Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

E-ISSN: 2320-7078
P-ISSN: 2349-6800
JEZS 2020; 8(1): 896-899

Received: 16-11-2019
Accepted: 20-12-2019

Abinash Kumar
M.V.Sc Scholar, Department of Veterinary Physiology and Biochemistry, College of Veterinary Science & AH., CGKV, Anjora, Durg, Chhattisgarh, India

Nitin E Gade
Assistant Professor, Department of Veterinary Physiology and Biochemistry, College of Veterinary Science & AH., CGKV, Anjora, Durg, Chhattisgarh, India

OP Mishra
Professor and Head, Department of Veterinary Physiology and Biochemistry, College of Veterinary Science & AH., CGKV, Anjora, Durg, Chhattisgarh, India

JR Khan
Professor, Department of Veterinary Physiology and Biochemistry, College of Veterinary Science & AH., CGKV, Anjora, Durg, Chhattisgarh, India

SA Dhenge
Assistant Professor, Department of veterinary physiology and biochemistry college of veterinary science and animal husbandry, Udgir, Maharashtra, India

SK Yadav
M.V.Sc Scholar, Department of veterinary surgery and radiology, College of Veterinary Science & AH., CGKV, Anjora, Durg, Chhattisgarh, India

Anupam Soni
M.V.Sc Scholar, Department of livestoc production and management, College of Veterinary Science & AH., CGKV, Anjora, Durg, Chhattisgarh, India

Corresponding Author:
Abinash Kumar
M.V.Sc Scholar, Department of Veterinary Physiology and Biochemistry, College of Veterinary Science & AH., CGKV, Anjora, Durg, Chhattisgarh, India

Effects of graphene quantum dots on physiological characteristics of Caprine oocyte maturation

Abinash Kumar, Nitin E Gade, OP Mishra, JR Khan, SA Dhenge, SK Yadav and Anupam Soni

Abstract
Present study was conducted in department of Veterinary Physiology and Biochemistry at campus of College of Veterinary Science and Animal Husbandry, Anjora, Durg (C.G.). The study was main aim to evaluate the effects of different concentrations of graphene oxide quantum (GQ) dots on oocyte maturation. Epididymal sperms were collected and incubated with different concentrations of GQ dots nanoparticles (0 µg/ml, 10 µg/ml, 50 µg/ml and 100 µg/ml). Caprine oocytes were collected, graded and cultured *in vitro* in maturation media supplemented with different concentrations of GQ dots nanoparticles (0 µg/ml, 10 µg/ml, 50 µg/ml and 100 µg/ml) and oocytes were observed microscopically and by BCB staining for maturation after 24 hr. Groups IV have least number of matured oocytes & significantly lower number of matured oocytes as compared to control. Increasing concentration of GQ dots has detrimental effects on *in vitro* maturation of oocytes.

Keywords: oocyte, maturation, caprine, media, graphene quantum

Introduction
Nanotechnology is small size of particles ranges from1 nm to 100 nm radius, it possesses own physio-chemical properties. At present, nanomaterials and devices are used in different fields such as textile (Ali *et al*., 2016) [1], cosmetics (Wiechers and Musee, 2010) [2], environment and space research (Nikalje, 2015) [3], food industry (Chaudhry *et al.*, 2008) [4] and agricultural (Agrawal and Rathore, 2014) [5]. It has potential applications in the biomedical research like chemotherapy, disease diagnosis, biosensors, tissue culture and regeneration (Gannon *et al*., 2008) [6], and it also play important role in separation and purification of biological molecules and cells (Molday *et al*., 1982) [7].
In vivo tracking of sperm and their behaviour in the oviductal lumen can be studied with these techniques (Holt and Fazeli, 2016; Knox, 2016; Suarez & Pacey, 2006) [8, 9, 10]. Carbon nanotubes, carbon nanohorns, carbon nanofibers and graphene are mostly used in biomedical sciences especially in tissue engineering (Nishida *et al*., 2016) [11]. These nanomaterials are extensively investigated in a wide range of biomedical applications, in particular regenerative medicine and tissue engineering.
Development of nano biosensors it helps for detection of physiological or altered reproductive status (Monerris *et al*., 2012) [12] and production of metal nanoparticle for fertility control applications (Jha *et al*., 2014) [13] and nano devices for secure cryopreservation of gametes and embryo (Wang *et al*., 2014) [14] are recent advances. Application of nanoparticles are also in the field of reproductive technologies for inducing oocyte maturation, improving the survival and development of oocytes after cryopreservation, gene knockdown in oocytes, delivering antibodies into oocytes, sustained surge of gonadotropins and enhanced reproductive output, and nanoparticle-based semen purification (Hen *et al*., 2017; Li *et al*., 2016) [15, 16].
After culture of rat ovarian preantral follicle in media containing 12.5 µg/ml, 25 µg/ml and 50 µg/ml TiO_2 nanoparticles, and with the increase of TiO2 concentration, the significantly decreases the survival rate of follicles and mature oocytes decreased significantly (Hou *et al*., 2009) [17]. Nano Particles can affect both primary and secondary follicles by disturbing their development. A irregular shape follicular antrum were observed (Gao *et al*., 2012) [18].
Zhao *et al*., (2013) [19] also reported that ovary injury and fertility reduction due to TiO_2NPs are closely associated with disturbances in the sex hormone equilibrium, and alterations in

inflammation-related or follicular atresia-related cytokine expression. The Cerium oxide nanoparticles (CeO_2 NPs) improve the developmental ability of *in vitro*-matured prepubertal ovine oocytes (Ariu *et al*., 2016) [20]. In bovine, developmental of competence of oocytes with increasing concentrations of nano-copper and nano- zinc particles during *in vitro* maturation (Abdel-Halim *et al*., 2018) [21]. The main aim of this study to effect of graphene quantum dots on oocyte maturation

Materials and Methods

Present investigation was conducted in the Department of Veterinary Physiology and Biochemistry at College of Veterinary Science and A.H., Anjora, Durg, Chhattisgarh. Experimental design employed and methods executed for different parameters are explained in this section. The study was stated in April month with proper arrangement of all instruments which were required during experimental procedures.

Experimental Design: Total three groups with different concentration of GQ dots (10 µg/ml, 50µg/ml and 100µg/ml) were prepared to evaluate their effects on *in vitro* maturation of Caprine oocytes and compared with control (0µg/ml). Oocytes were *cultured in vitro* for maturation in maturation media (Wahjuningsih *et al*., 2014) [22] along with three different concentrations GQ dots.

Evaluation of cytotoxic effects of GQ dots on *in vitro* maturation of Caprine oocytes: Caprine ovaries were collected from local abattoir Supela, Durg and transported to the laboratory in normal saline within 2 hr. Ovaries were washed thrice with Dulbecco's phosphate buffer solution (DPBS) at 37^0 C. Oocytes were aspirated from follicle by using an 16-G needle attached to a 10 ml syringe containing 1 ml oocyte collection media. Oocytes were graded on the basis of morphology and number of the intact cumulus cell layers. Only good quality grade oocytes with intact cumulus layers and homogeneous cytoplasm were selected for culture. Culturable oocytes were subjected to *in vitro* maturation experiment. After grading oocytes were washed thrice in the OCM followed by final washing in maturation media and cultured at 38.5°C in 5% CO2 in maturation medium with different concentrations of GQ dots.

Microscopic Evaluation: Cultured oocytes were observed for microscopically for physiological changes during *in vitro* maturation. The expansion of cumulus cell layers and release of polar body was observed using inverted microscope (Nikon Diphot 300) and the recorded images of different treatment groups were compared.

Statistical Analysis: Data obtained from study were analyzed by one way and two way analysis of variance described by Snedecor and Cochran (1989) [23].

Results and Discussions

Effects of different concentrations of GQ dots on *In vitro* maturation of Caprine oocytes

Oocytes were collected from ovaries by aspiration method and graded on the basis of number of cumulus cell layers. Only good quality of oocytes with complete cumulus layers and homogeneous cytoplasm were selected. After grading of oocytes, 25 to 30 oocytes with cumulus-oocyte-complexes were cultured in maturation medium by hanging drop method for 24 hr with 5% CO_2 in air at 38.5°C. *In vitro* maturated oocytes were observed for expansion of cumulus cell complex and release of first polar body. Further BCB staining was performed and BCB+ oocytes with expanded COCs were counted. The matured oocytes have well expanded cumulus oophorus complexes and cytoplasm took homogenous BCB stain. However immature oocytes showed less or no expansion of COCs and cytoplasm remains unstained with BCB dye. All treatment groups have significantly lower percentage of *in vitro* matured oocytes as compared to control group. Group IV have lowest number of matured oocytes and it differed significantly from group II and III also. Group II and III showed comparable number of matured oocytes. There were significant differences in percentage of matured oocytes among different concentration of graphene quantum dots nanoparticle (Table 1). This study showed that exposure to GQ dots gives significant effect on Caprine oocytes. As increased concentration of GQ dots nanoparticles decreased the maturation efficiency of oocytes.

Table 1: Effects of different concentrations of GQ dots NPs on *in vitro* maturation of caprine oocytes

Group I	Group II	Group III	Group IV
71.00 ± 0.57^x	65.33 ± 0.88^y	64.00 ± 2.51^y	53.33 ± 0.88^z

Oocyte maturation in ovarian follicle precedes the process of ovulation and oocyte becomes developmentally competent at the time of fertilization. Series of events during the process of maturation are regulated by the cumulus oophorus cells complex. It modulates the timings of resumption of meiotic division process prior ovulation. *In vitro* maturation becomes more critical due to precise simulation of *in vivo* conditions during this process. Molecular and genomic alterations lead to cellular organelle reorganizations before fertilization (Fulka *et al.*, 1998; Mao *et al.*, 2014) [24, 25]. Microenvironment alterations directly influence the developmental competence of oocytes. Duration of maturation and quality of matured oocyte is sensitive toxicological parameter and it depends on the culture conditions for *in vitro* maturation. In the present study the functional parameters of oocyte maturation, the cumulus cell expansion and quality of matured oocytes were assessed.

Significant difference is recorded in treatment groups exposed to various concentrations of GQ dots. Similar results were reported in porcine oocytes co-incubated with gold-silver alloy nanoparticle (with 80% or more silver molar fraction) which showed significant decrease in maturation rate. Similarly, Ag+ ions (added as $AgNO_3$ at 12.5ug/ml) has completely arrested maturation in porcine oocytes. However gold nanoparticles of different sizes (6nm and 20nm) and different concentration (10 µg/ml and 30 µg/ml) have no effect on oocyte maturation (Tiedemann *et al*., 2014) [26]. In Zebra fish where Ag NPs (30 mg/mL) and AgNO3 (10 mg/mL) was added during oocyte maturation resulted in apoptotic changes in surrounding granulosa cells leading to reduced maturation rate (Chen *et al.*, 2017) [27]. TiO2 (25 nm) at different concentrations (25 µg/ml or above) significantly inhibited the follicular development and *in vitro* oocyte maturation in rats (Juan *et al.*, 2009) [28].

CdTe/ZnTe QDs-Tf bioconjugates also have reprotoxic effects on follicle development and oocyte maturation. Oocyte maturation rate along with release of first polar body was affected in treatment groups (Xu *et al.*, 2012) [29]. Previously also similar combinations of QDs are reported to reduce the oocyte maturation rate in mice by disturbing the signal interaction between cumulus cells and germ cell (Lin *et al.*, 2009) [30]. In mice at high concentrations of CeO_2 NPs (10 and

100 mg/L) significant changes are observed in follicular cells which were unable to prevent the mature oocytes from oxidative stress and DNA damage (Courbiere *et al*., 2013) [31]. *In vitro* maturation of oocytes is highly sensitive parameter to evaluate the repo toxicity of nanoparticles because depends on slight variations in microenvironment of culture conditions. It can be concluded that GQ dots have dose dependent cytotoxic effects on Caprine oocytes and their increasing concentration have detrimental impact on the maturation of oocytes.

Conclusion

It can be concluded that in different concentration of GQ dots shows that oocyte maturation affected at irregular rate. The oocyte maturation is severely influenced by the GQ dots nanoparticles even at lower concentration (≥10µg/ml).

References

1. Ali KY, Qu H, Manbanchi A, Butt B, Dokmeci MR, Hinestroza JP *et al*. Nanotechnology in Textiles. ACS Nano. 2016; 10(3):3042-3068.
2. Wiechers JW, Musee N. Engineered inorganic nanoparticles and cosmetics: Facts, issues, knowledge gaps and challenges. J Biomed. Nanotech. 2010; 6(5):408-431.
3. Nikaje AP. Nanotechnology and its applications in medicine. Med. Chem. 2015; 5(2):81-89.
4. Chaudhry Q, Scotter M, Blackburn J, Ross B, Boxall A, Castle L *et al*. Applications and implications of nanotechnologies for the food sector. Food Addit. Contam. 2008; 25(3):241-258.
5. Agrawal S, Rathore P. Nanotechnology pros and cons to agriculture: A review. Int. J Curr. Microbiol. App. Sci. 2014; 3(3):43-55.
6. Gannon CJ, Patra CR, Bhattacharya R, Mukherjee P, Curley SA. Intracellular gold nanoparticles enhance non-invasive radiofrequency thermal destruction of human gastro intestinal cancer cells. J Nanobiotechnology. 2008; 6:2.
7. Molday RS, Mackenzie D. Immunospecific ferromagnetic iron dextran reagents for the lebeling and magnetic separation of cells. J Immunol Methods. 1982; 52:353-367.
8. Holt W, Fazeli A. Sperm selection in the female mammalian reproductive tract. Focus on the oviduct: Hypotheses, mechanisms, and new opportunities. Theriogenology. 2016; 85(1):105-112.
9. Knox RV. Artificial insemination in pigs today. Theriogenology. 2016; 85(1):83-93.
10. Suarez SS, Pacey AA. Sperm transport in the female reproductive tract. Human Reproduction Update. 2006; 12(1):23-37.
11. Nishida E, Miyaji H, Takita H, Kanayama I, Tsuji M, Akasaka T *et al*. Graphene oxide scaffold accelerates cellular proliferative response and alveolar bone healing of tooth extraction socket. Int. J Nanomed. 2016; 11:2265-2277.
12. Monerris MJ, Arévalo FJ, Fernández H, Zon MA, Molina PG. Integrated electrochemical immunosensor with gold nanoparticles for the determination of progesterone. Sens Actuators B Chem. 2012; 166/167:586-592.
13. Jha RK, Jha PK, Chaudhury K, Rana SVS, Guha SK. An emerging interface between life science and nanotechnology: present status and prospects of reproductive healthcare aided by nano- biotechnology. Nano Rev. 2014; 5:22762.
14. Wang T, Zhao G, Liang XM, Xu Y, Li Y, Tang H *et al*. Numerical simulation of the effect of super paramagnetic nanoparticles on microwave rewarming of cryopreserved tissues. Cryobiology. 2014; 68:234-243.
15. Hen SX, Yang XZ, Deng Y, Huang J, Li Y, Sun Q. Silver nanoparticles induce oocyte maturation in zebrafish (Danio rerio). Chemosphere. 2017; 70:51-60.
16. Li R, Jin Z, Gao L, Liu P, Yang Z, Zhang D. Effective protein inhibition in intact mouse oocytes through peptide nanoparticle- mediated antibody transfection. Peer J. 2016; 4:1849.
17. Hou J, Wan X, Wang F, Xu G, Liu, Z, Zhang T. Effects of titanium-dioxide nanoparticles on development and maturation of rat preantral follicle. Academic Journal of Second Military Medical University. 2009; 29(8):869-73.
18. Gao G, Ze Y, Li B, Zhao X, Zhang T, Sheng L *et al*. Ovarian dysfunction and gene-expressed characteristics of female mice caused by long term exposure to titanium dioxide nanoparticles. J Hazard Mater. 2012; 243:19-27.
19. Zhao X, Ze Y, Gao G, Sang X, Li B, Gui S *et al*. Nanosized TiO2-induced reproductive system dysfunction and its mechanism in female mice. PLoS One. 2013; 8:e59378.
20. Ariu F, Bogliolo L, Pinna A, Malfatti L, Innocenzi P, Falchi L *et al*. Cerium oxide nanoparticles (CeO2 NPs) improve the developmental competence of *in vitro*-matured prepubertal ovine oocytes. Reprod Fertil Dev. 2016; 29(5):1046-1056.
21. Abdel-Halim BR, Moselhy WA, Helmy NA. Developmental competence of bovine oocytes with increasing concentrations of nano- copper and nano-zinc particles during *in vitro* maturation. Asian Pacific Journal of Reproduction. 2018; 7(4):161.
22. Wahjuningsih S, Isnaini N, Costa N. Supplementation of goat follicular fluidin TCM 199 based medium on *In Vitro* mature oocytes. American-Eurasian Journal of sustainable Agriculture. 2014; 8(7):39-43.
23. Snedecor GW, Cochran WG. Statistical Methods, 8thed. Oxford and IBH Publishing Company, New Delhi, 1989.
24. Fulka J, First NL, Moor RM. Nuclear and cytoplasmic determinants involved in the regulation of mammalian oocyte maturation. Mol. Hum. Reprod. 1998; 4:41-49.
25. Mao L, Lou H, Lou Y, Wang N, Jin F. Behaviour of cytoplasmic organelles and cytoskeleton during oocyte maturation. Reprod. Biomed. 2014; 28:284-299.
26. Tiedemann D, Taylor U, Rehbock C, Jakobi J, Klein S, Kues WA *et al*. Reprotoxicity of gold, silver, and gold-silver alloy nanoparticles on mammalian gametes. Analyst. 2014; 139(5):931-42.
27. Chen SX, Yang XZ, Deng Y, Huang J, Li Y, Sun Q *et al*. Silver nanoparticles induce oocyte maturation in zebrafish (Daniorerio). Chemosphere. 2017; 170:51-60.
28. Juan H, Ying WX, Fei W, Gui Feng, X Zhen L, Tian Bao Z. Effects of titanium dioxide nanoparticles on development and maturation of rat preantral follicle *in vitro*. Academic Journal of Second Military Medical University. 2009; 30(8):869-873.
29. Xu G, Lin S, Law WC, Roy I, Lin X, Mei S *et al*. The Invasion and Reproductive Toxicity of QDs-Transferrin Bioconjugates on Preantral Follicle *in vitro* Theranostics. 2012; 2(7):734-745.
30. Lin XT, Xu GX, Yong KT, Roy I, Qu JL, Wang XM. Visualization of reproduction toxicity of QDs for *in vitro*

oocytes maturation. Proceedings of SPIE. 2009; 7519:75191.
31. Courbiere B, Auffan M, Rollais R, Tassistro V, Bonnefoy A, Botta A *et al*. Ultrastructural interactions and genotoxicity assay of cerium dioxide nanoparticles on mouse oocytes. Int J Mol Sci. 2013; 14:21613-21628.