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Effect of Taurine on Turkey (*Meleagris gallopavo*) Spermatozoa Viability and Motility

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ABSTRACT

Slanina T., Miškeje M., Tirpák F., Błaszczyk M., Stawarz R., Massányi P. (2018): **Effect of taurine on turkey (*Meleagris gallopavo*) spermatozoa viability and motility**. Czech J. Anim. Sci., 63, 127–135.

The effect of taurine on the turkey spermatozoa motility and viability during the *in vitro* incubation was assessed. Experimental samples were prepared by diluting the raw semen in nine different concentrations of taurine – from 10 mg/ml to 0.078125 mg/ml. The motility parameters were evaluated by the CASA system (Computer Assisted Semen Analyser) using the program Sperm Vision® and for spermatozoa viability assessment the eosin-nigrosin staining was performed. Selected parameters were evaluated at six time periods: 0, 1, 2, 3, 4, and 5 h at 5°C and 41°C. At 5°C, a significantly lower percentage of motility and progressive motility was detected only in the samples with the highest concentration of taurine (10 mg/ml) at time 0 and 1. After 2 h of incubation a significant preventive effect of taurine on spermatozoa parameters was observed. The tendency of the taurine effect on motility parameters was different during the *in vitro* incubation at 41°C. Significantly lower values of motility parameters were detected in all experimental samples in comparison to the control after 5 h. The analysed concentrations of taurine did not significantly affect viability of turkey spermatozoa during all time periods. A higher percentage of dead spermatozoa were observed at 41°C (4.87–9.90%) if compared to 5°C (2.12–4.88%). The results indicated that the addition of taurine (from 2.5 to 7.5 mg/ml) to turkey spermatozoa positively affected the monitored spermatozoa parameters incubated at 5°C.

Keywords: amino acid; liquid storage; *in vitro*; CASA system; membrane integrity; fowl

Taurine, also known as 2-aminoethanesulfonic acid, is one of the most widely distributed low molecular compounds in humans as well as in animals. Taurine takes part neither in protein synthesis nor in metabolic pathways. However, some physiological functions of taurine were proved – osmoregulation, calcium modulation, anti-oxidation, membrane stabilization, energy storage, xenobiotic conjugation, and anion balance (Yang et al. 2010a).

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As a semi-essential amino acid, taurine is highly contained in various tissues and can be biosynthesized by many tissues such as central nervous system, liver, kidneys, retina, and mammary gland. In addition, in male reproduction system taurine was detected in Leydig cells, blood vessel endothelium cells, and other interstitial cells of testicles and epithelial cells of efferent ducts in rats (Lobo et al. 2000). Li et al. (2006) discovered that taurine can be biosynthesized by male reproductive organs. Taurine was identified as the major free amino acid of spermatozoa and semen plasma. Therefore, taurine may act as an antioxidant, capacitation factor, membrane stabilization factor, and factor of spermatozoa motility. It can also stimulate secretion of testosterone in both *in vivo* and *in vitro* conditions (Yang et al. 2010b).

The anti-oxidative action of taurine is described as either detoxification of reactive intermediate metabolites (as hypochlorous acid, nitric oxide, hydrogen peroxide), or intercalation into the plasmatic membrane (Dawson and Wallace 1992). In the study by Yun et al. (2013) the taurine antioxidative action was demonstrated by the protection of mitochondria against excessive production of superoxide radical when taurine regulated synthesis of mitochondrial proteins.

Taurine and hypotaurine are necessary compounds for spermatozoa capacitation, fertilization, and development of embryo. Hypotaurine has a protective function against peroxidative damage. Hypotaurine and taurine were quantified in spermatozoa, semen, and in secretion of oviducts in various animals (Guerin et al. 1995).

Taurine is present in spermatozoa and seminal fluid in numerous species and it is known that taurine has a positive effect on mammalian spermatozoa. It was also pointed out that taurine has an important function in the maintenance and stimulation of spermatozoa motility and in the stimulation of capacitation and acrosome reaction in *in vivo* and *in vitro* conditions. Taurine can also inhibit lipid peroxidation in rabbit spermatozoa and prevent the loss of motility (Alvarez and Storey 1983).

Taurine, a non-enzymatic antioxidant, has a positive effect on spermatozoa during cryoconservation due to reduced damage on male gametes. Taurine inhibits lipid peroxidation and protects cells against reactive oxygen species (ROS) accumulation (Chhillar et al. 2012). The addition of taurine results in improved inceptive motility of rat spermatozoa after thawing with ongoing effect (Sanchez-Partida et al. 1997). In recent years, taurine has been used as an additive to cryopreservation media for buffalo (Reddy et al. 2010; Kumar and Atreja 2012), boar (Gutierrez-Perez et al. 2009; Hu et al. 2009), ram (Bucak and Tekin 2007), and dog (Martins-Bessa et al. 2009) spermatozoa with the purpose to improve particular spermatozoa characteristics after thawing (Chhillar et al. 2012).

The aim of this study was to determine the most efficient taurine concentration which would stimulate the motility of turkey spermatozoa without a negative effect on their viability. The reason is to find a new, effective, and cheaper supplement of turkey semen extenders.

MATERIAL AND METHODS

Biological material. In this study, semen from sexually mature turkeys (n = 30) line of Big 6 (British United Turkeys (BUT) Ltd., Chester, UK) was used. The semen was collected by stimulating the copulatory organ to protrude by massaging the abdomen and the back over the testes. Semen samples were collected with an aspirator and used as a mixture of several groups of identical individual turkeys (Slanina et al. 2012). In this way heterosperm consisting of 5–7 different semen samples was prepared as is generally used in artificial insemination on farms.

Sample preparation. The semen was diluted in a ratio of 1 : 100. The fresh turkey spermatozoa was diluted in one of 9 taurine solutions of different concentration (Taurine ≥ 99%; Sigma-Aldrich, USA) prepared with physiological saline (NaCl 0.9% w/v intravenous infusion (Bieffe Midetal S.p.A., Italy)) (in mg/ml): I: 0.078125, H: 0.15625, G: 0.3125, F: 0.625, E: 1.25, D: 2.5, C: 5, B: 7.5, A: 10. The samples of each concentration were prepared and divided into two experimental groups, based on the incubation temperature: 5°C and 41°C (I–A vs IT–AT). Control samples (sample K and/or KT) were prepared by diluting the fresh semen with physiological solution.

Motility analysis. Motility was assessed at six time intervals: 0, 1, 2, 3, 4, and 5 h after spermatozoa collection and experiments were realized in six repetitions. The samples were evaluated

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using the CASA system (Computer Assisted Semen Analyser) with the Sperm Vision® program (Minitube, Germany) equipped with a microscope Olympus BX 51 (Olympus Corp., Japan) to assess the spermatozoa motility (Massanyi et al. 2008; Krockova et al. 2012; Slanina et al. 2015a). Each sample (10 µl) was placed into Makler Counting Chamber® (depth 10 µm) (Sefi Medical Instruments Ltd., Germany). Using the turkey-specific set-up, the total motile spermatozoa (MOT; %) and progressively motile spermatozoa (PRO; %), beat cross frequency (BCF), curvilinear velocity (VCL), and amplitude of lateral head displacement (ALH) were selected. Within each of the measurements by the CASA system motility parameters from minimum seven fields of the Makler Counting Chamber were evaluated (Blaszczyk et al. 2013; Slanina et al. 2015b).

Viability analysis. The spermatozoa viability was evaluated using eosin-nigrosin staining methods (Slanina et al. 2016). The membrane integrity was determined by the samples containing the highest concentration of taurine – samples A/AT, B/BT, C/CT, D/DT, E/ET and in the control sample – K/KT. From all the samples smears were prepared. Experimental samples A–I and the control sample were diluted in the ratio 1 : 2 : 2 with 5% eosin (Eosin Y) and 10% nigrosin (Nigrosin) solution (both Sigma-Aldrich). For each slide 300 cells were counted under a light microscope (1000×, Leica DMIL LED; Leica Microsystems CMS GmbH, Germany) and classified as viable (intact membrane) and dead (damaged membrane). The experiment was realized in six replicates. The results of viability evaluation were expressed as the percentage of viable and dead spermatozoa (in %).

Statistical analysis. Obtained data were statistically analyzed using MS Excel program and a statistics package GraphPad Prism 5 (GraphPad Software, Inc., USA) using one-way ANOVA with Dennett's post-test. Statistical significance was indicated by *P*-values < 0.05, 0.01, and 0.001.

RESULTS

Spermatozoa motility

Incubation temperature 5°C. During the 5°C incubation temperature, spermatozoa motility (MOT; Figure 1) fluctuated in the range from 58.48 to 35.27% during the entire incubation time. Only the sample with the highest taurine concentration (A) at time intervals 0 and 1 h showed a significant decrease ($P < 0.001$) in spermatozoa MOT in comparison with the control sample. Positive effect of taurine was observed after 2 h of incubation in sample C ($P < 0.01$) where even higher effect of taurine was detected after further incubation. MOT in experimental samples B, C, and D was significantly higher compared to control. Consider-

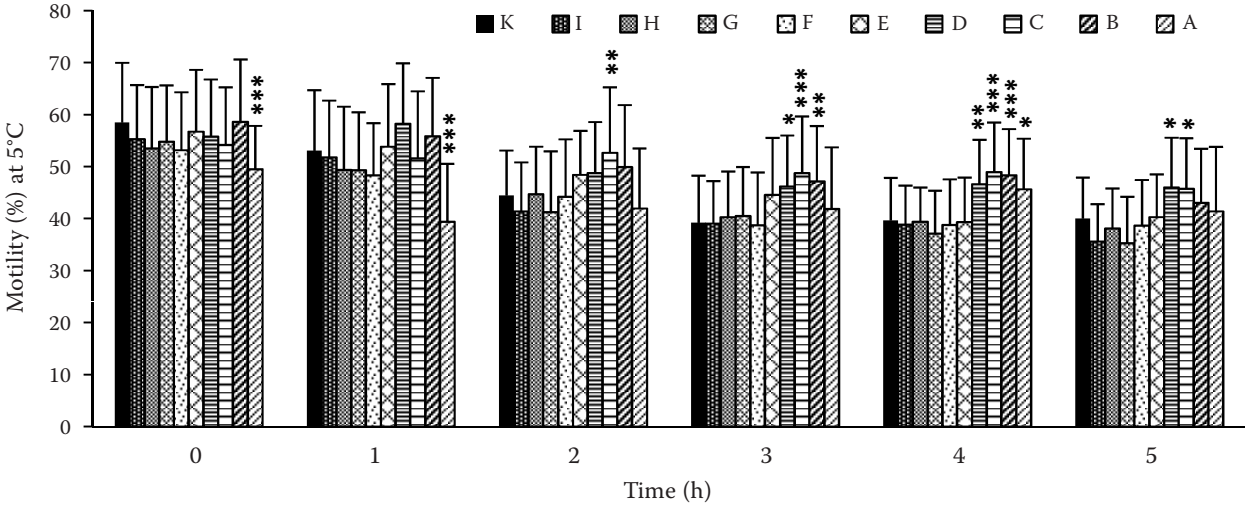

Figure 1. Spermatozoa motility (%, mean values) after incubation at 5°C

K = control, I = 0.078125 mg/ml, H = 0.15625 mg/ml, G = 0.3125 mg/ml, F = 0.625 mg/ml, E = 1.25 mg/ml, D = 2.5 mg/ml, C = 5 mg/ml, B = 7.5 mg/ml, A = 10 mg/ml

error bars represent the standard deviation

significant differences: *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$

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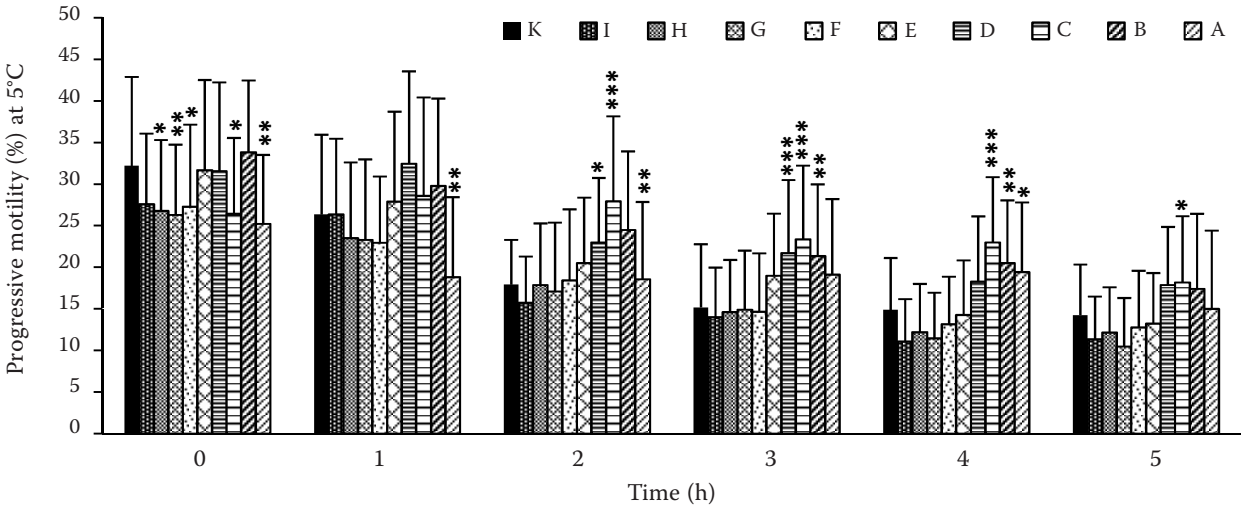

Figure 2. Spermatozoa progressive motility (%, mean values) after incubation at 5°C
K = control, I = 0.078125 mg/ml, H = 0.15625 mg/ml, G = 0.3125 mg/ml, F = 0.625 mg/ml, E = 1.25 mg/ml, D = 2.5 mg/ml, C = 5 mg/ml, B = 7.5 mg/ml, A = 10 mg/ml
error bars represent the standard deviation
significant differences: $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$

ing progressive motility (PRO; Figure 2), negative effect of concentrations in samples A, C, F, G and H was recorded at the beginning of incubation. According to MOT results, a significant increase in numbers of progressively moving spermatozoa in samples B, C, and D was noted when compared to K after 2–5 h of incubation.

Negative effect in inceptive incubation intervals was found also in curvilinear line velocity (VCL, Figure 3) parameter. Significantly lower values were obtained in samples G ($P < 0.05$) and A ($P < 0.001$). Positive effect was assessed at the highest taurine concentrations (C, B, and A) after 2 h of incubation and persisted for the remaining

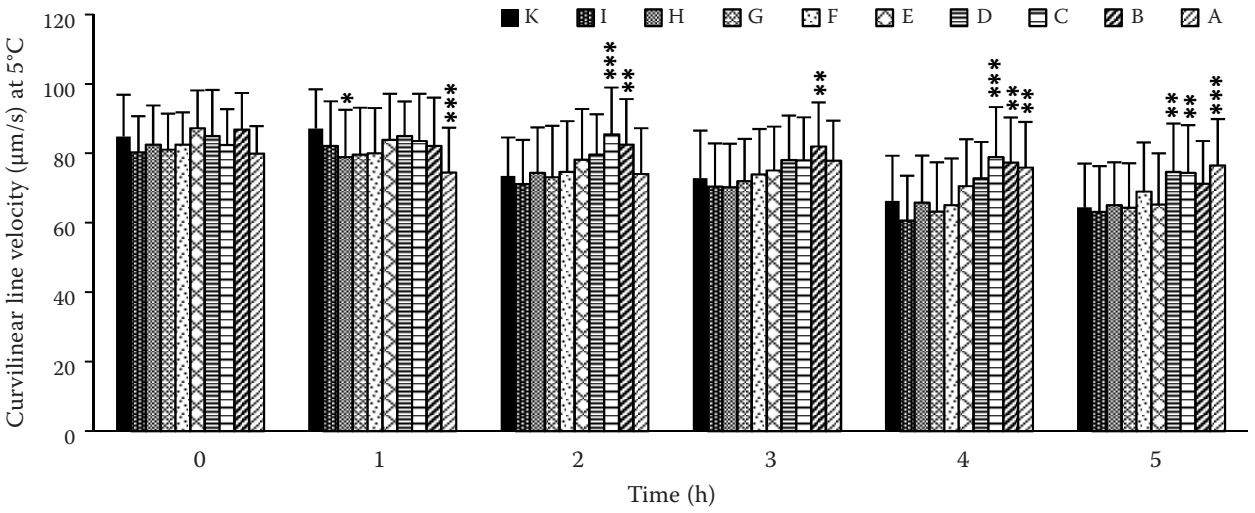

Figure 3. Curvilinear line velocity (µm/s, mean values) after incubation at 5°C
K = control, I = 0.078125 mg/ml, H = 0.15625 mg/ml, G = 0.3125 mg/ml, F = 0.625 mg/ml, E = 1.25 mg/ml, D = 2.5 mg/ml, C = 5 mg/ml, B = 7.5 mg/ml, A = 10 mg/ml
error bars represent the standard deviation
significant differences: $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$

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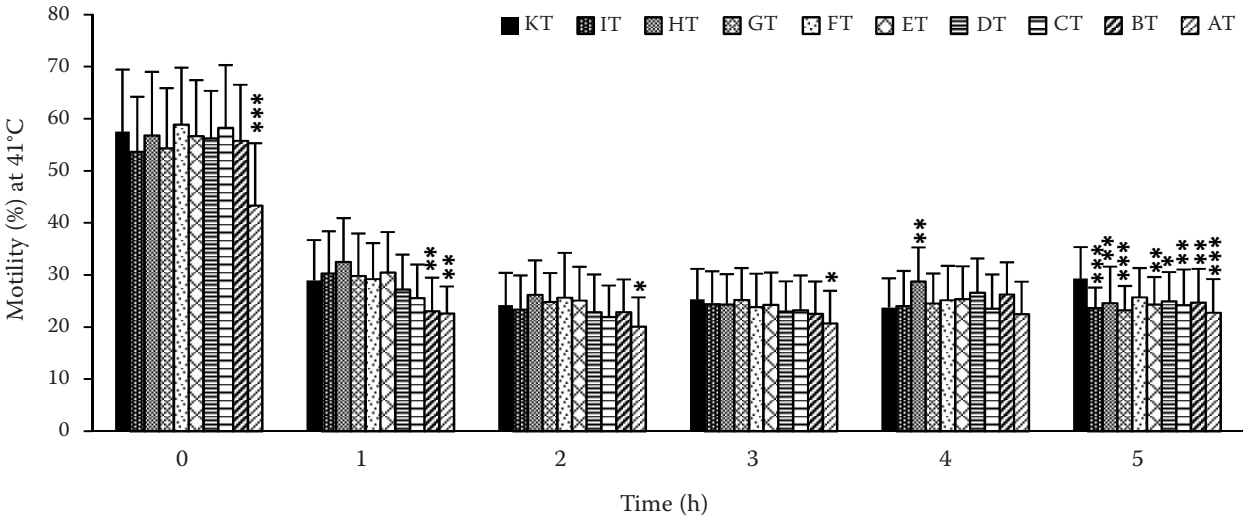

Figure 4. Spermatozoa motility (%, mean values) after incubation at 41°C
KT = control, IT = 0.078125 mg/ml, HT = 0.15625 mg/ml, GT = 0.3125 mg/ml, FT = 0.625 mg/ml, ET = 1.25 mg/ml, DT = 2.5 mg/ml, CT = 5 mg/ml, BT = 7.5 mg/ml, AT = 10 mg/ml
error bars represent the standard deviation; significant differences: $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$

time intervals. While analyzing the amplitude of lateral spermatozoa head displacement (ALH) and beat cross frequency (BCF), fairly equal results were recorded. Negative effect between any of the tested concentrations and control was observed considering these two parameters. Contrariwise, samples C and B after 4 h showed significantly increased ALH compared to control. Four hours of incubation resulted in significantly increased BCF in C and the same level of difference ($P < 0.01$) was found for concentrations A and D at the end of *in vitro* incubation.

Incubation temperature 41°C. Incubation at higher temperature caused extensive decline in MOT even after 1 h of *in vitro* incubation. MOT decreased minimally by 20.75% in sample AT and maximally by 28.57% in control sample (Figure 4). Experimental sample AT, enriched with the high-

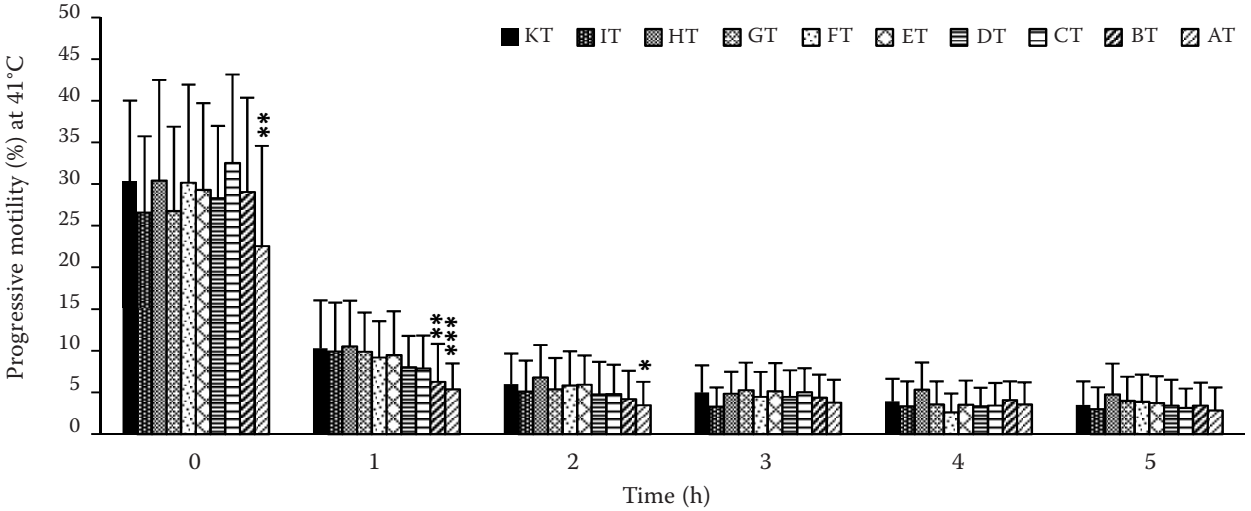

Figure 5. Spermatozoa progressive motility (%, mean values) after incubation at 41°C
KT = control, IT = 0.078125 mg/ml, HT = 0.15625 mg/ml, GT = 0.3125 mg/ml, FT = 0.625 mg/ml, ET = 1.25 mg/ml, DT = 2.5 mg/ml, CT = 5 mg/ml, BT = 7.5 mg/ml, AT = 10 mg/ml
error bars represent the standard deviation; significant differences: $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$

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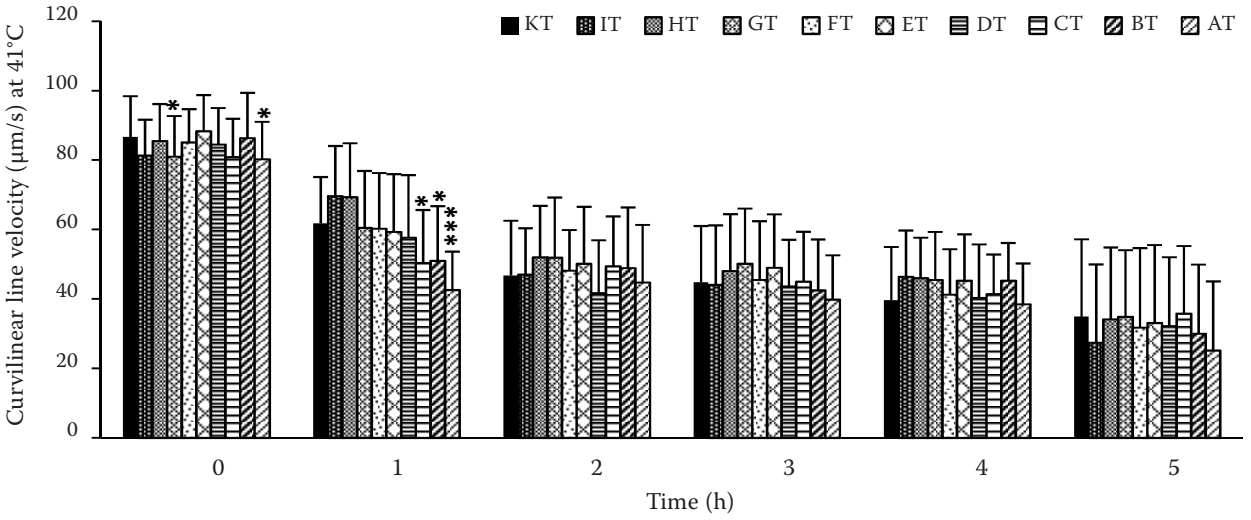

Figure 6. Curvilinear line velocity (μm/s, mean values) after incubation at 41°C
KT = control, IT = 0.078125 mg/ml, HT = 0.15625 mg/ml, GT = 0.3125 mg/ml, FT = 0.625 mg/ml, ET = 1.25 mg/ml, DT = 2.5 mg/ml, CT = 5 mg/ml, BT = 7.5 mg/ml, AT = 10 mg/ml
error bars represent the standard deviation; significant differences: $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$

est concentration of taurine, showed significantly lower percentage of motile spermatozoa in comparison with control during the whole incubation. Positive effect ($P < 0.01$) was detected in group HT after 4 h of incubation. At the end of the *in vitro* incubation all tested samples compared with control indicated significantly decreased MOT. PRO was not positively stimulated during the whole incubation process (Figure 5). The highest taurine concentrations (AT) even caused decrease of PRO at time intervals 0, 1, 2 h and sample BT after 1 h of culture.

Significant difference favouring control sample over experimental samples was noted also in the first two time intervals of incubation also for VCL parameter (Figure 6). AT and GT concentrations

Table 1. Effect of selected taurine concentration on viability of turkey spermatozoa (%; values are means ± SD) during 0–5 h of *in vitro* incubation at 5°C and 41°C

Taurine 5°C	K	E	D	C	B	A
0 h	94.63 ± 0.86	95.47 ± 0.77	95.94 ± 0.44	94.38 ± 0.71	95.66 ± 0.33	94.80 ± 0.81
1 h	94.74 ± 1.16	94.19 ± 1.09	93.69 ± 1.68	92.16 ± 1.22	93.97 ± 2.19	93.55 ± 1.48
2 h	92.75 ± 2.45	93.49 ± 1.68	93.43 ± 1.20	93.28 ± 2.26	91.97 ± 1.33	92.27 ± 1.39
3 h	88.85 ± 1.95	90.50 ± 1.84	91.75 ± 2.02	91.09 ± 1.37	90.22 ± 1.68	91.09 ± 1.51
4 h	90.50 ± 3.03	90.72 ± 2.14	92.02 ± 2.63	90.26 ± 1.83	90.19 ± 0.74	90.80 ± 2.51
5 h	89.75 ± 2.11	92.15 ± 2.65	92.22 ± 3.10	92.26 ± 3.03	92.06 ± 3.10	92.04 ± 3.79
Taurine 41°C	KT	ET	DT	CT	BT	AT
0 h	92.95 ± 0.84	94.36 ± 0.22	92.50 ± 1.00	95.13 ± 1.58	91.48 ± 1.16	93.75 ± 1.59
1 h	92.31 ± 1.82	91.90 ± 0.66	90.82 ± 0.83	91.54 ± 1.82	90.82 ± 1.11	91.80 ± 0.96
2 h	88.37 ± 2.64	89.22 ± 1.70	90.00 ± 1.22	91.40 ± 1.94	90.22 ± 1.40	89.50 ± 1.61
3 h	88.85 ± 3.70	86.87 ± 0.98	88.66 ± 2.32	86.70 ± 0.94	88.41 ± 0.78	87.62 ± 1.84
4 h	84.86 ± 0.53	88.64 ± 3.28	88.88 ± 2.61	89.29 ± 1.78	86.76 ± 1.64	85.57 ± 1.89
5 h	86.31 ± 4.36	85.69 ± 0.95	87.63 ± 3.18	88.00 ± 2.79	85.25 ± 0.52	83.85 ± 1.12

K = control, I = 0.078125 mg/ml, E = 1.25 mg/ml, D = 2.5 mg/ml, C = 5 mg/ml, B = 7.5 mg/ml, A = 10 mg/ml, KT = control, ET = 1.25 mg/ml, DT = 2.5 mg/ml, CT = 5 mg/ml, BT = 7.5 mg/ml, AT = 10 mg/ml
significant differences: $^{*}P < 0.05$,$^{**}P < 0.01$, $^{***}P < 0.001$

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in incipient time interval caused decline ($P < 0.05$) and 1 h of incubation resulted in significant differences ($P < 0.001$ for AT and $P < 0.05$ for BT and CT) in the case of curvilinear line velocity of spermatozoa. Very similar values were reported by the analysis of ALH. No significant differences were detected between samples with taurine addition and the control sample. BCF was significantly increased only at the first time interval – IT, GT, and FT groups showed lower beat cross frequency in comparison with control.

Spermatozoa viability. Determination of the membrane integrity of turkey spermatozoa during *in vitro* incubation using eosin-nigrosin method showed that the monitored concentrations of taurine (samples A–E) have no significant effect on viability of the stored spermatozoa (Table 1). A higher percentage of dead spermatozoa were observed for the incubation temperature of 41°C in comparison to the lower temperature (5°C) and with increasing time of incubation. Therefore, our results confirmed negative effect of higher incubation temperature on this parameter. During the 5 hours of *in vitro* incubation higher percentage of spermatozoa with damaged membrane integrity were detected at 41°C. At this temperature the number of spermatozoa with the intact membrane integrity was in the range from 83.85 ± 1.12% (AT) to 88.00 ± 2.79% (CT) while at 5°C the number of these spermatozoa was in the range from 89.75 ± 2.11% (K) to 92.26 ± 3.03% (C).

DISCUSSION

Taurine as an antioxidant helps maintain normal integrity of acrosome (Sariozkan et al. 2009) and stabilizes cytoplasmic membrane, thereby it enhances spermatozoa motility in mammals. Taurine is able to react with many reactive forms of oxygen whereby it protects mammalian spermatozoa against oxidative stress and by that sustains spermatozoa motility in *in vitro* conditions (Bucak et al. 2007). Taurine plays important roles in male reproduction mainly in older individuals. Results of Yang et al. (2010a) demonstrated that taurine can stimulate excretion of luteinizing hormone and testosterone, enhances the level of testicular enzymes, testicular antioxidation, improves quality of rat spermatozoa, and can significantly increase the amount and motility of spermatozoa in adult rat. This beneficial effect of taurine has been demonstrated in their *in vivo* study where taurine was administered in water to male rats of different ages. The present study is the first to provide information about the effect of taurine on avian spermatozoa storage in *in vitro* conditions.

Spermatozoa motility is a critical factor in the maintenance of fertility. In birds, the vaginal portion of oviduct regulates spermatozoa entry and only motile spermatozoa are able to traverse the vagina and enter into the hen's spermatozoa storage tubules (King et al. 2000). As described by Froman and McLean (1996), spermatozoa motility is a primary determinant of fertility in the fowl. On the basis of results, a graded relationship was predicted between fertility and spermatozoa motility. When fertility was plotted as a function of spermatozoa motility, data points approximated a skewed logistic function. The hypothesis that vaginal immunoglobulins constitute an immunological barrier to spermatozoa transport was tested and rejected.

No previous studies of taurine effects on fowl spermatozoa are available, but there are several studies on mammalian spermatozoa. Perumal et al. (2013) studied the effect of taurine on spermatozoa qualitative parameters of domesticated gayal (*Bos frontalis*) during liquid storage at 5°C. Ejaculate was diluted in TEYC extender (Tris egg yolk citrate extender) with taurine added at concentrations 25, 50, and 100 mmol/l. The addition of taurine caused a significant decrease in the occurrence of dead spermatozoa, abnormal spermatozoa, and acrosome abnormalities in comparison with control group which consisted explicitly of TEYC extender and ejaculate. Furthermore, compared to other taurine concentrations, 50 mmol/l taurine concentrated diluent significantly increased spermatozoa quality. Results of the study pointed out on the potential protective effect of taurine on various spermatozoa parameters as the result of higher activity of antioxidant enzymes, inhibition of cholesterol efflux from cell membranes, and decreased production of malondialdehyde. The protective effect of taurine against the loss of cholesterol from cell membranes is important because cholesterol along with phospholipids is essential for cellular integrity (Witte and Schafer-Somi 2007).

In cattle breeding, cryoconservation of spermatozoa is considered to be the dominant biotechnological method. During this process, spermatozoa experience numerous physiological and biochemical changes which affect their fertilization abilities. Chhillar et al. (2012) described the effect of taurine on thawed bull spermatozoa quality. Fresh bull semen was diluted in TEYC extender enriched with

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taurine (50 mmol/l). Results of the thawed semen analysis showed that taurine significantly increased motility, viability, and membrane integrity of spermatozoa, which is in accordance with our results reached during liquid storage of turkey spermatozoa at 5°C in *in vitro* condition. The positive effect of taurine described also in this study was proved not only in cattle breeding but in fish breeding, too. Research of Martinez-Paramo et al. (2013) pointed out that spermatozoa cryopreservation of European seabass (*Dicentrarchus labrax*) treated with 1 mmol/l of taurine and 1 mmol/l of hypotaurine improved selected semen parameters after thawing. Better motility and lower DNA damage in comparison with control sample was reported. According to Yang et al. (2010a) the cryoprotective properties of taurine on ram spermatozoa may be attributed rather to its osmoregulation abilities than to its antioxidative potential. However Partyka et al. (2017) demonstrated that substances such as L-carninite and taurine are antioxidants that improve cryopreserved chicken semen. Their study showed that taurine addition showed best results in sperm motility, viability, and mitochondrial activity and reduced spermatozoa apoptosis and DNA damage. Tested substances contributed to a cryoprotective effect, suppressing lipid peroxidation in chicken spermatozoa membranes. The positive effect of taurine on poultry spermatozoa motility is also confirmed by our study. The motility-stimulating effect was also observed during the short-term *in vitro* liquid storage of turkey spermatozoa.

The positive effect of taurine was detected also in human spermatozoa. Yun et al. (2013) in their study assessed a combination of several antioxidants (taurine, cysteine, glutathione) and their effect on motility and morphological changes of human spermatozoa. Taurine concentrations of 1, 5, 10 mmol/l significantly improved spermatozoa motility during *in vitro* incubation. The effect of taurine was even augmented by a parallel treatment with cysteine and glutathione. Concerning morphological abnormalities, taurine did not cause sperm cell damage either during sperm processing or during incubation with cysteine and glutathione. A combined treatment with taurine, cysteine, and glutathione improved human spermatozoa motility. Potential use of this combination could find exploitation in semen processing in centres of assisted reproduction. Appropriate choice of other antioxidants and their combination with taurine play a significant role in the development and efficiency of methods for spermatozoa quality enhancing in *in vitro* conditions.

CONCLUSION

To conclude, the analysis of the effect of taurine on turkey spermatozoa motility revealed that taurine concentrations higher than 10 mg/ml negatively impacted spermatozoa preserved in liquid form both at 5°C and 41°C at the incubation time 0 and 1 h. Contrarily, a positive effect of taurine was proved at concentrations of 7.5, 5, and 2.5 mg/ml when significantly higher values of selected spermatozoa motility parameters compared to control were detected in samples B, C, and D, respectively. Overall, taurine did not significantly affect viability of the stored spermatozoa. Therefore, we propose the use of taurine as an appropriate component of semen extenders with a potential effect on improving turkey fertility.

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