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Effect of Zinc and Nano Zinc on Developmental Competence of Buffalo Oocytes

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Abstract

This study aimed to investigate the impact of zinc sulfate and nano zinc oxide on the *In-vitro* maturation (IVM) of oocytes and on the *In-vitro* embryo developmental competence of buffaloes. Ovaries were obtained from the abattoir. Good quality oocytes (excellent & good) were matured in tissue culture medium -199 (TCM-199) vs. TCM-199 +10-6M zinc sulfate vs. TCM-199 +10-6 M nano zinc oxide enriched by fetal calf serum 10% (FCS) + 10 µg/ml follicle-stimulating hormone + 50 µg/ml gentamicin. The oocyte maturation was done in the incubator for 22 h in a humidified environment with CO_2 5% and 38.5°C. Frozen-thawed semen was used to fertilize Mature oocytes, which were then incubated for 18 hours, before being cultured by synthetic oviduct fluid (SOF) for 7 days. The obtained results showed that supplementing maturation medium with 10-6 M zinc sulfate and 10-6 M nano zinc oxide resulted in a significant ($P<0.05$) rise in GIII cumulus cell expansion of buffalo oocytes by 52.93 %, and 59.75%, respectively, as compared to oocytes cultured in free medium (36.8%). G0 cumulus cell expansion showed a significant ($P<0.05$) decrease in zinc sulfate and nano zinc oxide groups (7.85, 3.29 %, respectively) when compared with oocytes cultured in free medium (14.73 %). The rate of maturation of oocytes with polar bodies was significantly greater in the zinc sulfate and nano zinc oxide groups (86.98, and 92.43%, respectively) when compared with those matured in free medium (80.11%). The hatching (cleavage) rate was significantly greater ($P<0.05$) in the zinc sulfate and nano zinc oxide groups (83.17, 87.66 respectively %) when compared with the TCM-199 (free medium) group (78.60%). The transferable embryos (blastocyst & morula) rates significantly raised ($P<0.05$) in the zinc sulfate (17.28 & 19.87 %, respectively) and nano zinc oxide groups (21.23 and 26.21%, respectively) when compared with TCM-199 group (11.19 &13.75 %, respectively). In conclusion, *in vitro* maturation rate and transferable embryo rates in buffaloes improve by adding zinc sulfate and nano zinc oxide to the medium of maturation.

KEYWORDS
In vitro embryo development, Zinc, Nano zinc, Buffaloes.

INTRODUCTION

Buffalo is an essential source of income in most nations, their reproductive capacity is still above average, though. Modern biotechnologies have been used to boost the ability of these animals to reproduce. The process of *in vitro* culture is mostly affected by the cells' exposure to greater oxygen concentrations and the subsequent creation of free radicals, which is thought to be the primary factor of cell degradation and the subsequent cell activity loss (Chwa *et al*., 2006). The major goal of enhancing culture operations through the addition of antioxidants to the media used for culture is to protect oocytes from oxidative damage during *in vitro* maturation and fertilization. The oxidant/antioxidant equilibrium in cells is kept in check by antioxidants (Oteiza *et al*., 2000; Song *et al*., 2009).

The deficiency of Zn causes oxidative stress in cells cultured *in vitro*. In the context of embryo culture, zinc can also enhance cell development. Zn is an essential component of enzymes that are present in the ovum and embryonic stages of development. It participates in all phases of the development of oocytes and embryos (Picco *et al*., 2010). Additionally, zinc is produced from the mitochondria during the oxidation reaction as a form of glutathione (Rice *et al*., 2016) and serves as an adjunct protein connected to DNA (Cathomen and Joung, 2008). The reproductive sciences now utilize nanotechnology more frequently than ever before, including *in vitro* maturation, fertilization, and culture processes in buffaloes. Because they are tunable in size, have a greater surface area, stable, and mutual interactions at fluid interfaces as well as label-free characterization techniques, nanoparticles exhibit physical characteristics that differ from microparticles and bulk materials (Albanese *et al*., 2012).

In this study, nano-zinc was used as a promising way to increase the positive properties of zinc. According to the authors' knowledge, there are few investigations into how nano-zinc oxide affects the developmental capacity of buffalo oocytes and embryos.

Therefore, the aims of this investigation were to study the effect of zinc sulfate and zinc oxide nanoparticles on the IVM rate of oocytes and on *In-vitro* embryo development in buffaloes.

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MATERIALS AND METHODS

Ethical approval

This investigation was conducted in accordance with standard protocols, with no pain or injury to the buffalo. Additionally, the procedures of experiments were approved by the Ethics Committee of the National Research Centre, Cairo, Egypt (NRC, ID: 19/145).

Unless otherwise specified, all the compounds utilized in this investigation were bought from Sigma-Aldrich. The Santa Barbara, California, United States company Particle Sizing Systems, Inc. was used to evaluate the zinc oxide nanoparticle's (ZnO-NP) size and zeta potential.

In vitro oocyte maturation

Ovaries of buffaloes were harvested from Awlad Bakry slaughterhouse in Qalyubia Governorate in Egypt (2021-2022) and transferred to the Embryo and genetic resources conservation bank in National Research Centre in a tank filled with normal saline solution (NSS, 0.9% NaCl with100 µg/ml streptomycin and 100 IU penicillin). ovaries were cleaned many times in pre-heated (37°C) NSS and then maintained at this temperature till aspiration. By 18-gauge needle connected to a sterile syringe filled with 3 ml of phosphate-buffered saline (PBS) + 6 mg/ ml bovine serum albumin F-V+50 µg/ml gentamicin, oocytes were aspirated from follicles with a diameter of 2 to 8 mm. Following aspiration, follicular fluid was placed in a Falcon tube and left for settling down for 15 minutes at 37°C water bath. COCs were examined by a stereo microscope at a magnification of 90 and washed three times in an aspiration medium (Ismael *et al*., 2016).

According to Kandil *et al*. (1999), buffalo oocyte quality was assessed. Depending on the cumulus investment and equally granulated ooplasm, there were four groups of COCs under a stereomicroscope (90x) as follows:

Excellent: Oocytes with at least five layers of fully developed cumulus cells (CC) and evenly granulated dark ooplasm.

Good: One to four layers of cumulus cells and evenly granulated dark cytoplasm.

Fair: Oocytes are incompletely encircled by cumulus cells, and the ooplasm has little granulation.

Denuded: Oocytes were covered by zona pellucida and had no cumulus cells.

Good-quality oocytes (excellent & good) were placed in TCM-199 vs.TCM-199+zinc sulfate (10-6M) vs. TCM199 + nano zinc oxide (10-6M) enriched with FCS 10%+10 µg/ml FSH+50 µg/ml gentamicin. Maturation of the oocytes was done in the incubator for 22 h a humidified environment with 5% CO_2 and 38.5°C.

According to the extent of cumulus-cell development, the cytoplasmic maturation of buffalo oocytes was evaluated and divided into 4 grades (Kandil *et al*., 1999).

G0: without expansion.

GI: with slight expansion.

GII: moderate expansion is moderate.

GIII: complete expansion

Oocytes' nuclear maturation was determined by the presence of the 1st polar body (PB) in the perivitelline space. The PB was found using an inverted microscope at 200X magnification. The cumulus expansion and nuclear maturation rate were determined according to Ismail *et al*. (2016).

In vitro embryo production according to Kandil et al. (1999)

In brief, Matured oocytes that had fully expanded cumulus cells and the first PB were washed in a fertilization medium (Fert-TALP enriched with six mg/l BSA). Frozen thawed sperm was placed on the top of 2 layers of Percoll density gradient (90% and 45%) and centrifuged for thirty min at 1800 rpm. The percoll and supernatant were discarded, and the semen pellet was mixed with five ml sperm-TALP medium enriched by ten µg/ml heparin and four mg/ml BSA, then centrifugation once more for ten minutes at 1800 rpm. The supernatant was discarded, and the semen pellet was re-mixed with Fert- TALP medium enriched with ten µM/ml hypotaurine, twenty µM penicillamine (PHE) + one µg/ml heparin and six mg/ml BSA. The concentration of the perm was elevated to 1×10^6 spermatozoa /ml before being placed in a four-well plate. The semen and oocytes were incubated together for eighteen h at 38.5°C with 5% CO_2 in a humid atmosphere. After at least three washings, the presumptive zygotes were cultured in culture medium (IVC, mSOFaa medium) enriched with 5 mg/ml BSA, 5 µg/ml insulin, and 50 µg /ml gentamycin and kept in an incubator at 38.5°C with 5% CO_2. On Days 2, 5, and 7, the hatching rate and embryo development to blastocyst & morula stages (transferable embryos) were assessed. The culture medium was changed every forty-eight hours. Detection of the cleavage rate and transferable embryo rate according to El-Sanea *et al*. (2021).

Statistical analysis

Mean±standard error (SE) was used to express the data. By using the analysis of variance (ANOVA) followed by a hoc test, the significant differences were evaluated. Statistical analyses were done by SPSS for windows, V 25.0, SPSS Inc., Chicago, IL, USA.

RESULTS

Analysis of Zn Sulfate and nano-zinc oxide (ZnO-NP)

The intensity-weighted Gaussian distributions analysis means±SD in nano zinc oxide (ZnO-NP) was 744.4 nm with 0.076 variances while in zinc sulfate was 1316.3 nm with variance 0.771 (Fig. 1). The stability of dispersion (average Zeta potential) was -17.47 mV and 19.78 mV in zinc oxide nanoparticles (ZnO-NP) and zinc sulfate respectively (Fig. 2).

Table 1. Effect of zinc sulfate and nano zinc oxide on buffalo oocytes' cytoplasmic maturation rate (cumulus expansion).

Media	No. of Ovaries	No. of Oocytes	GIII		GII		GI		G0	
			No.	%	No.	%	No.	%	No.	%
TCM	102	296	106	36.80±1.68^{c}	53	17.34±1.71^{b}	93	31.10±2.57^{a}	44	14.73±2.01^{a}
TCM + Zinc sulfate	118	354	189	52.93±1.26^{b}	106	30.29±1.13^{a}	29	8.91±1.48^{b}	30	7.85±1.50^{b}
TCM + Nano zinc oxide	150	439	264	59.75±2.09^{a}	142	32.80±2.24^{a}	18	4.04±0.74^{b}	15	3.29±0.97^{c}

*Replicates = 10; Values with different superscript letter ($^{a, b, c}$) within the same column differ significantly ($P<0.05$).

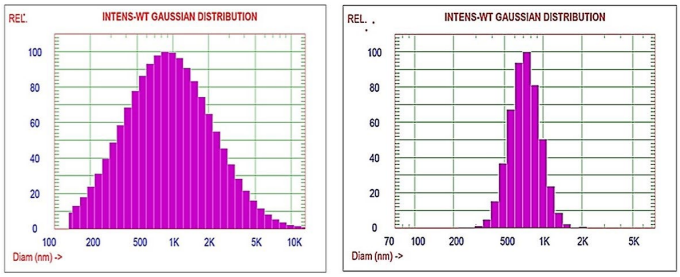

Fig. 1. Intensity weighted Gaussian Distribution Analysis (Solid Particle).

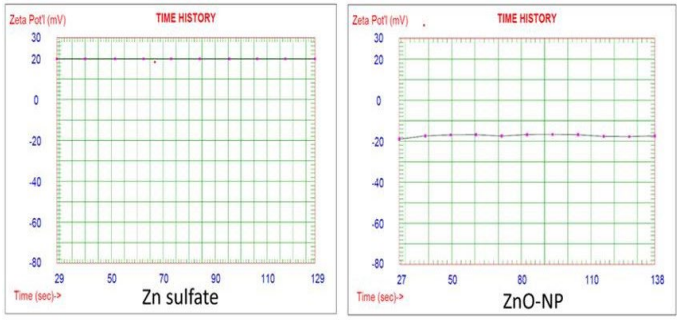

Fig. 2. Average Zeta Potential of Zn sulfate and ZnO-NP particles.

Effect of zinc sulfate and nano zinc oxide on in vitro maturation of buffalo oocytes

Effect of zinc sulfate and nano zinc oxide on cytoplasmic maturation rate (cumulus expansion) of buffalo oocytes

Results (Table1 and Fig. 3A) showed that adding 10-6 M zinc sulfate and 10-6 M nano zinc oxide to maturation media resulted in a significant (P<0.05) rise in GIII cumulus cell expansion of buffalo's oocytes (52.93±1.26 % and 59.75±2.09%, respectively), as compared with oocytes cultured in free medium (36.80±1.68%). G0 cumulus cell expansion showed a significant (P<0.05) decrease in zinc sulfate and nano zinc oxide groups (7.85±1.50,3.29±0.97 %, respectively) when compared with oocytes cultured in free medium (14.73±2.01%). There is a significant rise (P<0.05) in GIII expansion of buffalos' oocytes in the nano zinc group in comparison with the zinc sulfate group. Also, the zinc sulfate group showed a significant decrease in G0 cumulus cell expansion in comparison with the nano zinc group. There is no significant difference in GII & G I between zinc sulfate and zinc oxide nano–particles groups.

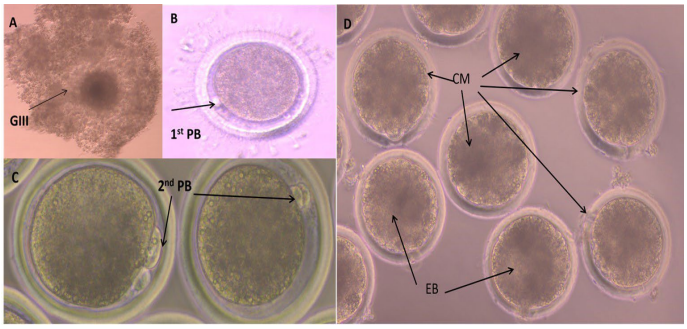

Fig 3. In vitro matured oocytes and embryos of buffalo, oocytes with full cumulus expansion GIII (A), buffalo's oocyte with first poplar body (1st PB) (B), fertilized oocyte with the 2nd polar body (2nd PB) (C), buffalo compact morula (CM) and early blastocyst (EB) (D).

Effect of zinc sulfate and nano zinc oxide on nuclear maturation rate (the 1st polar body) of buffalo oocytes

The maturation rate (Table 2 and Fig. 3B) of buffalo oocytes with the 1st PB was significantly (P<0.05) greater in zinc sulfate and nano zinc oxide groups (86.98±1.48,92.43±1.04 %, respectively) when compared with those matured in free medium (control) (80.11±1.33%). Compared with the zinc sulfate group, there was a significant rise (P<0.05) in the nuclear maturation rate of buffalos' oocytes with the 1st PB in the nano zinc oxide group.

Effect of zinc sulfate and nano zinc oxide on embryo developmental competence in Buffaloes

The data (Table 3, and Fig. 3C, D) showed that the hatching rate was significantly greater (P<0.05) in zinc sulfate and nano zinc oxide groups (83.17±1.07, 87.66±0.94 %, respectively) when compared to TCM-199 (free medium) group (78.60±1.17%). The morula and blastocyst (transferable embryo) rate significantly elevated (P<0.05) in the zinc sulfate (19.87±0.82, 17.28±0.94 respectively) and nano zinc oxide groups (26.21±1.34 %, 21.23±1.34%, respectively) when compared with TCM-199 group (13.75±0.98 % and 11.19±0.90%) respectively. Compared to the zinc sulfate group, there was a significant rise (P<0.05) in the cleavage rate and transferable embryo rate in the nano zinc oxide group.

Table 2. Effect of zinc sulfate and nano zinc oxide on nuclear maturation rate (1st polar body) of buffalo oocytes.

Media	No. of ovaries	No. of Oocytes	1st pb		Without 1st pb	
			No.	%	No.	%
TCM	102	296	238	80.11±1.33^{c}	58	20.99±1.23^{a}
TCM + Zinc sulfate	118	354	309	86.98±1.48^{b}	45	13.01±1.48^{b}
TCM + Nano zinc oxide	150	439	404	92.43±1.04^{a}	35	7.56±1.04^{c}

*Replicates = 10; Values with different superscript letters ($^{a, b, c}$) within the same column differ significantly (P<0.05).

Table 3. Effect of zinc sulfate and nano zinc oxide on embryo developmental competence in buffaloes.

Media	No. of mature ocytes	Cleavage rate		2-4 cell stage		8-16 cell stage		Morula		Blastocyst	
	No.	No.	%	No.	%	No.	%	No.	%	No.	%
TCM	238	186	78.60±1.17^{c}	73	39.94±1.40^{a}	65	35.10±1.37^{a}	27	13.75±0.98^{c}	21	11.19±0.90^{c}
TCM + Zinc sulfate	309	255	83.17±1.07^{b}	87	33.73±2.17^{b}	76	29.10±2.17^{b}	50	19.87±0.82^{b}	42	17.28±0.94^{b}
TCM + Nano zinc oxide	404	354	87.66±0.94^{a}	79	22.08±1.66^{c}	101	28.14±1.20^{b}	91	26.21±1.34^{a}	73	21.23±1.34^{a}

*Replicates = 10; Values with different superscript letters ($^{a, b, c}$) within the same column differ significantly (P<0.05).

DISCUSSION

This investigation indicated that zinc sulfate significantly (P<0.05) increases both the cytoplasmic and nuclear maturation rates of buffalo oocytes. This is in line with the findings of Khalil *et al*. (2021) who revealed that the proportion of buffalo oocytes that reach the metaphase II (MII) stage was significantly (P< 0.05) higher in the IVM medium enriched with zinc compared with the control medium. In cattle, Zn addition to the IVM medium reduced DNA damage and apoptosis in the CC and enhanced superoxide dismutase (SOD) activity, but it had no effect on cumulus expansion (Anchordoquy *et al*., 2014). Yak oocyte maturation is improved by Zn supplementation during IVM due to increased GSH and SOD activity and decreased ROS (Xiong *et al*., 2018). However, Jeon *et al*. (2014) recorded no appreciable differences between the control and zinc groups in the rates of matured porcine oocytes. These discrepancies could be a result of differences in the concentrations of supplements, the original quality of the oocytes, the genotype, physiology, and the reproductive status of animals whose ovaries were harvested.

Intracellular GSH plays a crucial part in cytoplasmic maturation in oocytes and is a useful indication of it. Several molecular processes are involved in the maturation of the oocyte cytoplasm, such as the creation of biochemical substances, protein phosphorylation, and the stimulation of specific metabolic pathways. Embryo development and cumulus cell growth *in vitro* are both impacted by GSH (Luberda, 2005) According to Xiong *et al*. (2018), after zinc addition to IVM media, the glutathione level in yak oocytes considerably increased.

There is a relationship between zinc and oxidative stress. It acts as a catalyst for several enzymes that reduce cellular ROS and is necessary for several cellular processes. (Kloubert and Rink, 2015). During IVM of bovine oocytes, insufficient zinc has a deleterious impact on the intracellular GSH concentration and DNA integrity of cumulus cells, which has an adverse effect on growing preimplantation embryos. The appropriate circumstances for oocyte IVM depend on having enough zinc (Picco *et al*., 2010). Cumulus cells (CC) with Zn insufficiency show increased DNA damage and apoptosis. A sufficient zinc concentration in the culture medium reduces the proportion of apoptotic to normal cells in CC and protects DNA integrity (Anchordoquy *et al*., 2011).

Due to its ability to lower mitochondrial oxidative stress, zinc exerts vital antioxidative and antiapoptotic effects on the development of buffalo oocytes (Khalil, *et al*., 2021). Additionally, zinc causes a decrease in ROS generation in cell cultures (Szuster-Ciesielska *et al*., 2000). Zinc also inhibits the expression of the apoptotic protease caspase-3 (Truong-Tran *et al*., 2001), maintains the structure of the DNA repair proteins and p53 (Chai *et al*., 1999), and consequently decreases apoptosis. It is also concerned with the upregulation of BCL2 and BCLXL, which are antiapoptotic genes, and the upregulation of BID and BAX, which are proapoptotic genes. BCL2 and BAX interactions control the release of cytochrome c (Cyt c) from mitochondria and set the stage for baseline sensitivity to apoptotic stimuli (Khalil *et al*., 2021).

The current investigation confirms that adding nano zinc oxide (10-6 M) to IVM medium significantly(P<0.05) enhances IVM rate in buffalo when compared to zinc. This is consistent with the results of Abdel-Halim and Helmy (2017) who postulated that bovine oocytes' maturation percentage dramatically enhanced when nano-zinc oxide (NZn-O) particles were added to their maturation media in comparison to the control group.

The current study showed that adding zinc to the *in vitro* maturation media significantly (P < 0.05) increased hatching and blastocyst rates. These findings are supported by Xiong *et al*. (2018), who postulated that Zn supplementation to IVM media elevated blastocyst rates significantly after *in vitro* fertilization and did not significantly alter cleavage rates in the yak. Anchordoquy *et al*. (2014) recorded that the addition of Zn to IVM media in cattle did not modify the cleavage rate after IVF but enhanced embryo growth up to the blastocyst stage and improved the quality of the blastocyst.

Jeon *et al*. (2014) found that adding zinc to IVM media in porcine significantly (P< 0.05) increased fertilization and blastocyst formation. They also indicated that sufficient Zn concentrations during IVM improved the developmental capacity of pig embryos by controlling the concentration of intracellular glutathione, level of ROS, and expression of a transcription factor. According to Barakat *et al*. (2015), adding zinc to the IVM medium improved the fertilization rate of mouse oocytes and the development of fertilized oocytes.

Zinc is necessary for the synthesis of several proteins and the regulation of enzyme systems, including metalloenzymes, synthesis, and transport of DNA, and metabolism of free radicals (Townsend, *et al*., 1994). Zinc reduced ROS as too much ROS adversely affects gametes and impairs their function through lipid peroxidation, protein degradation, and breakage of DNA strands (Aitken *et al*., 2010). Zinc addition in the IVM medium considerably enhanced embryonic development in porcine by raising intracellular GSH levels (Jeon *et al*., 2014). Additionally, glutathione was critical for cumulus expansion *in vitro* and embryo protection throughout different developmental phases. Also, elevated concentrations of GSH during IVM had several effects on embryos: first, they enhanced their capacity for development; second, they increased the percentage of embryos that reached the blastocyst stage (Sarkar *et al*., 2015).

This study showed that adding nano zinc oxide to the *in vitro* maturation media significantly (P < 0.05) improved hatching and blastocyst rates when in comparison with zinc. These findings agreed with those of Abdel-Halim *et al*. (2018), who found that adding zinc oxide nanoparticles to IVM media significantly raised the proportion of blastocysts rate in cattle. Moreover, Abdel-Halim and Helmy (2017) postulated that the percentage of transferable embryos was significantly (P < 0.05) greater in the medium enriched with nano-zinc oxide than in the control group in bovine.

Nanomaterials ranged in size from 1 to 100 nm and have large surface areas. They may be a useful technique for transporting chemicals into gametes and embryos because of their elevated loading capacity, stability, and selective affinities (Barkalina *et al*., 2014). Due to their characteristics, nanoparticles may prolong the half-life of loaded bioactive antioxidant compounds and enhance their advantageous effects *in vitro* by preventing their deterioration. These active carriers can enable the regulated release of carried biomolecules (antioxidants) and the transfer of chemicals to the target areas with lower doses (Conte *et al*., 2017). The additional fundamental benefit of using nanoparticles as carriers is their capability to make hydrophobic compounds more soluble, improving the efficacy of the active ingredient's dissolution (Długosz *et al*., 2020). *In vitro* culture of gametes and embryos might therefore greatly benefit from the use of these carriers, which would also increase the efficacy of ART in both humans and animals.

Because of their increased surface area, nanomaterials (nano-zinc oxide) may enhance antioxidant activity by preventing the formation of free radicals, creating more active sites for scavenging free radicals (Konvičná *et al*., 2015). According to Jahanbin *et al*. (2021), adding Zn nanoparticles to IVM media improve embryo development by increasing SOD activity in CC, reducing DNA damage, and apoptosis in COC. Catalase (CAT) and SOD enzymes stimulate the transformation of superoxide into oxygen and hydrogen peroxide. SOD regulates the amount of ROS and reactive nitrogen species, thus reducing their potential toxicity (Wang et. al 2018). An increase in the intracellular glutathione level of CC during *in vitro* maturation of bovine COCs in the presence of zinc oxide nanoparticles led to better embryo development (Abdel-Halim *et al*., 2018). Cumulus cells' intracellular glutathione levels and DNA integrity were both improved by ZnO nanoparticles as they were added to IVM media in bovine COCs matured *in vitro* (Abdel-Halim and Helmy, 2017). The management of oxidative stress depends on elevated glutathione levels in cultured cells (Adeoye *et al*., 2018).

CONCLUSION

Supplementation of *in vitro* maturation media with Zinc sulfate or nano Zinc Oxide improves the maturation and transferable embryo rate in buffalo.

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CONFLICT OF INTEREST

The authors affirm that they do not have any conflicts of interest.

REFERENCES

Abdel-Halim, B.R., Helmy, N.A., 2017. Effect of nano-selenium and nano-zinc particles during *in vitro* maturation on the developmental competence of bovine oocytes. Animal Production Science 58, 2021-2028.

Abdel-Halim, B.R., Moselhy, W.A., Helmy, N.A., 2018. Developmental competence of bovine oocytes with increasing concentrations of nano-copper and nano-zinc particles during *in vitro* maturation. Asian Pacific Journal of Reproduction 7, 161.

Adeoye, O., Olawumi, J., Opeyemi, A., Christiania, O., 2018. Review on the role of glutathione on oxidative stress and infertility. JABRA Assisted Reproduction 22, 61– 66.

Aitken, R.J., De Iuliis, G.N., Finnie, J.M., Hedges, A., McLachlan, R.I., 2010. Analysis of the relationships between oxidative stress, DNA damage and sperm vitality in a patient population: development of diagnostic criteria. Human Reproduction 25, 2415-2426.

Albanese, A., Tang, P.S., Chan, W.C., 2012. The effect of nanoparticle size, shape, and surface chemistry on biological systems. Annual Review of Biomedical Engineering 14, 1-16.

Anchordoquy, J.M., Anchordoquy, J.P., Sirini, M.A., Picco, S.J., Peral-García, P., Furnus, C., 2014. The importance of having zinc during *in vitro* maturation of cattle cumulus-oocyte complex: role of cumulus cells. Reproduction in Domestic Animals 49, 865-874.

Anchordoquy, J.M., Picco. .SJ., Seoane, A., Anchordoquy, J.P., Ponzinibbio, M.V., Mattioli, G.A., Peral-García, P., Furnus, C.C., 2011. Analysis of apoptosis and DNA damage in bovine cumulus cells after exposure *in vitro* to different zinc concentrations. Cell Biology International 35, 593–597.

Barakat, I.A., Al-Shammari, J.O., Khandeal, S.A., Al-Eisa, N.A., 2015. Influence of Media Supplementation with Zinc and/or Thymoquinone on *in vitro* Fertilization and Mouse Oocytes Development. Pakistan Journal of Zoology 47, 1133-1140

Barkalina, N., Charalambous, C., Jones, C., Coward, K., 2014. Nanotechnology in reproductive medicine: emerging applications of nanomaterials. Nanomedicine: Nanotechnology, Biology and Medicine 10, 921-938.

Cathomen, T., Joung, J.K., 2008. Zinc-finger nucleases: the next generation emerges. Molecular Therapy 16, 1200–1207.

Chai, F., Truong-Tran, A.Q., Ho, L.H., Zalewski, P.D., 1999. Regulation of caspase activation and apoptosis by cellular zinc fluxes and zinc deprivation: a review. Immunol Cell Biol. 77, 272–278.

Chwa, M., Atilano, S.R., Reddy, V., Jordan, N., Kim, D.W., Kenney, M.C., 2006. Increased stress-induced generation of reactive oxygen species and apoptosis in human keratoconus fibroblasts. Investigative Ophthalmology and Visual Science 47, 1902-1910.

Conte, R., Marturano, V., Peluso, G., Calarco, A., Cerruti, P., 2017. Recent advances in nanoparticle-mediated delivery of anti-inflammatory phytocompounds. International Journal of Molecular Sciences 18, 709.

Długosz, O., Szostak, K., Staroń, A., Pulit-Prociak, J., Banach, M., 2020. Methods for reducing the toxicity of metal and metal oxide NPs as biomedicine. Materials 13, 279.

El-Sanea A.M., Abdoon A.S., Kandil O.M., El-Toukhy N.E., Abo El-maaty M.A., Ahmed H.H., 2021. Effect of oxygen tension and antioxidants on the developmental competence of buffalo oocytes cultured *in vitro*. Veterinary World 14, 78-84.

Ismail, E.A., Elsayed M.A.I, Hemeida N.A., Abdoon A.S.S., Kandil O.M., 2016. Effect of Insulin-Like Growth Factor-I (IGF-1) on oocyte competence and embryo development of buffaloes (*Bubalus bubalis*), International Journal of Chem. Tech. Research 9, 738-748.

Jahanbin, R., Yazdanshenas, P., Rahimi, M., Hajarizadeh, A., Tvrda, E., Nazari, S.A., Ghanem, N., 2021. In vivo and *in vitro* evaluation of bull semen processed with zinc (Zn) nanoparticles. Biological Trace Element Research 199, 126-135.

Jeon, Y., Yoon, J.D., Cai, L., Hwang, S.U., Kim, E., Zheng, Z., Hyun, S.H., 2014. Supplementation of zinc on oocyte *in vitro* maturation improves preimplantation embryonic development in pigs. Theriogenology 82, 866-874.

Kandil, O.M., Abdoon, A.S., Murakami, M., Otoi, T., Suzuki, T., 1999. New technique, using a portable CO_2 incubator, for the production of *in vitro* fertilized Egyptian buffalo embryos. J. Reprod. Develop. 45, 315-320.

Khalil, W., Yang, C., El-Moghazy, M., El-Rais, M., Shang, J., El-Sayed, A., 2021. Effect of zinc chloride and sodium selenite supplementation on *in vitro* maturation, oxidative biomarkers, and gene expression in buffalo (*Bubalus bubalis*) oocytes. Zygote 29, 393-400.

Kloubert, V., Rink, L., 2015. Zinc as a micronutrient and its preventive role of oxidative damage in cells. Food Function 6, 3195–3204.

Konvičná, J., Vargová, M., Paulíková, I., Kováč, G., Kostecká, Z., 2015. Oxidative stress and antioxidant status in dairy cows during prepartal and postpartum periods. Acta Veterinaria Brno. 84, 133-140.

Luberda, Z., 2005. The role of glutathione in mammalian gametes. Reprod Biol. 5, 5–17.

Nasr, M.A.F., Awad, A., El Araby, I.E., 2016. Associations of leptin and pituitary-specific transcription factor genes' polymorphisms with reproduction and production traits in dairy buffalo. Reproduction in Domestic Animals 51, 597-603.

Oteiza, P.I., Clegg, M.S., Zago, M.P., Keen C.L., 2000. Zinc deficiency induces oxidative stress and AP-1 activation in 3T3 cells. Free Radic Biol Med. 28, 1091-1099.

Picco, S.J., Anchordoquy, J.M., De Matos, D.G., Anchordoquy, J.P., Seoane, A., Mattioli, G.A. and Furnus, C.C., 2010. Effect of increasing zinc sulfate concentration during *in vitro* maturation of bovine oocytes. Theriogenology 74, 1141-1148.

Rice, J.M., Zweifach, A., Lynes, M.A., 2016. Metallothionein regulates intracellular zinc signaling during CD4þ T cell activation. BMC Immunol. 17, 1–14.

Sarkar, B., Bhattacharjee, S., Daware, A., Tribedi, P., Krishnani, K.K., Minhas, P.S., 2015. Selenium nanoparticles for stress-resilient fish and livestock. Nanoscale research letters 10, 1-14.

Song, Y., Leonard, S.W., Traber, M.G., Ho, E., 2009. Zinc deficiency affects DNA damage, oxidative stress, antioxidant defenses, and DNA repair in rats. Journal of Nutrition 139, 1626-1631.

Szuster-Ciesielska, A., Stachura, A., Słotwińska, M., Kamińska, T., Śnieżko, R., Paduch, R., Abramczyk, D., Filar, J., Kandefer-Szerszeń, M., 2000. The inhibitory effect of zinc on cadmium-induced cell apoptosis and reactive oxygen species (ROS) production in cell cultures. Toxicology 145, 159–171.

Townsend, S.F., Briggs, K.K., Krebs, N.F., Hambidge, K.M., 1994. Zinc supplementation selectively decreases fetal hepatocyte DNA synthesis and insulin-like growth factor II gene expression in primary culture. Pediatr. Res. 35, 404–408.

Truong-Tran, A.Q., Carter, J., Ruffin, R.E., Zalewski, P.D., 2001. The role of zinc in caspase activation and apoptotic cell death. Biometals 14, 315-330.

Wang, Y., Branicky, R., Noë, A., Hekimi, S., 2018. Superoxide dismutase: Dual roles in controlling ROS damage and regulating ROS signaling. The Journal of Cell Biology 217, 1915–1928.

Xiong, X., Lan, D., Li, J., Lin, Y., Zi, X., 2018. Effects of zinc supplementation during *in vitro* maturation on meiotic maturation of oocytes and developmental capacity in the yak. Biological Trace Element Research 185, 89-97.