulated almost in all experimental groups (Kročková et al., 2013).

CONCLUSION

In this experiment, we studied the effect of taurine on the structure of rabbit testes. The results of our work show that per oral application of taurine caused a statistically significant increase of the diameter of the seminiferous tubules (E1, E2, E3), the height of the seminiferous epithelium (E3), the diameter of the lumen of seminiferous tubules (E1), the area seminiferous tubules (E3) and the perimeter of the seminiferous tubules (E3). It is important to say, that the effect of taurine is

dose-dependent, as the most significant changes were detected at its highest dose. We may conclude, that application of taurine caused a significant increase of the selected morphological parameters, which finally points to the positive effect of taurine on the sperm production. At the same time, we may suggest further experiments to study the effects of taurine on other organ systems.

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