Role of taurine against toxic effects of nanosilver

To evaluate the preservative role of taurine against toxic effects of nanosilver, ovine epididymal sperm were exposed to different concentrations (0. 05, 0. 2, 0. 4 M) of taurine (T) and nanosilver colloid (N) (1 ppm) for 120 min in vitro and then, the viability and motion parameters of sperm were assessed. The percentage of live sperm in taurine–treated groups did not differ in comparison with control (P > 0. 05). The percentage of live sperm in T0. 2/N1-group was higher than control (P > 0. 05) and other treated groups (P < 0. 05), Most motion parameters of sperm did not changed in taurine–treated groups except in T0. 4-group which was decreased in compared to other groups (P < 0. 05). In comparison of taurine/nanosilver groups, the parameters of motile sperm, VCL, VSL, VAP, BCF, LIN, WOB, ALH, MAD and STR in control, T0. 05/N1- and T0. 2/N1-groups were more than N1- and T0. 4/N1-groups (P < 0. 05). The progressive motility of sperm did not differ between control, T0. 2/N1- and T0. 4/N1-groups but it was decreased in N1- and T0. 05/N1-groups compared to other groups (P < 0. 05). It is concluded that many concentrations of taurine could neutralize adverse effects of nanosilver on viability and motion parameters of ovine sperm, although taurine does not have considerable benefits on the ovine sperm individually.

Key words: taurine, nanosilver, ovine sperm, motion parameters.

1. Introduction

Silver is a heavy metal which was applied a long history as a medicinal drug and antibacterial agent (1). Recently, Metallic silver is engineered into ultra-fine particles, which is called nanosilver. Nanosilver is silver particle with at least one dimension less than 100 nm (2). Nanosilver colloid, a well-

dispersed and stabilized silver nanoparticle solution will be more adhesive on bacteria and fungus, and so have enhanced antibacterial activity. Colloidal silver can disable oxygen metabolism enzymes in virus, fungus, bacterium or any other single cell pathogen (3). Recently nanoparticles have been in the center of attention in order to create analytical tools in biotechnology and life science. In spite of the wide application of nanoparticles, there are many studies about the effect of these elements on human health and environment (2, 4), and the mechanism of their toxicity is not completely clear. Small sizes of nanoparticles allow their penetration of cell membranes, making distribution easy in the body. Distribution of nanoparticles could lead to increased interactions with proteins, leading to biological and physiological changes of cells (2). Silver nanoparticles can also deplete the antioxidant defense mechanism, leading to reactive oxygen species (ROS) accumulation (5).

Taurine (2-aminoethane sulphonic acid) is a low-molecular-weight organic component in human and many animals. It differs from most biological amino acids as it is a β-amino acid (as sulfonic acid) rather than a α-amino acid and never incorporated into proteins. It has been considered as a conditionally essential amino acid having antioxidant properties and is the principal free amino acid in many animal tissues (6). It is thought to be an osmoregulator of cell volume; it stabilizes cell membrane, modulates excitatory neurotransmission and intracellular calcium levels (7). Taurine is abundant in many tissues, and tissues such as liver, central nervous system, kidney and mammary gland can biosynthesized it (7). In the male reproduction, taurine has been found in the interstitial cells of testis (Leydig

cells, vascular endothelial cells), and epithelial cells of vasa deferens in rats (7, 8). It has also been confirmed that male reproductive organs can biosynthesized taurine. In addition, taurine is determined as the main free amino acid of sperm and semen (7, 9). Taurine protect many of the body's tissues against toxicity and oxidative stress induced by harmful chemicals (10, 11), although the underlying mechanism remains to be elucidated. Severe decrease in taurine levels of tissues may inï¬¬uence their antioxidant power and susceptibility to oxidative damage (12). The objective of this study is to evaluate whether concentration-time dependent taurine could neutralize or even decrease the toxic effects of nanosilver on the kinematic parameters of ram sperm in vitro.

2. Materials and Methods

2. 1. Preparation of spermatozoa

Seven epididymides from healthy adult ram were prepared from an abattoir and conveyed to the laboratory on ice under 2 h. The sperm cells were extracted from cauda epididymal segment as described by Hassanpour et al. (2011) (13) and transferred into a solution containing 2 ml of equilibrated Hepes-TALP (114 mM NaCl, 0. 2 mM sodium pyruvate, 0. 3 mM NaH_2PO_4, 10 mM sodium lactate, 0. 4 mM $MgCl_2$, 3. 1 mM KCl, 10 mM Hepes, 5 mg/ml bovine serum albumin, 2 mM $NaHCO_3$, 0. 7 mg/L Pen/Strep and 2. 1 mM $CaCl_2$). All samples were incubated at 38°C and 5% CO_2 in air (13).

The aliquots of sperm suspension in Hepes-TALP medium (100 µl, containing 5×10^{7} cells), were incubated for 120 min exposing to 0. 05 M taurine (T0. 05) (Sigma, UK), 0. 2 M taurine (T0. 2), 0. 4 M taurine (T0. 4), 1 ppm

nanosilver colloid (N1)(Nanocid, Iran), 0. 05 M taurine + 1 ppm nanosilver colloid (T0. 05/N1), 0. 2 M taurine + 1 ppm nanosilver colloid (T0. 2/N1), 0. 4 M taurine + 1 ppm nanosilver colloid (T0. 4/N1) and an equal volume of HEPES-TALP medium (control).

2. 2. Assessment of sperm viability

The samples of sperm suspension from each group was assessed for viability after the 120 min The staining was done with eosin-nigrosin as described by Bjorndahl et al. (14).

2. 3. Assessment of motion parameters

Sperm motility parameters were assessed by CASA (Hooshmand Fanavar, Iran), with the subsequent settings: speed of image collection, 20 frames per sec; analysis time for each frame, less than 15 sec; sperm velocity that can be analysed, 0-180 µm/s; vision fields number, 6/samples; microscopic magnifying power (object lens), ×4; Makler chambers with 20 µm depth were used for measurement. Sperm motility parameters were analysed after 120 min. The motility of sperm was assessed as motile sperm and progressive sperm motility, all in percentages. The analysed motion parameters can be explained as follows: straight line velocity (VSL), which shows the average velocity of sperm in a straight line from the outset to the end of one path in micrometer per sec; the straightness (STR) determines the proximity of the straight line of cell’s pathway corresponding to the desirable straightness in percentage; The curvilinear velocity (VCL), that is the mean velocity assessed over the actual point to-point track in micrometers per sec; the average path velocity (VAP), that is the average velocity of the smoothed

cell’s pathway in micrometers per sec; the amplitude of lateral head displacement (ALH) which is represented as micrometer; the beat cross frequency (BCF) is the sub-alternation at which head of the sperm passes its mean pathway in Hertz; the linearity (LIN) which accounts linearity of a cycloid path in percentage; the wobble (WOB), that is the oscillation in the real path about the mean trajectory. The mean angular displacement (MAD), that is the time mean of absolute amounts of the momentary rotating angle of the sperm head along its cycloid track in degree (15).

2. 4. Statistical analysis

All results are presented as means ± SEM. The statistical analysis was carried out using Sigmastat3. 1software (Systat Software Inc., Point Richmond, USA). The treatments and control were compared using one way ANOVA. Differences were considered significant at a $P < 0.05$.

3. Results

3. 1. Sperm viability

Sperm viability exposed to nanosilver / taurine was evaluated by eosin-nigrosin exclusion and is presented in figures 1 and 2. The percentage of live sperm in taurine–treated groups did not differ in comparison with control ($P > 0.05$) while the percentage of live sperm in T0. 05-group was significantly higher than T0. 4-group ($P < 0.05$) (Fig. 1). The percentage of live sperm in N1-T0. 05/N1- and T0. 4/N1-groups were significantly lower than control, wherever this parameter in T0. 2/N1-group was higher than control ($P > 0.05$) and other treated groups ($P < 0.05$) (Fig. 2).

3. 2. Motion parameters of sperm

The results of nanosilver / taurine effects on the motion parameters of sperm after 120 min of incubation are shown in Tables 1 and 2 and figures 3 and 4. The parameters of motile sperm, VCL, VSL, VAP, BCF, LIN, WOB (Table 1) and progressive motility (Fig. 3) were decreased at T0. 4-group compared to other groups ($P < 0.05$) while ALH and STR parameter were not significant between different groups ($P > 0.05$). MAD parameter was increased in T0. 2-group compared to other group ($P < 0.05$) (Table 1).

In comparison of N1-, T0. 05/N1-, T0. 2/N1-, T0. 4/N1-groups and control, the parameters of motile sperm, VCL, VSL, VAP, BCF, LIN, WOB, ALH, MAD and STR were significantly decreased at N1- and T0. 4/N1-groups compared to other groups ($P < 0.05$) while this parameters between T0. 05/N1-, T0. 2/N1-groups and control were not significant ($P > 0.05$) (Table 2). The progressive motility of sperm were decreased at N1- and T0. 05-groups compared to other groups ($P < 0.05$). The percentage of this parameter was not significant between control and T0. 2/N1-, T0. 4/N1-groups ($P > 0.05$) (Fig. 4).

4. Discussion

In the present research, the effect of taurine on the motion parameters and viability of ovine epididymal sperm was evaluated in vitro which its beneficial effects of was not considerable, of course high concentration of taurine showed adverse effect on those parameters. The presence of taurine have been confirmed in sperm cells and seminal fluid of most animals and are known to have useful effects on sperm properties of mammals (7, 16). It has

been reported that taurine play critical roles in the maintenance and improvement of sperm motility, acrosome reactions and capacitation in vitro and in vivo. Taurine could also attenuate lipid peroxidation in spermatozoa and preserve the loss of motility (7). Taurine also improved the initial post-thaw motility and duration of motility in frozen–thawed ram sperm. It is suggested that this effects of taurine is probably due to its osmoregulation more than to its antioxidant characteristics (17-19). Anyway, contrary results of our study about in vitro effect of taurine on motion parameters could be due to variance between the species and different conditions of study such as duration of sperm exposing to taurine and its used concentration. On the other hand, our study confirmed that many concentrations of taurine could preserve spermatozoa against adverse effects of nanosilver colloid, and insignificant variations in viability and motion parameters of sperm exposed to taurine and nanosilver colloid could be its evidence. Mirshokraei et al. (2011) found that nanosilver colloid has adverse effects on the most kinematic parameters of sperm (such as progressive motility, VCL, VSL, STR, MAD, ALH, WOB, VAP and LIN) in a time-concentration dependent manner which is agreed with our data (20). Costa et al., (2010) reported that nanoparticles of silver may eviscerate the antioxidant defense mechanism, leading to ROS accumulation. The accumulation of ROS can initiate an destruction of the mitochondria and oxidative phosphorylation (5). Therefore, nanosilver may damage the main source of energy (i. e., oxidative phosphorylation) for sperm motility (21) and decrease motion parameters of sperm. However, simultaneous application of taurine and nanosilver in our study eliminated the harmful effects of nanosilver on sperm function. It has been determined that taurine contribute in the regulation of many

physiological functions such as osmoregulation, calcium modulation, antioxidation, radioprotection, energy storage, xenobiotic conjugation (7, 22). In the sperm, many functions of taurine (membrane-stabilized factor, motility factor, antioxidant and capacitating agent) have been also confirmed (7, 11, 17, 23). However, our data showed that this effects of taurine would be prominent when there is a harmful factor such as nanosilver. It must be noticed that taurine in high concentration may be toxic as our data showed. The reason of this toxicity is unclear which requires more studies.

In conclusion, our data show that many concentrations of taurine could neutralize adverse effects of nanosilver on viability and motion parameters of ovine sperm, although taurine does not have considerable benefits on the ovine sperm individually.