NATIONAL AGRICULTURAL AND FOOD CENTRE
RESEARCH INSTITUTE FOR ANIMAL PRODUCTION NITRA

Slovak J. Anim. Sci., 48, 2015 (2): 49–56

ISSN 1337-9984

EFFECT OF TAURINE ON BOVINE SPERMATOZOA MOTILITY PARAMETERS FOLLOWING CRYOPRESERVATION

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ABSTRACT

The use of artificial insemination in animal production and especially in cattle breeding is an important intensification factor. For the purpose of maximal use of genetic potential, importance is always put on the creating conditions for effective insemination. The aim of this work was to analyze the effect of the taurine addition to bovine ejaculates in the process of cryopreservation on the spermatozoa motility.
Taurine was dissolved in a saline to obtain various concentrations (50, 100, 200, 400 and 600 mM) and added to the extender. Fresh ejaculates collected from six breeding bulls were used in the experiment. Ejaculates were placed to the prepared extender. The control sample consisted of the extender only. After four hours of exposure at 4 °C, the ejaculates were put into straws with a volume of 0.5 ml. The semen was then exposed to freezing temperature of nitrogen vapour for 10 min. The straws were stored for one month in liquid nitrogen at -196 °C. Thereafter, the spermatozoa motility parameters were analyzed using the CASA method. This assay was repeated four times in the intervals of 0, 30, 60 and 90 min at 39 °C. The correlation between the time after thawing and spermatozoa motility shows a regressive character in the curve. In semen doses with taurine at 200 mM significant increase in the motility and progressive motility, in comparison to the control sample, was recorded. The results show that the addition of taurine increases total motility and progressive motility of bovine spermatozoa.

Key words: spermatozoa; cryopreservation; taurine; CASA; bull; artificial insemination; motility

INTRODUCTION

The spermatozoa is a male gamete almost devoid of cytoplasm with large nucleus, containing haploid amount of highly condensed chromosomes, an acrosome, responsible for interaction and penetration of oocyte and series of mitochondria located at the anterior region of the flagellum (Eddy and O´Brien, 1994). Mitochondria produce ATP mainly for the purpose of maintaining motility of the spermatozoa, while Golgi apparatus and endoplasmic reticulum work on maintaining integrity of the cell membrane (Medeiros *et al*., 2002). Not more than 7 % of spermatozoa are present in the ejaculate. Spermatozoa are protected and nourished by seminal plasma. Bull seminal plasma originates from the urethral glands, the ampullary glands and the seminal vesicles (Rothschild and Barnes, 1954). According to Massányi *et al.* (2003), there are significant differences in composition of seminal plasma among different animals due to differences in structure and function of reproductive system.

Artificial insemination (AI) is the mostly used biotechnology method in cattle reproduction (Foote *et al.*, 2002). Use of the AI has a great potential in breeding of domestic animals. Its benefits come out of potential use of genetic material from small number of superior sires (Watson, 2000; Maxwell and Watson, 1996). AI allows crossbreeding, which results in hardening traits in milk and meat production (Unal *et al*., 2006).

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Received: April 24, 2015
Accepted: May 19, 2015

Cryopreservation is a method of storing cells and tissues in liquid nitrogen, which have use in different fields of biology, medicine and agriculture (Andrabi and Maxwell, 2007). The use in agriculture is focused on genetic improvement of domestic species and preserving rare plant species and rare breeds of animals (Holt, 1997). Arav *et al*. (2002) state that vitrification is the most beneficial method of cryopreservation of spermatozoa because of decreasing cold shock by rapid freezing. Cryopreservation is a method which slows the cellular metabolic activity but restarts it after thawing (Medeiros *et al*., 2002; Mazur, 1984). Intracellular ice crystals are formed during cryopreservation, what results in different damages to spermatozoa, such as a cytoplasm fracture, abnormalities in the cytoskeleton and in genome-related structures (Isachenko, 2003). According to Del Maestro (1980), decline in motility, functional integrity of spermatozoa membranes and fertility can be attributed to the action of reactive oxygen species (ROS), namely hydrogen peroxide (H_2O_2) and superoxide anion radical (O_2-). Protection against ROS and improved spermatozoa motility after addition of an antioxidant like taurine to the extender was proved by Bucak and Tekin (2007).

Extenders for semen cryopreservation must have suitable osmolality, adequate pH and buffering capacity to protect spermatozoa from cryogenic injury (Salamon and Maxwell, 2000). Cryoprotectants used as extenders are either penetrating or non-penetrating the cell membrane. Taurine is, therefore, classified as a non-penetrating and acts extracellularly (Barbas and Mascarenhas, 2009; Purdy, 2006).

Taurine is an organic acid, which contains sulphur. Molecular structure of taurine is very similar to γ-aminobutyric acid (GABA), which is the main neurotransmitter in brain (Huxtable, 1992). Taurine has cytoprotective abilities which emerge from the ability to detoxicate, osmoregulate and maintain calcium homeostasis (Devi *et al*., 2008). Sinha *et al*. (2008) mention that mechanisms of taurine cytoprotective abilities are still not well-investigated, however taurine can be perceived as an antioxidant due to its efficiency in efflux of free radicals along with maintaining the cell membrane permeability exposed to ROS.

The aim of this study was to determine spermatozoa motility in semen doses with various concentrations of taurine in comparison to conventionally produced semen doses without taurine.

MATERIAL AND METHODS

Animals and semen collection

Semen samples were obtained from 6 breeding bulls. All bulls were held and maintained under usual housing and feeding conditions. Ejaculates were collected to artificial vagina maintained at temperature of 38 – 40 °C. Consistency of ejaculates was determined spectrophotometrically.

Semen processing

The amount of extender added to ejaculates was calculated according to its consistency to reach at least $20x10^6$ spermatozoa per each insemination dose. Extender, consisted of egg yolk, glycerine, fructose, citric acid, Tris, *aqua pro injectione*, antibiotics Norostrep® a Linco-Spectin® and taurine (Taurine ≥ 99 %, Sigma Aldrich, Bratislava, Slovakia) dissolved in physiological solution, was added to experimental samples. Concentrations of taurine in physiological solution were 50 mM, 100 mM, 200 mM, 400 mM and 600 mM. Control sample (without taurine) was labeled with letter X and experimental samples were marked according to increasing taurine concentration with letters A, B, C, D, E. Diluted ejaculates were cooled down to 4 °C for four hours. Ejaculates were filled into straws with the volume of 0.5 ml. Consecutive cooling of straws at 4 °C lasted for 10 min. Subsequent freezing involved 10 min exposure to liquid nitrogen vapor, afterwards the straws with sperm samples were stored in liquid nitrogen for a one month.

Semen evaluation

Semen analyses were performed using the CASA method with SpermVision software (Minitub, Tiefenbach, Germany) and the microscope Olympus BX 51 (Olympus, Japan). Five straws of control group and five straws of each experimental concentration were analyzed to achieve the most authentic results. After thawing samples were placed to thermostat at the temperature of 39 °C and afterward it were transferred to Makler counting chamber (10μm, Sefi–Medical Instruments, Germany). Measurements of spermatozoa motility were repeated three-times every half an hour (Time 0, 30, 60, 90) and the tested samples were stored in a thermostat at 39 °C. The following spermatozoa characteristics were assessed: motility (MOT), progressive motility (PRO), beat cross frequency (BCF), curvilinear velocity (VCL) and amplitude of lateral head displacement (ALH).

Statistical analysis

For the comparison of the CASA results in certain time intervals with the focus on effects of extenders, ANOVA and Dunnett´s comparative test were applied using GraphPad Prism 5 (GraphPad Software Inc., USA). Control sample was considered to be 100 % and, at the same time, a basic comparison value for all experimental samples was set. All statistical tests were carried out at levels of significance at $p < 0.05$, $p < 0.01$ and $p < 0.001$.

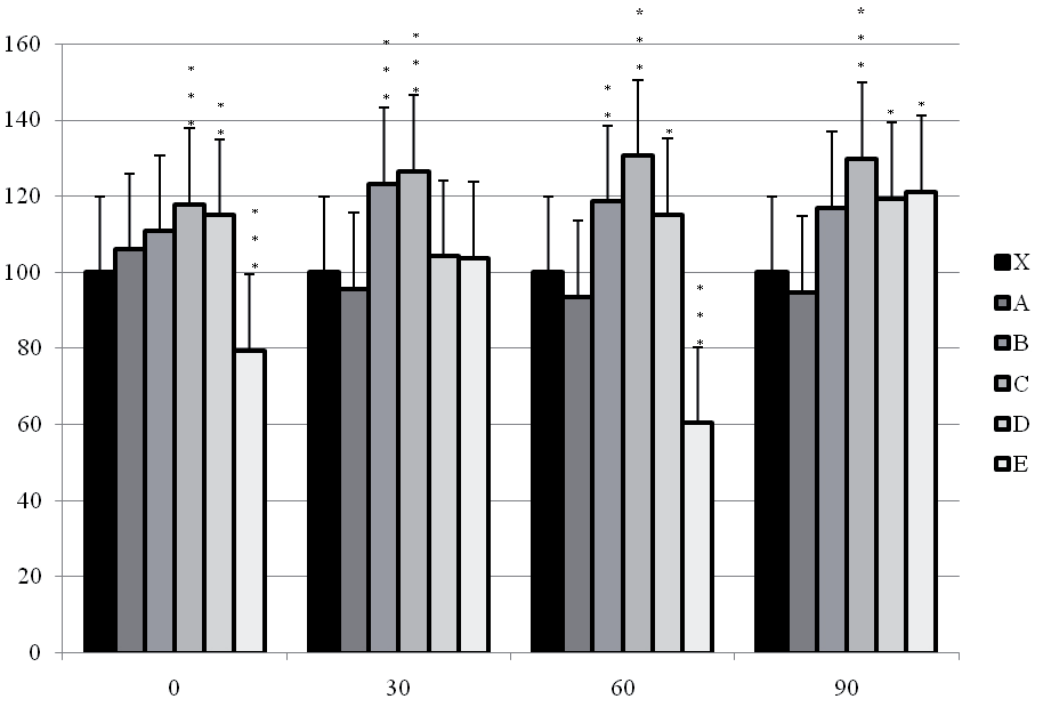

Fig. 1: Spermatozoa motility [control = 100 %] in different taurine concentrations at various time intervals (min.) (*p ≤ 0.05; **p ≤ 0.01; ***p ≤ 0.001)

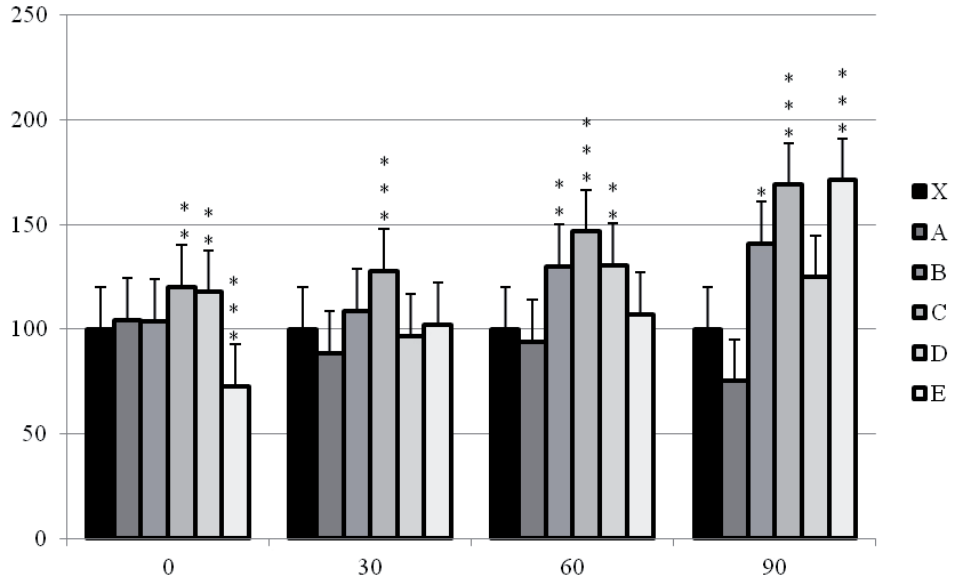

Fig. 2: Spermatozoa progressive motility [control = 100 %] in different taurine concentrations at various time intervals (min.) (*p ≤ 0.05; **p ≤ 0.01; ***p ≤ 0.001)

RESULTS

Post-thawing differences between conventionally protected spermatozoa and 5 various taurine concentrations of experimentally protected spermatozoa were assessed using CASA method.

Spermatozoa motility (Figure 1), measured immediately after thawing, was in control sample lower than in experimental samples, except for the E group (the highest taurine concentration). Significance difference was found for the C ($p < 0.001$) and D ($p < 0.01$) concentration. After 30 min incubation

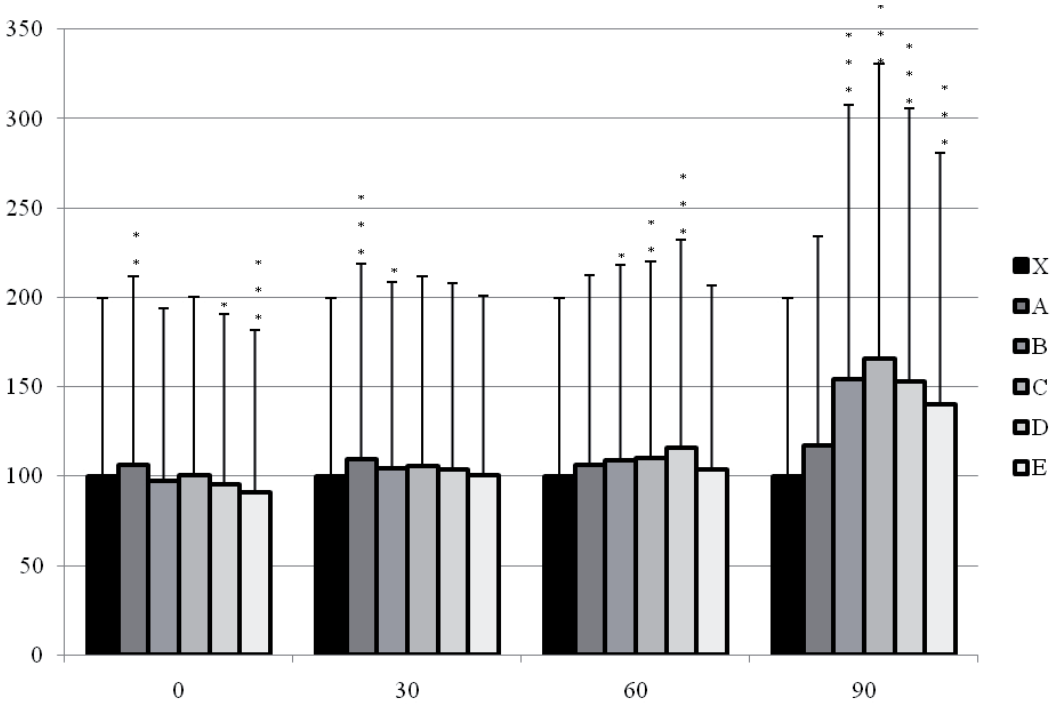

Fig. 3: Beat cross frequency [control = 100 %] in different taurine concentrations at various time intervals (min.) (*p ≤ 0.05; **p ≤ 0.01; ***p ≤ 0.001)

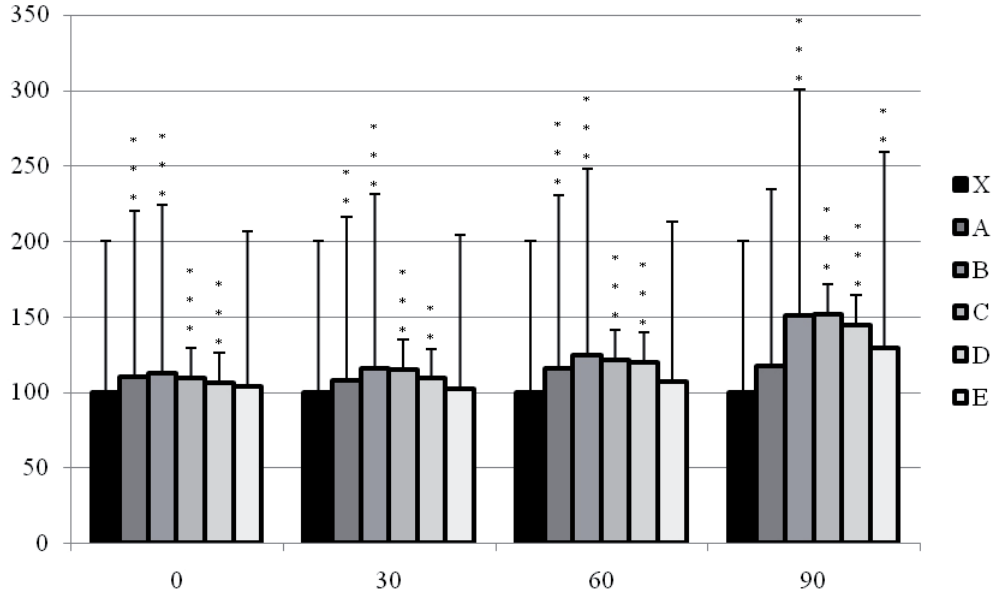

Fig. 4: Curvilinear line velocity [control = 100 %] in different taurine concentrations at various time intervals (min.) (*p ≤ 0.05; **p ≤ 0.01; ***p ≤ 0.001)

significantly higher motility ($p < 0.01$) was detected in samples B and C. Significant increase in the motility was found for concentrations B ($p < 0.01$), C ($p < 0.001$) and D ($p < 0.05$) 60 min after thawing. After 90 min of incubation a higher motility was recorded for C ($p < 0.001$), D and E ($p < 005$) groups.

Spermatozoa progressive motility (Figure 2) at the beginning of the assessment reflected results of spermatozoa motility. Only E concentration showed lower progressive motility compared to control ($p < 0.001$). Higher progressive motility ($p < 0.01$) was recorded for C and D groups. After 30 min incubation a significant increase was recorded only for C concentration ($p < 0.001$). After 60 min significantly higher spermatozoa progressive motility was found at B ($p < 0.05$), C and E concentrations ($p < 0.001$). The further 30 min incubation at 39 °C resulted in significantly higher progressive motility in groups B ($p < 0.05$), C and E ($p < 0.001$).

Beat cross frequency (Figure 3) at initial time showed positive effect of taurine on spermatozoa in sample B ($p < 0.01$). Negative effect was proven in samples D ($p < 0.05$) and E ($p < 0.001$). Following 30 min incubation higher BCF values in comparison to control were recorded for A ($p < 0.001$) and B ($p < 0.05$) concentrations. The further 30 min of incubation resulted in higher BCF at concentrations B ($p < 0.05$), C ($p < 0.01$) and D ($p < 0.001$). After 90 min incubation significantly higher values were recorded for samples C ($p < 0.001$), D ($p < 0.05$) and E ($p < 0.05$).

Velocity of spermatozoa in curvilinear line (VCL; Figure 4) was higher in all experimental samples compared to the control sample for all time periods. Significant differences with control group ($p < 0.001$) during the first assessment of VCL was found in samples A, B, C, D. Incubation in a thermostat for 30 min positively affected all experimental samples. Incubation for 90 min resulted in significantly higher VCL in samples B (+41 %; $p < 0.001$), C (+69 %; $p < 0.001$), D (+25 %; $p < 0.001$) and E (+71 %; $p < 0.01$).

Amplitude of lateral head displacement (ALH; Figure 5) copied previous spermatozoa motility parameters with significant differences (sample B – $p < 0.05$; C – $p < 0.001$). After 30 min incubation significant increase was detected for the group B ($p < 0.05$) and C ($p < 0.05$). No significant differences were found after 60 min of incubation. After 90 min incubation positive effect of taurine was recorded for samples B (+24 %; $p < 0.01$), C (+26 %; $p < 0.001$) and D (+27 %; $p < 0.001$).

DISCUSSION

Differences between fresh and frozen-thawed semen are significant, what is reflected in reduced fertility (Salamon and Maxwell, 1995). It is also proved that frozen-thawed semen contains only 50 % of motile spermatozoa in comparison with fresh semen (Salamon and Maxwell, 2000).

Massányi *et al*. (2011) analyzed effect of various additives on bull spermatozoa motility and, on the basis of CASA results, determined that substances with

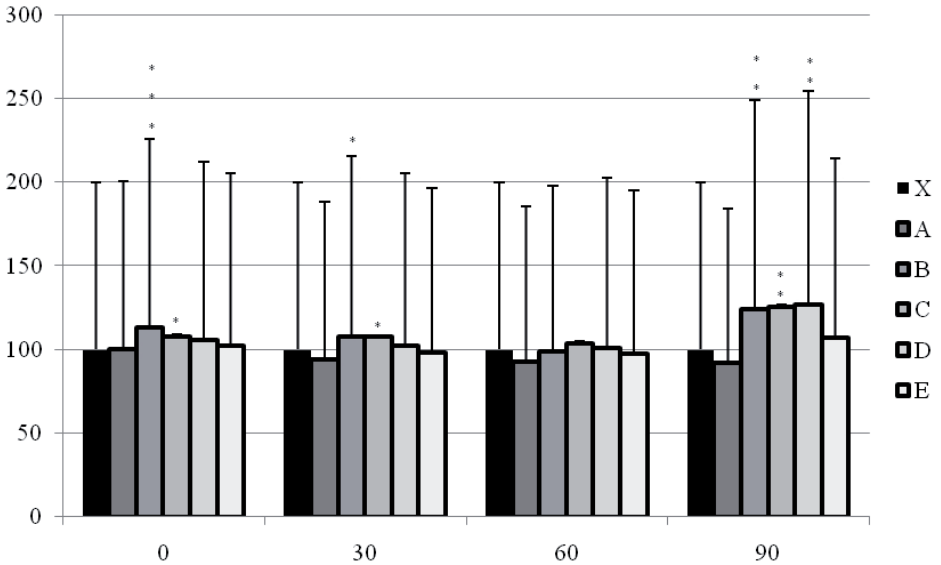

Fig. 5: Amplitude of lateral spermatozoa head displacement [control = 100 %] in different taurine concentrations at various time intervals (min.) (*p ≤ 0.05; **p ≤ 0.01; ***p ≤ 0.001)

antioxidant action have positive impact on spermatozoa motility, what is in accordance with our data. Bucak and Tekin (2007) studied effect of taurine on ram semen during cryopreservation. Experimental sperm samples, enriched with 50 or 100 mM taurine showed, that taurine contributes to creation of an ideal environment for the spermatozoa, in comparison with conventional TRIS-based egg yolk extender. Motility parameters measured at time intervals of 0, 6, 24 and 30 hours are in concert with our data on bovine spermatozoa recorded following 0, 30, 60, 90 min after thawing. Although the time intervals are different, the trend of higher motility in taurine protected spermatozoa was proved. Significant differences in taurine-treated cryopreserved semen were demonstrated also by Chhillar *et al*. (2012). In addition to motility analysis membrane integrity and intracellular calcium tests were performed. The results show that taurine antioxidant action may be the reason of higher motility in experimental sperm samples.

Taurine has multiple biological and metabolic functions as an antioxidant that conjugates biliary acids, detoxifies some xenobiotics and modulates intracellular calcium levels. Taurine 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. 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 spermatozoa characteristics in mammals. Previous study investigated the effect of taurine on rabbit spermatozoa motility in vitro (Kročková *et al*., 2013). Total spermatozoa motility and progressive motility were evaluated immediately after samples preparation, after 2 hours of incubation and after 24 hours of incubation. The results confirm that the addition of taurine increases motility and progressive motility of rabbit spermatozoa. With the increase of its concentration and the length of incubation the parameters of motility were stimulated almost in all experimental groups (Kročková *et al*., 2013). Also, significant amounts of taurine and hypotaurine were found in spermatozoa, seminal plasma and epididymal flushing fluid (Buff *et al*., 2001).

Holmes *et al*. (1992) determined taurine and hypotaurine levels in human spermatozoa and seminal fluid. Sperm hypotaurine content was significantly correlated with the spermatozoa morphology, relative forward progression, the percentage of motile spermatozoa and the total number of spermatozoa in the ejaculate. Oppositely, sperm taurine content was negatively correlated with these parameters. Hypotaurine, as an antioxidant, may play an important role in protecting spermatozoa from reactive oxygen species. Higher concentrations of taurine in the spermatozoa of infertile men suggest that accelerated oxidation of hypotaurine to taurine may accompany the observed decline in other spermatozoa parameters. The results of our experiments show, that the addition of taurine increases total motility and progressive motility of bovine spermatozoa. The elevation of taurine concentrations and the length of incubation resulted in the stimulation of the sperm motility almost in all experimental groups.

ACKNOWLEDGEMENT

This study was supported by the projects VEGA 1/0760/15; 1/0857/14, APVV-0304-12 and KEGA 006/SPU-4/2015. This work was financially supported by the European Community under project No. 26220220180: Building Research Centre „AgroBioTech".

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