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IN VITRO EFFECT OF TAURINE ON RABBIT SPERM MOTILITY

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Abstract
Taurine (2-aminoethane sulphonic acid), a ubiquitous β-amino acid is conditionally essential in man, also it has multiple biological and metabolic functions: is an antioxidant, it conjugates biliary acids, detoxifies some xenobiotics and modulates intracellular calcium levels. Secretion of the taurine takes place also in mammalian reproductive tract, it preserves the motility of the spermatozoa, supports their capacitation, improve the chances of success of fertilization and the early embryonic development. In our study we investigated the effect of taurine on rabbit sperm motility *in vitro*. We used semen of six adult rabbits (New Zealand White rabbits, CVŽV Lužianky, Nitra, Slovakia). Samples were mixed together to create one heterospermic sample. We weighed 10 mg of taurine and dissolved it in 10 mL of saline. The taurine solution was added in various amounts to the semen which were created experimental groups (A, B, C, D, E). The control group (K) was no added taurine. The measurements were performed using the method a Computer Assisted Semen Analyzer (CASA) system – Sperm Vision. Total motile spermatozoa and progressively motile spermatozoa were evaluated at time 0 (immediately after samples preparation), at time 1 (after 2 hours of incubation) and at time 2 (after 24 hours of incubation). The results of our experiments shows that the addition of taurine increase motility and progressive motility of rabbit sperm. With the increase of its concentration and prolonging the period of cultivation the parameters of motility were stimulated almost in all experimental groups.

Key words: rabbit, spermatozoa, taurine, CASA

Introduction

Taurine (2-aminoethane sulphonic acid), a ubiquitous β-amino acid is conditionally essential in man. It is not utilized in protein synthesis but found free or in some simple peptides. Derived from methionine and cysteine metabolism, taurine is known to play a pivotal role in numerous physiological functions (Stapleton et al., 1998). Taurine has multiple biological and metabolic functions: is an antioxidant, it conjugates biliary acids, detoxifies some xenobiotics and modulates intracellular calcium levels. Moreover, taurine plays an important part in osmoregulation, neuromodulation and stabilization of the membranes. Taurine is looked upon as an "essential amino acid" in some particular situations associating inadequate intake or synthesis and major loss of biliary salts. Clinically, taurine has been used with varying degrees of success in the treatment of several pathologies (cardiovascular diseases, cystic fibrosis, alcoholism, retinal degeneration, hepatic disorders). Being found in the secretions of the mammalian reproductive tract, it preserves the motility of the spermatozoa, supports their capacitation, improves the chances of success of fertilization and the early embryonic development. This is why it can be found in some culture media for *in vitro* fertilization (Bidri and Choay, 2003; Guérin and Ménézo, 1995). Intracellular taurine is maintained at high concentrations in a variety of cell types and alteration of cell taurine levels is difficult. The role of taurine within the cell appears to be determined by the cell type. Plasma murine levels are also high, although decreases are observed in response co surgical injury and numerous pathological conditions including cancer and sepsis. Although commonly used as a dietary supplement in the Far East, the potential advantages of dietary taurine supplementation have

not as yet been fully recognized in the Western World (Stapleton et al., 1998). Recent and past studies suggested that taurine might be a pertinent candidate for use as a nutritional supplement to protect against oxidative stress, neurodegenerative diseases or atherosclerosis (Bouckenooghe et al., 2006). Fan et al. (2009) and Yang et al. (2010) reported that male accessory sex glands are able to synthesize taurine through the cysteine sulfinate decarboxylase (CSD) pathway. Also Li et al. (2006) reported that male genital organs have the function to produce taurine through the CSD pathway, although quantifying the relation of CSD expression to taurine synthesis and the exact functions of taurine in male genital organs still need to be elucidated in future studies. Taurine and hypotaurine have been found in spermatozoa and seminal plasma of numerous species and are known to have beneficial effects on sperm characteristics in mammals. Taurine is considered an essential dietary constituent in cats. Dietary deficiency has been associated with a range of serious clinical disorders. The concentrations of taurine measured in serum samples confirmed that the cats were not deficient in taurine. Significant amounts of taurine and hypotaurine were found in spermatozoa, seminal plasma and epididymal flushing fluid (Buff et al., 2001). Lobo et al. (2000) observed taurine distribution in male rat reproductive organs by immunohistochemical methods. In the testis, taurine was found in Leydig cells, vascular endothelial cells, other interstitial cells and epithelial cells from the intratesticular segments of the rete testis. The possible functional roles for taurine in these cells are discussed. Holmes et al. (1992) investigated taurine, and hypotaurine levels in human sperm and seminal fluid. Sperm hypotaurine content was significantly correlated with sperm morphology, sperm relative forward progression, the percentage of motile sperm, and the total number of sperm in the ejaculate. By contrast, sperm taurine content was negatively correlated with these parameters. Hypotaurine, an antioxidant, may play an important role in protecting sperm from reactive oxygen species. Higher concentrations of taurine in the sperm of infertile men suggest that accelerated oxidation of hypotaurine to taurine may accompany the observed decline in other sperm parameters. Das et al. (2012) investigated the protective effect of taurine against doxorubicin- induced testicular oxidative stress and apoptosis was investigated in rats. Authors found that taurine could effectively prevent nearly all of doxorubicin-induced testicular abnormalities, thereby proving to be an effective cytoprotectant.

Material and methods

In this study semen of six adult rabbits (New Zealand White rabbits, CVŽV Lužianky, Nitra, Slovakia) was evaluated. After sampling, the samples were transported to the laboratory and mixed together to create one heterospermic sample. We weighed 10 mg of taurine and dissolved it in 10 mL of saline. Then the taurine solution was added in various amounts to the semen which were created experimental groups (Table 1). The control group (K) was no added taurine. The measurements were performed using the method a Computer Assisted Semen Analyzer (CASA) system – Sperm Vision (Minitub, Tiefenbach, Germany) equipped with a microscope (Olympus BX 51, Japan). Each sample was placed into Makler Counting Chamber (depth 10 µm, Sefi-Medical Instruments, Germany). Using the rabbit specific set up the following parameters were evaluated – total motile spermatozoa and progressively motile spermatozoa at time 0 (immediately after samples preparation), at time 1 (after 2 hours of incubation) and at time 2 (after 24 hours of incubation). Incubation between each measurement was carried in conditions 2-5 °C. Obtained data were statistically analyzed with the help of the PC program Excel and a statistics package SAS 9.1 (SAS Institute Inc., USA) using Student`s t-test and Scheffe`s test. Statistical significance was indicated by *p* values of less than 0.05; 0.01 and 0.001.

Table 1

Experimental groups preparation.

Experimental group	Semen (µL)	Taurine solution (µL)	Saline solution (µL)
K	50	-	
A	50	18.75	131.25
B	50	37.5	112.5
C	50	75	75
D	50	112.5	37.5
E	50	150	-

Results

The measurements of the parameters of sperm motility (Fig. 1) at time 0 showed a significant increase in the experimental group C (84.45±4.15%), D (81.68±4.57%) and E (82.83±2.89%)

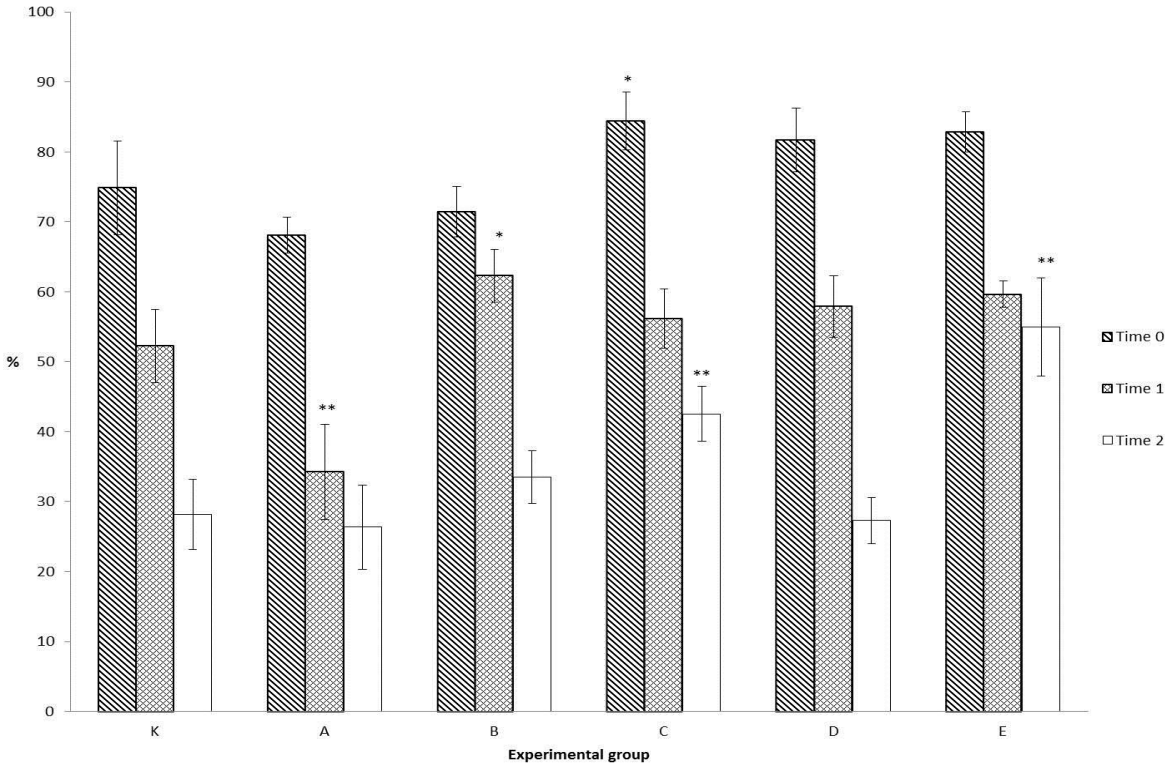

Fig. 1

Sperm motility after taurine addition

Legend: * $p < 0.01$, ** $p < 0.001$

with the addition of the highest concentrations of taurine. Significant difference was in the experimental group C ($p < 0.01$) compared with the control group (74.84±6.66%). At the time 1 the motility increased in all experimental groups except for group A (34.23±6.08%). Motility was significantly higher in group B (62.27±3.74%; $p < 0.01$) as compared with the control group (52.25±5.24%). At the time 2 we recorded marked motility increase in the experimental group C (42.53±3.90%; $p < 0.001$) and E (54.95±7.00%; $p < 0.001$) compared to the control group (28.18±5.00%). Progressive motility (Fig. 2) was similar to the motility. At time 0, there was a

significant increase in experimental group C (66.53±5.19%; $p < 0.01$), D (67.48±3.72%; $p < 0.01$) and E (71.27±3.64%; $p < 0.001$) compared with the control group (55.49±8.18%). At the time 1 progressive sperm motility increased in all experimental groups except for group A (13.21±3.78%) compared with the control group (28.72±6.08%). At the time 2 we recorded marked increase of progressive motility in the experimental group C (21.67±4.41%; $p < 0.001$) and E (19.73±5.43%; $p < 0.05$) compared to the control group (12.19±4.25%).

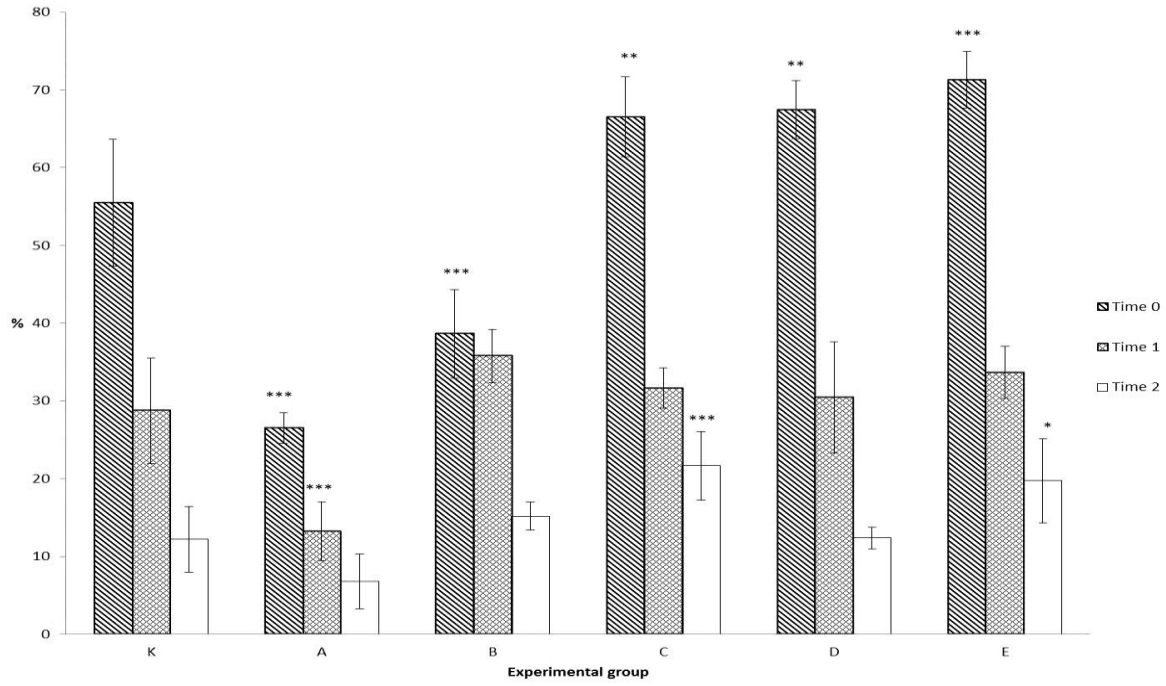

Fig. 2

Progressive sperm motility after taurine addition

Legend: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

Discussion

The results of our experiments shows that the addition of taurine increase motility and progressive motility of rabbit sperm. With the increase of its concentration and prolonging the period of cultivation the parameters of motility were stimulated almost in all experimental groups. Taurine and β-alanine (taurine transport inhibitor) were offered in water to male rats of different ages. The motility of spermatozoa was obviously increased by taurine supplement in adult rats. The numbers and motility of spermatozoa, the rate of live spermatozoa were significantly increased by taurine supplement in aged rats (Yang et al., 2010). Chhillar et al. (2012) studied the effects of taurine or trehalose supplementation on functional competence of cryopreserved Karan Fries semen. The results obtained clearly indicated that supplementation of taurine (50 mM) or trehalose (100 mM) to tris-egg yolk citrate (EYTC) extender prior to cryopreservation improves Karan Fries sperm quality. Martínez-Páramo et al. (2013) evaluated taurine and hypotaurine as potential additives to improve European sea bass (*Dicentrarchus labrax*) sperm quality after cryopreservation. For cryopreservation, three different extenders were used: control extender (NAM), supplemented with 1mM taurine or supplemented with 1mM hypotaurine, all of them containing 10% Me_2SO as cryoprotectant. To evaluate sperm quality of fresh and thawed sperm, motility (CASA), viability (SYBR Green/propidium iodide), lipid peroxidation (malondialdehyde level), protein oxidation (carbonyl content), glutathione peroxidase, glutathione reductase and superoxide dismutase activities and DNA fragmentation (comet assay) were quantified. The result demonstrated that 1mM hypotaurine supplemented extender increased total motility (30.1±3.2%), and that 1mM

taurine extender produced higher velocity (18.1±2.6µm/s) and linearity (46.0±4.8%) than the control extender (21.8±3.2%, 15.5±1.3µm/s, 41.8±2.4%, respectively). Cell viability, lipid peroxidation and protein oxidation were not statistically different between treatments. Similar results were obtained for glutathione peroxidase and superoxide dismutase activities. Only glutathione reductase showed differential activity before and after freezing, increasing its activity in thawed sperm. Regarding the comet assay results, taurine and hypotaurine significantly reduced DNA fragmentation (52.8±0.9% and 51.8±0.9%, respectively) in comparison to the control (55.7±0.8%). Authors reported that extenders supplemented with 1mM taurine and 1mM hypotaurine improved some parameters of sperm quality after thawing, resulting in better motility and lower DNA damage than the control, two very important factors related to fertilization success Alam et al. (2011) treated mice with taurine orally (100. mg/kg. b.wt.) for nine days. Treatment with taurine showed increases sperm count and motility, and decreases the incidence of sperm abnormalities. Das et al. (2009) reported that oral administration of taurine (at a dose of 100 mg/kg body weight for 5 days) to rats was found to be effective in counteracting arsenic-induced oxidative stress, attenuation of testicular damages and amelioration of apoptosis in testicular tissue. Taurine was also found to play similar beneficial role via mitochondrial dependent pathways in arsenic-induced testicular damages leading to apoptotic cell death. Cabrita et al. (2011) analyzed the effect of extender supplementation with taurine on post-thawed sperm motility, viability and DNA integrity of two commercial species, gilthead seabream (*Sparus aurata*) and European seabass (*Dicentrarchus labrax*). Sperm was cryopreserved in ten different extenders containing taurine (1 and 10. mM). Taurine slightly increased the percentage of motile cells and significantly reduced both DNA fragmentation parameters, protecting DNA against strand breaks.

Conclusion

At the end of our experiment, we observed that the addition of small amounts of taurine in rabbit sperm has a stimulating effect on sperm motility. The increasing of taurine concentrations and extension of its period of exposure, taurine has positive effect on sperm motion parameters.

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