



Original article

The effect of ZnO nanoparticles on rabbit spermatozoa motility and viability parameters *in vitro*

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ABSTRACT

Zinc plays a very important role in various biological activities of the body. Multifaceted role of zinc is also known in testes development, spermatogenesis, capacitation and has effect on spermatozoa motility. On the other hand, the growing industry of nanotechnology has created reasonable interest of the risk assessment for nanoparticles. The aim of this study was to evaluate *in vitro* effect of zinc oxide (ZnO) nanoparticles on rabbit spermatozoa. Fresh semen was collected from sexually mature New Zealand rabbits. Experimental groups were prepared by diluting semen with ZnO nanoparticles in seven different concentrations (6–391 mg/mL). The experimental groups were compared with control group. Semen was assessed using computer assisted semen analysis (CASA) at intervals of 0, 1, 2 and 3 h of incubation. The mitochondrial toxicity assay (MTT) assay was used to determine cell viability. The results of monitored motility parameters in experimental groups showed a decreasing trend during whole experiment. Significant decrease ($P < 0.001$) of motility and progressive motility was observed after 3 h of incubation in samples cultured with higher ZnO nanoparticles in comparison to the control group. After 3 h of incubation, viability of rabbit spermatozoa showed slightly increased values in group with the lowest concentration of ZnO nanoparticles, but in other groups viability showed non-significant decrease compared to control. Similar tendency was detected for spermatozoa membrane integrity. These original data show the negative dose-dependent effect of ZnO nanoparticles on spermatozoa motility and viability parameters.

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1. Introduction

Nanoparticles become widely used in life over the past two decades. Utilization of nanoparticles with one dimension at 1–100 nm range is in variety fields of industry and medicine (Barkhordari et al., 2013). Differences of nanoparticles and their larger samples are in their physical and chemical properties (Wiwanitkit et al.,

2009, Khan et al., 2020). Potential toxicity, ability to pass through cell membrane barriers and enter into individual cells is affected by size of nanomaterials (Bakand et al., 2016, Yilmaz and Soylak, 2016).

For the proper development of every organism, both plant and animal, minerals are needed. One of the necessary micronutrients is zinc. Zinc has a vital role for humans because it is present in many enzymes (e.g. polymerase DNA and RNA etc.), it influences the synthesis of proteins, hormones and red blood cells, as well as the proper functioning of the skin and mucous membranes (Evenson et al., 1993, Puzanowska-Tarasiewicz et al., 2009, Seddigi et al., 2016, Silici et al., 2016).

Zinc is delivered to the body, mainly via the alimentary canal and to a lesser extent through the respiratory system and skin. It has been shown that the presence of this element in the body

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affects the occurrence of various types of disorders in the proper course of various physiological processes, which may result in various types of lesions (Schlegel-Zawadzka et al., 2002).

Zinc plays a very important role in maintaining normal sexual function, especially in men (Isaac et al., 2017). Deficiencies of this element may lead to a reduction in the amount and quality of spermatozoa and hypogonadism. Zinc has a multi-directional protective effect on the semen (Zdrojewicz and Wisniewska, 2005). Multifaceted role of zinc is also known in testes development, spermatogenesis, capacitation and has effect on MOT (Riffo et al., 1992, Cologar et al., 2009, Kerns et al., 2018a). *In vitro* studies have shown that high levels of zinc in semen reversibly inhibit their motility (Barkhordari et al., 2013, Halo Jr. et al., 2018) and adequate zinc levels prevent premature acrosomal reactions (Zdrojewicz and Wisniewska, 2005). A significant part of the genes that participate in the spermatogenesis process are the so-called zinc fingers. DNA forms a complex with proteins in them and is stabilized by zinc ions. Zinc deficiencies can also affect the early stages of spermatogenesis (Razavi et al., 2019). This study was therefore designed to evaluate effect of ZnO nanoparticles on spermatozoa motility and viability.

2. Material and methods

2.1. Semen collection and processing

Fresh semen was collected from adult New Zealand White rabbits ($n = 8$) in the age of 1 year \pm 2 months at the experimental breeding and production centre (Experimental Station of the Animal Production Research Centre Nitra, Slovak Republic). A photoperiod 16L: 8D (minimum light intensity of 80 lx) was checked. Rabbits were bred in individual cages and fed with a commercial diet. The tap water was provided *ad libitum*. The average air temperature was 20 ± 2 °C and relative humidity of $70 \pm 5\%$. According to the experimental design the conditions of care, manipulations and other conditions corresponded to the instruction of EC no. 178/2002 as well as other related EC documents. The experiment was approved by the ethics committee of the National Agricultural and Food Centre, Research Institute for Animal Production in Nitra. Semen samples in five replicates were collected (early in the morning) three times per week with the help of artificial vagina. Ejaculates were pooled to avoid differences among the individuals. Only ejaculates with 80% of MOT were used for experiments. Later the spermatozoa were incubated in thermostat (37 °C) with various concentrations of ZnO nanoparticles in the form of dispersion < 100 nm particle size, 20 wt% in H₂O (Sigma-Aldrich, 3050 Spruce Street, Saint Louis, MO 63103, USA) dissolved in physiological solution. Fresh ejaculates were diluted with physiological solution (NaCl 0.9% Braun, B. Braun Melsungen AG, Germany) in ratio 1:7 for the control group (CON). Experimental samples were prepared according to the same dilution rate with seven different ZnO nanoparticles concentrations (in mg/mL): O – 6; N – 12; M – 24; L – 49; K – 98; J – 195 and I – 391 mg/mL which were calculated according to previous reports (Barkhordari et al., 2013, Jahanbin et al., 2020, Pikula et al., 2020). After dilution, the samples were stored in the thermostat (37 °C) and were analysed immediately.

2.2. Computer-assisted semen analysis

Semen analyses were carried out by Computer-assisted semen analysis (CASA) method with SpermVision software (Minitube, Tiefenbach, Germany) and the microscope Olympus BX 51 (Olympus, Japan). Diluted semen samples were placed into Makler counting chamber (Sefi-Medical Instruments, Germany) with volume of

10 μ l heated to 37 °C for each analysis. Evaluation of spermatozoa was performed in four time periods (0, 1, 2, 3 h). A specific set up for rabbit semen was used and the selected parameters were analysed – total motility (MOT; %), progressive motility (PRO; %), distance curved line (DCL; μ m) and velocity curved line (VCL; μ m/s). Every single output of the CASA system is the result of different 8 fields of Makler Counting Chamber. (Massányi et al., 2008, Halo Jr. et al., 2019).

2.3. Viability analysis – MTT test

Viability of rabbit spermatozoa incubated with ZnO nanoparticles was evaluated by the metabolic activity (MTT) assay after 3 h of culture. This colorimetric assay measures the conversion of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT; Sigma-Aldrich, St. Louis, USA) to purple formazan particles by mitochondrial succinate dehydrogenase of intact mitochondria of living cells. Formazan was measured spectrophotometrically by a microplate ELISA reader (Multiskan FC, ThermoFisher Scientific, Finland). The data were expressed in percentage of control (Slanina et al., 2016, Jambor et al., 2017).

2.4. Eosin-nigrosin

The spermatozoa viability was evaluated using eosin-nigrosin staining methods (Slanina et al., 2018). From all the samples smears were prepared after 3 h of culture. Experimental samples 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, St. Louis, USA). For each slide 300 cells were counted under a light microscope (1000 \times , Leica DMIL LED; Leica Microsystems CMS GmbH, Germany) and classified as viable (intact membrane) and dead (damaged membrane). The experiment was realized in five replicates. The results of viability evaluation were expressed as the percentage of viable and dead spermatozoa (%). All methods were performed in accordance with relevant guideline and regulations' in the manuscript.

2.5. Statistical analysis

For statistical analysis a comparison between the controls group and experimental groups were used. For the analysis the GraphPad Prism program (version 3.02 for Windows; GraphPad Software, La Jolla California USA) was used. Later, analysis the descriptive statistical parameters (mean, standard deviation) were analysed. The One-way ANOVA with Dunnett's post-test was selected for statistical evaluations. For the statistical significance different values were estimated according to the results *** ($P < 0.001$), ** ($P < 0.01$) and * ($P < 0.05$). Results are interpreted as means and finally expressed with SD.

3. Results

3.1. Motility parameters

The initial MOT (Time 0) showed decreased values for all doses of ZnO nanoparticles in comparison to the control group. Similar tendency was observed after one hour of incubation. After 2 h of incubation statistically significant decrease was observed ($P < 0.05$) in the sample L ($33.57 \pm 21.43\%$), K ($34.3 \pm 28.17\%$), J ($32.27 \pm 23.04\%$) and I ($25.18 \pm 18.75\%$). After 3 h of incubation significantly decreased ($P < 0.001$) of MOT in comparison to the control group was observed in groups I, J and K. At the same time a statistically significant ($P < 0.05$ and $P < 0.01$) decrease were observed in groups M and L (Fig. 1).

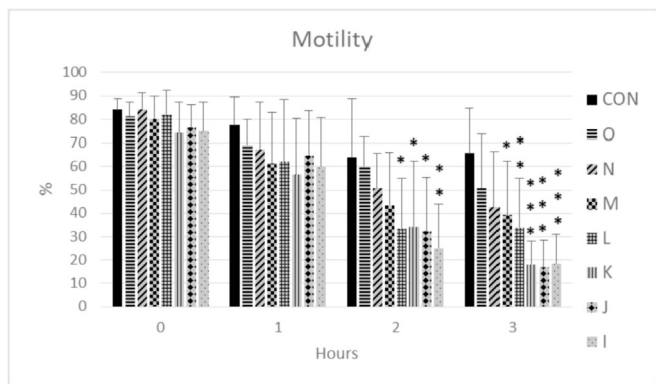


Fig. 1. The effect of ZnO nanoparticles on the total spermatozoa motility (%). CON – 0; O – 6; N – 12; M – 24; L – 49; K – 98; J – 195 and I – 391 mg/mL of ZnO nanoparticles. The level of significance was set at *** (P < 0.001), ** (P < 0.01) and * (P < 0.05).

ZnO nanoparticles negatively affected PRO of rabbit spermatozoa during whole experiment. Significantly lower values were obtained (P < 0.05) in the sample L (18.66 ± 22.16%), K (21.93 ± 28.31%), J (18.23 ± 22.92%) and also, I (12.28 ± 15.41%) after 2 h of incubation. After 3 h of *in vitro* incubation significantly decreased (P < 0.001) of MOT in comparison to the control group was observed in groups I, J and K. Further a statistically significant (P < 0.05 and P < 0.01) decrease were observed in groups M and L (Fig. 2).

The spermatozoa velocity curved line at all time periods in control group was 118–132.1 μm.s⁻¹. VCL was negative affected by the ZnO nanoparticles addition, but the differences were not significant (Fig. 3).

Spermatozoa DCL confirm the negative effect of ZnO nanoparticles. At the Time 3 statistically significant decrease was observed (P < 0.05) in the sample I (26.54 ± 19.86%) (Fig. 4).

3.2. Viability and membrane integrity

After 3 h of incubation, viability of rabbit spermatozoa showed slightly increased values in group O with the lowest dose of Zinc oxide nanoparticles, in other groups viability showed decreased values in comparison to the control group, but the differences were not significant (Fig. 5).

The values of membrane integrity showed very similar tendency in all experimental groups with no significant difference (Fig. 6).

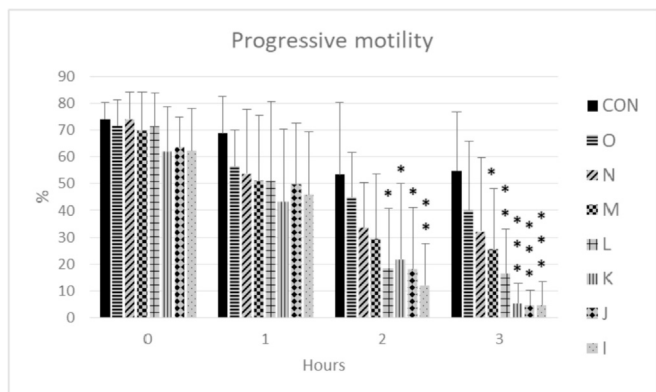


Fig. 2. The effect of ZnO nanoparticles on the progressive motility (%). CON – 0; O – 6; N – 12; M – 24; L – 49; K – 98; J – 195 and I – 391 mg/mL of ZnO nanoparticles. The level of significance was set at *** (P < 0.001), ** (P < 0.01) and * (P < 0.05).

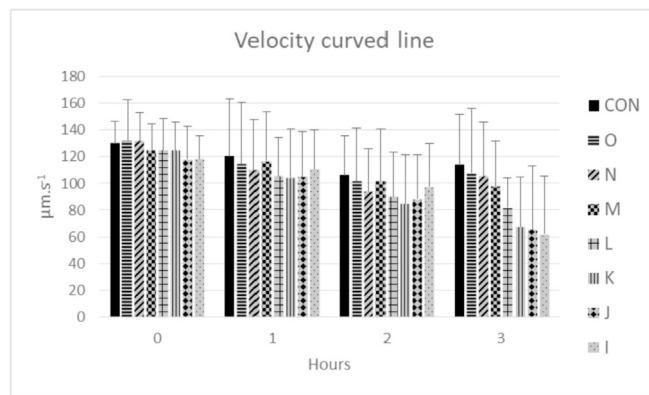


Fig. 3. The effect of ZnO nanoparticles on the velocity curved line (μm.s⁻¹). CON – 0; O – 6; N – 12; M – 24; L – 49; K – 98; J – 195 and I – 391 mg/mL of ZnO nanoparticles. The level of significance was set at *** (P < 0.001), ** (P < 0.01) and * (P < 0.05).

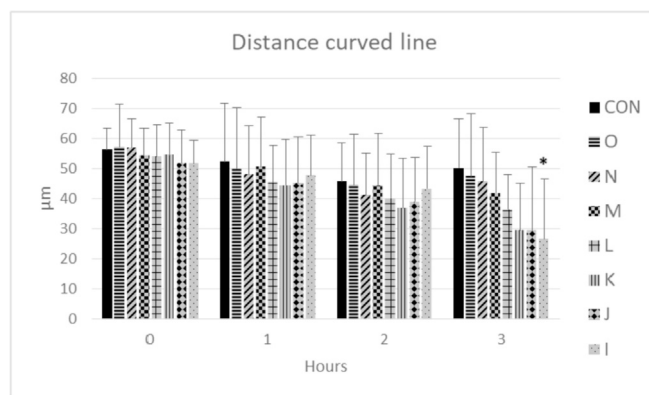


Fig. 4. The effect of ZnO nanoparticles on the distance curved line (μm). CON – 0; O – 6; N – 12; M – 24; L – 49; K – 98; J – 195 and I – 391 mg/mL of ZnO nanoparticles. The level of significance was set at *** (P < 0.001), ** (P < 0.01) and * (P < 0.05).

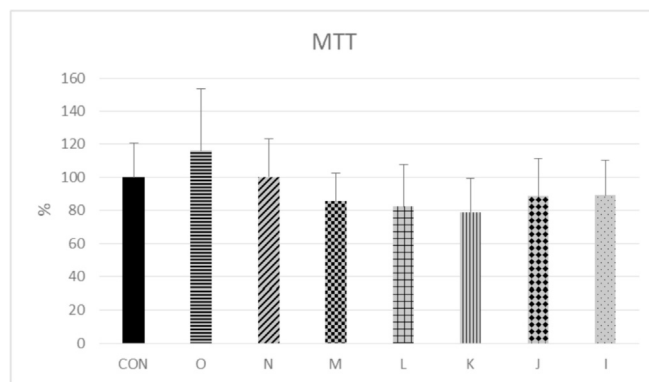


Fig. 5. The effect of ZnO nanoparticles on the viability (%) of rabbit spermatozoa after 3 h of incubation. CON – 0; O – 6; N – 12; M – 24; L – 49; K – 98; J – 195 and I – 391 mg/mL of ZnO nanoparticles.

4. Discussion

Our observations indicated that ZnO nanoparticles in higher concentrations decreased the spermatozoa MOT, PRO, viability and cell membrane integrity in relation to previous findings that ZnO nanoparticles as a widely used emerging materials in agricultural and food-related fields can have potential safety hazards to public health and environment (Yan et al., 2020).

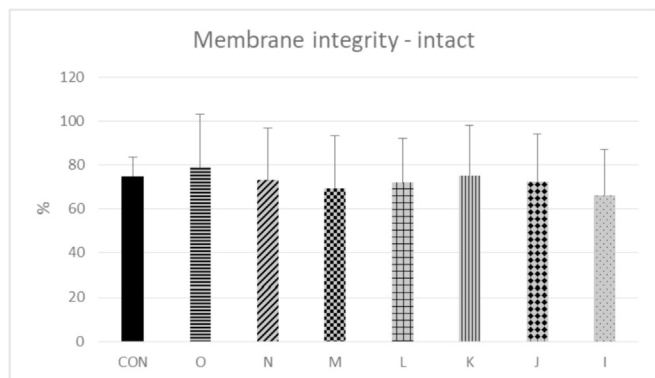


Fig. 6. The effect of ZnO nanoparticles on the membrane integrity (%) of rabbit spermatozoa after 3 h of incubation. CON – 0; O – 6; N – 12; M – 24; L – 49; K – 98; J – 195 and I – 391 mg/mL of ZnO nanoparticles.

Bilateral effect of zinc was confirmed in studies of many researchers. On one hand, the positive impact relates to testes development (Merker et al., 1997), spermatogenesis (Yamaguchi et al., 2009), also has a role in regulation of capacitation and the acrosome reaction of sperm (Fallah et al., 2018). On the other hand, negative effect was associated with higher concentration in seminal plasma and lower MOT (Henkel et al., 2006).

Recently, it was demonstrated *in vitro* that ZnO nanoparticles used in cryopreservation of human semen had beneficial effect on MOT and PRO. Monitored parameters were assessed after thawing and at 24 h of incubation. In both time intervals were recorded positive effect of ZnO nanoparticles for lower concentration, but time dependent effects were not studied. In study of Isaac et al. (2017), human semen was cryopreserved with 100 µg/mL of ZnO nanoparticles, based on their previous studies this concentration was found to be the optimum. Further positive effect of ZnO nanoparticles was recorded (Sanjay et al., 2014), where membrane protective activity in albino Wistar rat homogenates tissues was demonstrated.

Zinc as an essential trace element plays an important role in various biological activities (Kerns et al., 2018a, Kerns et al., 2018b, Sutovsky et al., 2019). As reported in previous study (Halo Jr. et al., 2018), the samples of semen with different forms (ZnCl₂, ZnSO₄·7H₂O) were analyzed (4.883–78.125 mg Zn/L) related to concentration. Final data show motility parameters (motility, progressive motility and velocity curved line) and decreasing tendency in zinc samples. Negative effect of zinc in each concentration on spermatozoa motility parameters and viability was confirmed by detected data. The results clearly describe that ZnCl₂ added to rabbit semen show higher negative impact on rabbit spermatozoa motility and viability parameters compared to ZnSO₄·7H₂O.

Previous study described the *in vitro* effect of zinc (1 or 3 mmol/L) in human semen on oxidative changes. Results clearly determined the effect of zinc participates on oxidative changes after ejaculation (Gavella et al., 2009). Positive protective effect on spermatozoa motility, lipid peroxidation and DNA fragmentation after *in vitro* administration of 10 µg/mL zinc (ZnCl₂) on human spermatozoa was observed (Talevi et al., 2013). The *in vitro* effect of zinc (1, 10, 100 and 1000 µM) on human sperm motility and the acrosome reaction were studied by Riffo et al. (1992). Similarly, to our results authors conclude that zinc (1 µM) may inhibit MOT and the acrosome reaction. Barbato et al. (2017) report protective effect of zinc (10 mg/mL) in combination with D-aspartate and co-enzyme Q10 against exogenous oxidative stress in bovine spermatozoa. In addition, embryos produced by spermatozoa and fresh oocytes affected by oxidative stress are better developed in this experimental culture media.

Negative impact of zinc on carp spermatozoa was reported with similar outcomes as our results. Chyb et al. (2000) evaluated the effect of zinc related to various concentrations on motility parameters. Carp semen with zinc addition (10–200 ppm) discovered significantly decreased for different motility parameters. Observation of different studies also suggest that extracellular zinc affects spermatozoa motility, but the final effect (positive and/or negative) clearly depends on species and the dose (Stoltenberg, 1997; Yamaguchi et al., 2009).

One of the main factors related of its toxicity is size and surface charge (Baek et al., 2011). Also, cytotoxic action of ZnO nanoparticles was observed on testicular germ cells of NMRI mice. Mice were treated daily for 35 days with 5, 50 and 300 mg/kg ZnO nanoparticles (Talebi et al., 2013).

Supplementation of 10 mg/kg/day ZnO nanoparticles to nicotine-exposed rats minimized adverse effect on histological and biochemical structure of testis and epididymis of adult rats. ZnO nanoparticles reduced oxidative stress, which suggests effect of Zn in male reproduction (Mohamed et al., 2019). Opposite effect of ZnO nanoparticles supplementation was recorded in study of Ibrahim et al. (2019). Adult albino rats were treated with 700 mg/kg of ZnO nanoparticles dispersed in distilled water by intraperitoneal single injection for 2 weeks and a significant decrease of spermatozoa motility was observed. However, the percentage of abnormal forms of spermatozoa was highly increased.

Cytotoxic effect was recorded on testis tissue of NMRI adult mice treated with three concentrations of zinc oxide nanoparticles (250, 500, 700 mg/kg/day) by intraperitoneal single injection (Mozaffari et al., 2015). Authors describe that cells such as A type spermatogonia were significantly decreased compared to the control group. The number of primary spermatocyte cells was significantly decreased compared to the control, showing some similar trends compared to our study. Also, a significant reduction in fibroblast cells a significant increase in the number of degenerated cells was found.

Mohammadi et al. (2017) reported that ZnO nanoparticles can prevent in testicular tissue changes in adult mice. It is interesting that Zn nanoparticles co-administration can prevent the luminal extension and epithelial disorganization of seminiferous tubuli induced by cyclophosphamide.

5. Conclusion

The results of this study showed significant effects of ZnO nanoparticles on MOT, viability and cell integrity *in vitro*. However, the results of the experiment showed decreased spermatozoa parameters generated by higher concentrations of ZnO nanoparticles. Spermatotoxicity of ZnO nanoparticles is clearly dose- and time- dependent.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

- Baek, M.K.K.M., Kim, M.K., Cho, H.J., Lee, J.A., Yu, J., Chung, H.E., Choi, S.J., 2011. Factors influencing the cytotoxicity of zinc oxide nanoparticles: particle size and surface charge. *J. Phys.: Conference Series*. 304, 012044. [10.1088/1742-6596/304/1/012044](https://doi.org/10.1088/1742-6596/304/1/012044)
- Bakand, S., Hayes, A., 2016. Toxicological considerations, toxicity assessment, and risk management of inhaled nanoparticles. *Int. J. Mol. Sci.* 17, 929. <https://doi.org/10.3390/ijms17060929>.
- Barbato, V., Talevi, R., Braun, S., Merolla, A., Sudhakaran, S., Longobardi, S., Gualtieri, R., 2017. Supplementation of sperm media with zinc, D-aspartate and coenzyme Q10 protects bull sperm against exogenous oxidative stress and improves their ability to support embryo development. *Zygote* 25, 168–175. <https://doi.org/10.1017/S0967199416000459>.
- Barkhordari, A., Hekmatimoghaddam, S., Jebali, A., Khalili, M.A., Talebi, A., Noorani, M., 2013. Effect of zinc oxide nanoparticles on viability of human spermatozoa. *Iran. J. Reprod. Med.* 11, 767.
- Chyb, J., Kime, D.E., Mikołajczyk, T., Szczepik, P., Epler, P., 2000. The influence of zinc on sperm motility of common carp—a computer assisted studies. *Fish & Aqua Life*. 8, 5–14.
- Colagar, A.H., Marzony, E.T., Chaichi, M.J., 2009. Zinc levels in seminal plasma are associated with sperm quality in fertile and infertile men. *Nut. Res.* 29, 82–88. <https://doi.org/10.1016/j.nutres.2008.11.007>.
- Evenson, D.P., Emerick, R.J., Jost, L.K., Kayongo-Male, H., Stewart, S.R., 1993. Zinc-silicon interactions influencing sperm chromatin integrity and testicular cell development in the rat as measured by flow cytometry. *J. Anim. Sci.* 71, 955–962. <https://doi.org/10.2527/1993.714955x>.
- Fallah, A., Mohammad-Hasani, A., Colagar, A.H., 2018. Zinc is an essential element for male fertility: a review of Zn roles in men's health, germination, sperm quality, and fertilization. *J. Reprod. Infertil.* 19, 69.
- Gavella, M., Lipovac, V., 2009. In vitro effect of zinc on oxidative changes in human semen. *Andrologia*. 30, 317–323. <https://doi.org/10.1111/j.1439-0272.1998.tb01177.x>.
- Halo Jr., M., Tirpák, F., Daňo, A., Zbyňovská, K., Kováčik, A., Ondruška, L., Greň, A., Lukáč, N., Massányi, P., 2018. Zinc affects rabbit spermatozoa in vitro: effects on motility and viability. *J. Microbiol. Biotech. Food. Sci.* 8, 901. <https://doi.org/10.15414/jmbfs.2018-19.8.3.901-904>.
- Halo Jr., M., Massányi, P., Greň, A., Lasak, A., Slanina, T., Ondruška, L., Muchacka, R., Galbavý, D., Ivanič, E.R., Formicki, G., 2019. Time and dose-dependent effects of viscum album quercus on rabbit spermatozoa motility and viability in vitro. *Physiol. Res.* 68, 955–972. <https://doi.org/10.33549/physiolres.934223>.
- Henkel, R., Maass, G., Schuppe, H.C., Jung, A., Schubert, J., Schill, W.B., 2006. Molecular aspects of declining sperm motility in older men. *J. Urol.* 175, 1828. [https://doi.org/10.1016/s0022-5347\(06\)00178-9](https://doi.org/10.1016/s0022-5347(06)00178-9).
- Ibrahim, A.I., Amira, F.A., Manal, M.M., 2019. Effect of zinc oxide nanoparticles on the structure of testis of adult albino rats and the possible protective role of naringenin. *Med. J. Cairo. Univ.* 87, 3469–3483. [10.21608/mjcu.2019.65644](https://doi.org/10.21608/mjcu.2019.65644)
- Isaac, A.V., Kumari, S., Nair, R., Urs, D.R., Salián, S.R., Kalthur, G., Adiga, S.K., Manikkath, J., Mutalik, S., Sachdev, D., Pasricha, R., 2017. Supplementing zinc oxide nanoparticles to cryopreservation medium minimizes the freeze-thaw-induced damage to spermatozoa. *Biochem. Biophys. Res. Com.* 494, 656–662. <https://doi.org/10.1016/j.bbrc.2017.10.112>.
- Jahanbin, R., Yazdanshenas, P., Rahimi, M., Hajarizadeh, A., Tvrdá, E., Nazari, S.A., Mohammadi-Sangcheshmeh, A., Ghanem, N., 2020. In vivo and in vitro evaluation of bull semen processed with zinc (Zn) nanoparticles. *Biol. Trace Elem. Res.* 10.1007/s12011-020-02153-4
- Jambor, T., Tvrdá, E., Tušimová, E., Kováčik, A., Bistaková, J., Forgacs, Z., Lukáč, N., 2017. In vitro effect of 4-nonylphenol on human chorionic gonadotropin (hCG) stimulated hormone secretion, cell viability and reactive oxygen species generation in mice Leydig cells. *Environ. Pollut.* 222, 219–225. <https://doi.org/10.1016/j.envpol.2016.12.053>.
- Kerns, K., Zigo, M., Drobnis, E.Z., Sutovsky, M., Sutovsky, P., 2018a. Zinc ion flux during mammalian sperm capacitation. *Nat. Commun.* 9, 1–10. <https://doi.org/10.1038/s41467-018-04523-y>.
- Kerns, K., Zigo, M., Sutovsky, P., 2018b. Zinc: A necessary ion for mammalian sperm fertilization competency. *Int. J. Mol. Sci.* 19, 4097. <https://doi.org/10.3390/ijms19124097>.
- Khan, W.A., Arain, M.B., Soylak, M., 2020. Nanomaterials-based solid phase extraction and solid phase microextraction for heavy metals food toxicity. *Food Chem. Toxicol.* 145, 111704. <https://doi.org/10.1016/j.fct.2020.111704>.
- Massányi, P., Chrenek, P., Živčák, J., Bulla, J., 2008. Comparison of different evaluation chambers for analysis of rabbit spermatozoa motility parameters using CASA system. *Slov. J. Anim. Sci.* 41, 60–66.
- Merker, H.J., Gunther, T., 1997. Testis damage induced by zinc deficiency in rat. *J. Trace Elem. Med. Biol.* 11, 19–22. [https://doi.org/10.1016/s0946-672x\(97\)80004-1](https://doi.org/10.1016/s0946-672x(97)80004-1).
- Mohamed, D.A., Abdelrahman, S.A., 2019. The possible protective role of zinc oxide nanoparticles (ZnONPs) on testicular and epididymal structure and sperm parameters in nicotine-treated adult rats (a histological and biochemical study). *Cell Tissue Res.* 375 (2), 543–558. <https://doi.org/10.1007/s00441-018-2909-8>.
- Mohammadi, T., Hoveizi, E., Khajehpour, L., Jelodar, Z., 2017. Protective effects of zinc oxide nanoparticles on testis histological structure in cyclophosphamide treated adult mice. *J. Maz. Uni. Med. Sci.* 26, 19–27.
- Mozaffari, Z., Parivar, K., Roodbari, N.H., Irani, S., 2015. Histopathological evaluation of the toxic effects of zinc oxide (ZnO) nanoparticles on testicular tissue of NMRI adult mice. *Adv. Stud. Biol.* 7, 275–291. [10.12988/ASB.2015.5425](https://doi.org/10.12988/ASB.2015.5425)
- Pikula, K., Zakharenko, A., Chaika, V., Em, I., Nikitina, A., Avtomonov, E., Tregubenko, A., Agoshkov, A., Mishakov, I., Kuznetsov, V., Gusev, A., Park, S., Golokhvast, K., 2020. Toxicity of carbon, silicon, and metal-based nanoparticles to sea urchin stronglycentrotus intermedius. *Nanomaterials* 10, 1825. <https://doi.org/10.3390/nano10091825>.
- Puzanowska-Tarasiewicz, H., Kuzmicka, L., Tarasiewicz, M., 2009. Funkcje biologiczne wybranych pierwiastków. III. Cynk – składnik i aktywny enzymów. *Pol. Merk. Lekarski.* 27, 419–422.
- Razavi, S.R., Khadivi, F., Hashemi, F., Bakhtiari, A., 2019. Effect of zinc on spermatogenesis and sperm chromatin condensation in bleomycin, etoposide, cisplatin treated rats. *Cell. J. (Yakhteh)*. 20, 521. <https://doi.org/10.1080/01635581.2020.1757128>.
- Riffo, M., Leiva, S., Astudillo, J., 1992. Effect of zinc on human sperm motility and the acrosome reaction. *Int. J. Androl.* 15 (3), 229–237. <https://doi.org/10.1111/j.1365-2605.1992.tb01343.x>.
- Sanjay, S.S., Pandey, A.C., Kumar, S., Pandey, A.K., 2014. Cell membrane protective efficacy of ZnO nanoparticles. *SOP Trans. Nano-Tech.* 1, 21–29. [10.15764/nano.2014.01003](https://doi.org/10.15764/nano.2014.01003)
- Schlegel-Zawadzka, M., 2002. Cynk-aspetyki zdrowotne i lecznicze, przyczyny i objawy niedoborów. *Farm. Pol.* 58, 452–459.
- Seddigi, Z.S., Kandhro, G.A., Shah, F., Danish, V., Soylak, M., 2016. Assessment of metal contents in spices and herbs from Saudi Arabia. *Toxicol. Ind. Health.* 32, 260–269. <https://doi.org/10.1177/0748233713500822>.
- Silici, S., Uluozlu, O.D., Tuzen, M., Soylak, M., 2016. Honeybees and honey as monitors for heavy metal contamination near thermal power plants in Mugla, Turkey. *Toxicol. Ind. Health.* 32 (3), 507–516. <https://doi.org/10.1177/0748233713503393>.
- Slanina, T., Kováčová, R., Lukáč, N., Massányi, P., 2016. Impact of tilmicosin on the rabbit spermatozoa motility and viability. *J. Microbiol. Biotech. Food. Sci.* 5, 53–56. <https://doi.org/10.15414/jmbfs.2016.5.special1.53-56>.
- Slanina, T., Miškeje, M., Tirpák, F., Błaszczak, M., Stawarz, R., Massányi, P., 2018. Effect of taurine on turkey (Meleagris gallopavo) spermatozoa viability and motility. *Czech. J. Anim. Sci.* 63 (No. 4), 127–135. <https://doi.org/10.17221/CJAS10.17221/CJAS-410.17221/79/2017-CJAS>.
- Stoltenberg, M., 1997. Autometallographic demonstration of zinc ions in rat sperm cells. *Mol. Hum. Reprod.* 3, 763–767. <https://doi.org/10.1093/molehr/3.9.763>.
- Sutovsky, P., Kerns, K., Zigo, M., Zuidema, D., 2019. Boar semen improvement through sperm capacitation management, with emphasis on zinc ion homeostasis. *Theriogenology* 137, 50–55. <https://doi.org/10.1016/j.theriogenology.2019.05.037>.
- Talebi, A.R., Khorsandi, L., Moridian, M., 2013. The effect of zinc oxide nanoparticles on mouse spermatogenesis. *J. Assist. Reprod. Genet.* 30 (9), 1203–1209. <https://doi.org/10.1007/s10815-013-0078-y>.
- Talevi, R., Barbato, V., Fiorentino, I., Braun, S., Longobardi, S., Gualtieri, R., 2013. Protective effects of in vitro treatment with zinc, d-aspartate and coenzyme q10 on human sperm motility, lipid peroxidation and DNA fragmentation. *Reprod. Biol. Endocrinol.* 11 (1), 81. <https://doi.org/10.1186/1477-7827-11-81>.
- Yamaguchi, S., Miura, C., Kikuchi, K., Celino, F.T., Agusa, T., Tanabe, S., Miura, T., 2009. Zinc is an essential trace element for spermatogenesis. *Proc. Natl. Acad. Sci.* 106 (26), 10859–10864. <https://doi.org/10.1073/pnas.0900602106>.
- Yan, Y.-u., Wang, G., Huang, J.-u., Zhang, Y., Cheng, X., Chuai, M., Brand-Saber, B., Chen, G., Jiang, X., Yang, X., 2020. Zinc oxide nanoparticles exposure-induced oxidative stress restricts cranial neural crest development during chicken embryogenesis. *Ecotoxicol. Environ. Saf.* 194, 110415. <https://doi.org/10.1016/j.ecoenv.2020.110415>.
- Yılmaz, E., Soylak, M., 2016. Preparation and characterization of magnetic carboxylated nanodiamonds for vortex-assisted magnetic solid-phase extraction of ziram in food and water samples. *Talanta* 158, 152–158. <https://doi.org/10.1016/j.talanta.2016.05.042>.
- Wiwanitkit, V., Sereemasun, A., Rojanathanes, R., 2009. Effect of gold nanoparticles on spermatozoa: the first world report. *Fertil. Steril.* 91 (1), e7–e8. <https://doi.org/10.1016/j.fertnstert.2007.08.021>.
- Zdrojewicz, Z., Wisniewska, A., 2005. Rola cynku w seksualności mężczyzn. *Advan. Clinical. Experimen. Med.* 14, 1295–1300.