

Physiological and histological effects of (zinc and iron) oxide nanoparticles on some fertility parameters in female mice

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Article info

Received
13/12/2015

Accepted
22/5/2016

ABSTRACT

Nowadays nanoparticles have widespread application in various industries because of their special and unique features, there are many studies in side effects of nanomaterial. This study done by 40 white female mice with every other day intraperitoneally injection of low and high doses of both of ZnO nanoparticles (20 and 150 µg/kg of body weight) and FeO nanoparticles (5 and 40 mg/kg). After a 15 days period, the mice were sacrificed and blood samples were collected for hormone analysis, and tissue samples for morphometric studies.

Statistical Analysis shows significant differences in LH, Estrogen, Progesterone hormone levels between groups, while there are insignificant differences in Follicle stimulating hormone (FSH) level between the groups compared with its level in the control group.

The results also show that the highest level of LH reach 7.2 mIU/ml in the groups treated with low dose of zinc oxide, the highest level of FSH reach 4.58 mIU/ml in the groups treated with low dose of zinc oxide, the highest level of Estrogen hormone reach 69.5 ng/ml in the groups treated with low of dose zinc oxide and the highest level of Progesterone reach 1.9 ng/ml in the groups treated with high dose iron oxide. We conclude from the results that the low doses of ZnO has benefits in increasing fertility through high level of reproductive hormones, while the high levels of nanoparticles reduce fertility and there is a relation between FeO nanoparticles and progesterone levels which may need more future studies.

Morphometric study of the ovary show increase in Follicular stages number range in the group treated with Low dose ZnO in compare with its range in the control groups. The lower range was belong to the group treated with the high dose of FeO. No significant differences has been found in the diameter mean of the different follicular phases between the group treated with low dose of ZnO NPs in compared with the control group. High dose of ZnO NPs cause significant increase in the diameter mean of Primordial follicles in compared with the control group. Low and high dose FeO NPs treated groups show significant reduction in the diameter mean of the different follicular phases in compared with the control group.

Keywords: Nanoparticles, fertility, hormones, mice.

الخلاصة

للجزيئات النانوية في الوقت الحالي تطبيقات متنوعة وفي مختلف الصناعات وذلك بسبب خصائصها الفريدة وقد استحدثت العديد من الدراسات التي تهتم بكشف التأثيرات الجانبية والتأثيرات الفسيولوجية لهذه الجزيئات. شملت الدراسة الحالية 40 من اناث الفئران البيض تم حقنها يوميا بمقدار 0.1 مليلتر في البريتون بجرعات قليلة وعالية من جزيئات اوكسيد الزنك النانوية (بتركيز 20 و 150 ميكروغرام /كيلوغرام من وزن الجسم) و جزيئات اوكسيد الحديد النانوية (بتركيز 5 و 40 ميكروغرام /كيلوغرام من وزن الجسم) و بعد 15 يوم من الحقن تم الحصول على عينات الدم من الفئران لغرض اجراء الاختبارات الهرمونية وعينات نسيجية لاجراء دراسات مورفومترية.

اظهرت الدراسات الاحصائية وجود فروقات معنوية في مستويات هرمونات (هرمون الاباضة LH و الاستروجين E2 والبروجسترون Prg) مقارنة مع مستوياتها في مجموعة السيطرة في حين لم توجد اي فروقات معنوية في مستوى الهرمون المحفز للجريبات FSH بالمقارنة مع السيطرة . وجد من خلال نتائج البحث ان اعلى مستوى لهرمون الاباضة بلغ 7.2 mIU/ml في المجاميع المعاملة بجرع واطئة لجزيئات اوكسيد الزنك النانوية و اعلى مستوى لهرمون محفز الجريبات بلغ 4.58 mIU/ml كان لدى المجموعة المعاملة بجرعة واطئة لجزيئات اوكسيد الزنك النانوية و اعلى مستوى لهرمون الاستروجين بلغ 69.5 ng/ml لدى المجموعة المعاملة بجرعة واطئة لجزيئات اوكسيد الزنك النانوية و اعلى مستوى لهرمون البرجسترون بلغ 1.9 ng/ml كان لدى المجموعة المعاملة بجرعة عالية لجزيئات اوكسيد الحديد النانوية. تشير هذه النتائج الى كون الجرعات الواطئة لجزيئات اوكسيد الزنك النانوية لها فائدة في زيادة معايير الخصوبة من خلال رفع مستويات هرمونات الخصوبة، بينما الجرعات العالية تسبب اختزال مستويات هذه الهرمونات. كما ان هناك علاقة بين التراكيز العالية لجزيئات اوكسيد الزنك النانوية ومستوى هرمون البروجسترون وهي حقيقة تحتاج لمزيد من الدراسة في المستقبل.

وجد من خلال دراسة المقاطع النسيجية لمبايض الفئران زيادة في اعداد المرحلة الجريبية لدى المجاميع المعاملة بجرعات واطئة من جزيئات اوكسيد الزنك النانوية وبالمقارنة مع اعدادها لدى مجموعة السيطرة واطل اعداد لهزه المرحلة وجدت لدى المجموعة المعاملة بجزيئات اوكسيد الحديد النانوية وبالمقارنة مع اعدادها لدى مجموعة السيطرة. لم تلاحظ فروقات معنوية في معدل قطر مختلف مراحل البيضة لدى المجموعة المعاملة بجرعة واطئة من جزيئات اوكسيد الزنك النانوية وبالمقارنة مع مجموعة السيطرة، في حين الجرعة العالية من جزيئات اوكسيد الزنك النانوية سببت زيادة معنوية في معدل قطر المرحلة الاولى للبيضة وبالمقارنة مع مجموعة السيطرة. أما الجرعات الواطئة والعالية لجزيئات اوكسيد الحديد النانوية سببت اختزالا معنويا في معدل قطر مختلف مراحل البيضة وبالمقارنة مع مجموعة السيطرة.

INTRODUCTION

Small size and high surface area of Nanoparticles make it a principle participant in all aspects of modern life application [1]. Because of size dependent chemical and physical properties, nanoparticles are considered an interesting candidates for applications in both *in vivo* and *in vitro* biomedical research. In the field of medicine, nanoparticles implicated mainly in targeted drug delivery, imaging, sensing, and artificial implants, use as antimicrobials to target highly pathogenic and drug resistant microbes [2]. The application of nanoparticles on health and environment needs to be assessed before their large-scale production and application in various fields [3-4].

Nanoparticles rapidly can enter the bloodstream and reach to the organs (including the brain, heart, kidneys) [5] Experiment some of injected nanoparticles show that they have negligible side effect [6]. High surface area and other features of nanoparticles cause it is very reactive and toxic and can damage human and animal cells by increasing oxidative stress mechanism [1].

Among the various metal oxides studied for their medicinal application, zinc oxide nanoparticles have been found to be highly toxic [7-8]. ZnO NPs are used in many commercial products such as cosmetics, paints, textiles, and sanitary products. ZnO NPs are used in nutrients as additives. They have anti-fungal property which are used as pesticides in

agriculture sectors and is also deployed in anticancer characteristics in medicines [9]. Iron with many other nanoparticles characterized by its magnetic properties and stability [10]. Iron oxide nanoparticles have widespread application for *in vivo* and *in vitro* research due to the physiochemical characteristics [11], and used for drug delivery in cancer therapy since 1970 [12-13]. Yet, there is a few studies have been done about the effects of nanoparticles on the reproductivity potential [14]. The goal of this study is to evaluate the effect of low and high doses of ZnO and FeO nanoparticles on fertility in female mice.

MATERIALS AND METHODS

Chemicals and kits:

- Zinc oxide nanoparticles (ZnO NPs) solution (Conc. Wt % in water, size 35 nm) was obtained from Sigma, USA.
- Iron oxide nanoparticles (Fe₂O₃ NPs) solution (Conc. 20 wt% in water, size 30 nm) was obtained from Sigma, USA.

Solutions preparations:

Nanoparticles solutions: The solutions of nanoparticles were prepared by distilled water with a minimum concentration (20 µg/kg) of ZnO for low dose group and with a maximum concentration (150 µg/kg) of ZnO for high dose group. FeO nanoparticles solutions prepared by the same way with distilled water (low dose concentration 5 mg/kg and high dose concentration 40 mg/kg) [15-16].

Phosphate buffer saline (PBS): (8g of NaCl, 0.2g of KCl, 1.44g of Na₂HPO₄, 0.24g of KH₂PO₄) .Dissolve the following in 1L distilled H₂O ,Adjust pH to 7.4 with HCl.

Experiment design: Fifty adult female mice were obtained from the animal house of National center for drug control and research and kept in the animal house of biotechnology Research Center, Al-Nahrain University ,where its placed in a separated cages at (25C^o) room temperature, and fed with suiTable diet in addition to water.

The experimental animals were randomly divided into five groups as follows:

- First group: Control group consisted of 10 animals were injected intraperitoneally with PBS for 15 days.
- Second group: consisted of 10 animals were injected intraperitoneally with 0.1 ml of ZnO nanoparticles solution (20 µg/kg) for 15 days., represent the low dose group.
- Third group: consisted of 10 animals were injected intraperitoneally with 0.1 ml of ZnO nanoparticles solution (150 µg/kg) for 15 days., represent the high dose group.
- Fourth group: consisted of 10 animals were injected intraperitoneally with 0.1 ml of FeO nanoparticles solution (5 mg/kg) for 15 days., represent the low dose group.
- Fifth group: consisted of 10 animals were injected intraperitoneally with 0.1 ml of FeO nanoparticles solution (40 mg/kg) for 15 days., represent the high dose group.

Specimen collection and processing:

Blood samples were collected for hormonal analysis. Serum was separated by centrifugation at 3000 rpm for 15 min and stored at - 20° C until analysis. Luteinizing hormones (LH), Follicle stimulating hormones (FSH), Estrogen, Progesterone hormones levels were estimated using ELIZA kit (Orgmetric Germany) in accordance with the manufacturer's recommendations.

The ovaries of three mice of each group were immediately excised and preserved in 10% formalin for morphometric study which is conducted according to the method used by [17]. Eight microns thick sections were cut and stained with hematoxylin and eosin for microscopic examination.

Morphometric study were achieves using ocular micrometer

Ovaries of three animals of each group besides the control were taken for the follicular studies. Sections of the ovary were examined under a light microscope and the general histologic appearance of the ovary was assessed. All sections of the ovary were counted for various stages of development of follicles as described by Junqueira *et al.*, 1992 [18]. Four classes of ovarian follicles were categorized as follows:

- Primordial follicle (PF): contained an oocyte surrounded by a partial or complete layer of squamous granulosa cells.

- Primary follicle: showed a single layer of cuboidal granulosa cells. "Growing" follicles had an oocyte surrounded by a multilayered, solid mantle of granulosa cells.
- Secondary follicles were surrounded by more than one layer of cuboidal granulosa cells and accumulations of follicular fluid appear between the granulosa cells.
- Antral follicles were characterized by a central oocyte enriched by a fluid-filled space and bordered by hundreds of layered granulosa cells.

By using these criteria, mean diameter of each follicle stage have been measured by using a calibrated ocular micrometer to avoid repeated counting. (see Figure 1).

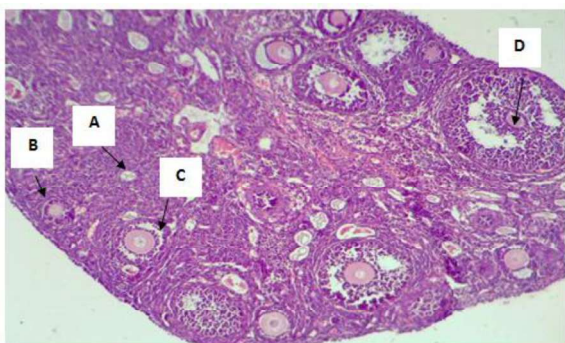


Figure 1: Histological section of mice ovary (20×) represents different stages of follicles. A:Primordial, B: Primary follicle C: Secondary follicle, D: Antral follicle.

Statistical Analysis: The Statistical Analysis System-SAS (2010) was used to study the effect of different factors in studied parameters. Least significant difference –LSD test was used to significant compare between means in this study. Results were expressed as mean±SE. Values were considered to be statistically significant at $P < 0.05$ [19].

RESULTS AND DISSCUSSION:

Reproductive hormones analysis:

Statistical Analysis of our results (see Table 1) shows significant differences in LH and estrogen levels between the groups and its levels in the control group.

The results also show that the highest level of LH reach 7.20 ± 0.34 mIU/ml in the groups treated with low dose zinc oxide, the highest level of FSH reach 4.58 ± 0.30 mIU/ml in the groups treated with low dose zinc oxide, the

highest level of Estrogen hormone reach 69.50 ± 2.10 ng/ml in the groups treated with low dose zinc oxide and the highest level of Progesterone reach 1.90 ± 0.08 ng/ml in the groups treated with high dose iron oxide.

Estrogen hormone reaches its lowest level in the group treated with high dose of ZnO. There are no significant differences noticed in the FSH and Progesterone levels between the groups and in compare with its levels in the control group.

These results agree with the results of (Esmacillou *et al.*, 2013) [20]. In this research, there is no alteration in serum sex hormone levels such as FSH, LH and estradiol in female Wistar rats after administration with 333.33 mg/kg zinc oxide NPs (ZnO-NPs).

Our study showed that low doses of ZnO nanoparticles cause increase in the reproductivity through the high levels of LH, FSH and estrogen, while the high dose causes low level of these hormones, this referred to decreased in reproductivity as mentioned by Espanani *et al.*, 2014 [21]. In his research, he confirmed that exposing to ZnO nanoparticles damaged to public health and reduced fertility potential. Puran *et al.*, 2013 [22] referred to the protective effect of zinc oxide nanoparticles through its antioxidant potential against the adverse effects of the anticancer drug, which is known as doxorubicin (DOX), one of its adverse effects is male infertility [23]. Administration of ZnO significantly improved DOX-induced changes in plasma total antioxidant power (TAP), Lipid peroxidation (LPO), plasma testosterone, LH, sperm count and DNA damage.

The zinc is one of the seven main trace elements besides to copper, iron, cobalt, iodine, manganese and selenium among these, iron (1.0-2.0 ppm) is the most abundant in serum followed by zinc (0.8-1.2 ppm) [24].

However, toxicological studies have shown that ZnO nanoparticles could be harmful to human and other species [25]. Han *et al.*, 2011 [26] reported neurotoxicity in rats after intraperitoneal administration of ZnO nanoparticles. Many studies have reported the cytotoxic and genotoxic effects of ZnO nanoparticles in various mammalian cell lines [27-28]. Results showed the benefits of using nanoparticles in low doses which may have a

protective effect through the antioxidant mechanisms, at the same time the high doses may have a harmful effects on many levels. This definitely requires more studies for the mechanisms that may cause this harm.

Zinc has an essential role for thyroid hormone secretion and function and other hormones such as testosterone, insulin, and growth hormone [29-30]. Also, zinc plays an essential role in sexual development. Copper and zinc play an important role in regulating progesterone production by luteal cells via involvement of superoxide dismutase [31]. Zinc is involved in there organization of ovarian follicles which are the source of progesterone. This occurs through the involvement of metalloproteinase-2 (MMP-2) enzyme, which is a member of zinc endopeptidase family [32].

Table 1: Hormones Concentrations between the different groups.

Group	Mean \pm SE			
	LH (mIU/ml)	FSH (mIU/ml)	Estrogen (ng/ml)	Progesterone (ng/ml)
Control	6.70 \pm 0.79 ab	4.45 \pm 0.37 a	69.00 \pm 2.48 a	1.52 \pm 0.28 a
Low dose ZnO	7.20 \pm 0.34 a	4.57 \pm 0.30 a	69.50 \pm 2.10 a	1.75 \pm 0.22 a
High dose ZnO	6.67 \pm 0.54 ab	4.32 \pm 0.39 a	35.00 \pm 2.34 b	1.57 \pm 0.21 a
Low dose FeO	6.35 \pm 0.46 ab	4.55 \pm 0.29 a	68.00 \pm 2.67 a	1.60 \pm 0.28 a
High dose FeO	5.60 \pm 0.25 b	4.16 \pm 0.24 a	67.66 \pm 3.39 a	1.90 \pm 0.08 a
LSD value	1.033 *	0.882 NS	16.752 *	0.579 NS
* (P<0.05), NS: Non-significant.				

*Significant

NS : Not Significant

After short-terms of exposure to zinc, histological alterations were observed in organs like the pancreas, pituitary gland, and adrenal gland of zinc-exposed mice fed with zinc sulphate diet [33]. Straube *et al.*. (1980) [34]

also examined the effects of excess dietary zinc in adult ferrets fed a diet of canned dog food (that contained 27 ppm zinc and 3.3 ppm copper). Animals in high doses between (1500 and 3000) ppm showed signs of severe toxicity represented by (macrocytic hypo-chromic anemia, increased reticulocyte count, diffuse nephrosis, and the presence of protein, glucose, blood, and bilirubin in the urine, increases in tissue zinc levels, decreases in copper levels, and decreased ceruloplasmin oxidase activity). Increasing use of iron oxide nanoparticles in medicine and environmental remediation has led to concerns regarding exposure of these nanoparticles to the public [35]. Besides the magnetic properties of iron (II, III) oxide nanoparticles (IONPs) it is also have an abundant potential in several biomedical and clinical applications, such as magnetic resonance imaging (MRI), tissue repair, as drug delivery, and magnetic hyperthermia cancer treatment [36].

However, possible risk on fertility hormones due to increasing application of Fe₂O₃ is not fully elucidated. Table 1 shows that no significant differences have been found between hormones levels in the low dose of FeO NPs treated group and control group. While high dose cause significant decrease in LH level in comparison with the control.

Recent studies reported that FeO NPs have the ability to induce genotoxicity which is likely to be mediated through ROS generation and oxidative stress in a dose-dependent manner, evident by depletion of glutathione lipid peroxidation [37]. However, in less than seven days the excess iron was either excreted or incorporated into the body [38]. (In the form of co-factors, hemoglobin, *etc.*).

Changes of oxidative stress were observed to be dependent on the body mass index (BMI) and the duration of infertility of the enrolled women. Levels of malondialdehyde (MDA) correlate negatively with LH concentration; meaning the increase in oxidative stress followed by regression in LH levels, suggesting that oxidative stress is involved in the pathophysiology of infertility in females, particularly through the induction of changes in gonadotrophin hormones [39].

Morphometric study of the ovary

Table 2 shows increase in Follicular stages number range in the group treated with Low dose ZnO in compare with its range in the control groups. Other groups' show a decrease in the Follicular stages number range and the lower range was belong to the group treated with the high dose of FeO, this may refer to the damaged caused by FeO precipitation in ovary tissue.

No significant differences has been found in the diameter mean of the different follicular phases between the group treated with low dose of ZnO NPs in compared with the control group, for example: (The diameter of primordial phase is $92.50 \mu\text{m} \pm 11.08$ in Low dose ZnO group in comparison to control group $95.00 \mu\text{m} \pm 20.20$). High dose of ZnO NPs cause significant increase in the diameter mean of Primordial follicles ($160.00 \mu\text{m} \pm 9.12$) in compared with the control group ($95.00 \mu\text{m} \pm 20.20$). Low and high dose FeO NPs treated groups show significant reduction in the diameter mean of the different follicular phases in compared with the control group as show in Table 3, for example: (The diameter of Antral follicle phase is $595.00 \mu\text{m} \pm 25.98$ in low dose FeO group in comparison to control group $710.00 \mu\text{m} \pm 102.14$).

Table 2: Number ranges of follicular stages in mice ovary in the different treated groups

Follicle stage	Groups				
	G1/Control	G2/Low dose ZnO	G3/High dose ZnO	G4/Low dose FeO	G5/High dose FeO
Primordial	9-18	11-20	5-7	5-7	1-2
Primary follicle	8-16	10-18	6-7	6-7	1-2
Secondary follicle	6-8	10-12	4-5	4-5	2-3
Antral follicle	1-3	2-3	1-2	1-2	0

Table 3: Follicles diameter (Micrometer) of different stages of mice ovary in the different groups

Group	Mean (μm) \pm SE			
	Primordial	Primary follicle	Secondary follicle	Antral follicle
Control	95.00 \pm 20.20 b	92.50 \pm 11.08 b	160.00 \pm 9.12 a	710.00 \pm 102.14 a
Low dose ZnO	92.50 \pm 11.08 b	286.67 \pm 18.55 a	343.33 \pm 23.92 a	736.67 \pm 86.85 a
High dose ZnO	160.00 \pm 9.12 a	288.00 \pm 15.27 a	367.50 \pm 13.67 a	693.33 \pm 82.93 a
Low dose FeO	80.00 \pm 9.12 b	166.67 \pm 24.03 c	345.00 \pm 45.55 a	595.00 \pm 25.98 b
High dose FeO	57.50 \pm 4.78 c	160.00 \pm 11.54 c	310.00 \pm 39.79 b	0.00 \pm 0.00 c
LSD value	22.194 *	39.613 *	29.850 *	72.893 *

* (P<0.05).

Control	95.00 \pm 20.20 b	290.00 \pm 32.14 a	366.67 \pm 32.99 a	710.00 \pm 102.14 a
Low dose ZnO	92.50 \pm 11.08 b	286.67 \pm 18.55 a	343.33 \pm 23.92 a	736.67 \pm 86.85 a
High dose ZnO	160.00 \pm 9.12 a	288.00 \pm 15.27 a	367.50 \pm 13.67 a	693.33 \pm 82.93 a
Low dose FeO	80.00 \pm 9.12 b	166.67 \pm 24.03 c	345.00 \pm 45.55 a	595.00 \pm 25.98 b
High dose FeO	57.50 \pm 4.78 c	160.00 \pm 11.54 c	310.00 \pm 39.79 b	0.00 \pm 0.00 c
LSD value	22.194 *	39.613 *	29.850 *	72.893 *

As noticed from the results, there's an obvious effect on the hormones levels, while the histological and morphometric study show no significant differences. Possibly, these disparities may due to differences in dosage, chemicals, particle size, and other factors related to the experimental design and the physio-chemical characteristics of the NPs investigated.

The issue concerning alterations in sex hormone levels caused by exposure to some NPs, has also been addressed by some *in vivo* studies which have provided conflicting results. In fact, serum levels of estradiol significantly increased, while progesterone, FSH, LH and testosterone levels diminished in female mice sub-chronically treated with TiO₂-NPs [40].

Nevertheless, the administration of zinc oxide NPs to female rats at a concentration of 333.33 mg/kg did not alter serum levels of FSH, LH and estradiol hormones [20]. Some nanoparticles like TiO₂-NP induce changes in hormone levels (increased estradiol and decreased progesterone, FSH, LH and testosterone) and cause ovarian injury in female mice may be related to alterations in ovarian gene expression, e.g., *Akr1c18*, *StAr*, *Cyp17a1*, and *Lgmn* genes were up-regulated. [41]. Little is known about the morphometric alterations induced by nanoparticles, a point to be clarified in the present study.

These results support an idea that iron could play a role in regulation of ovarian function, hormone release, proliferation and apoptosis. [42]. Iron is necessary in many cellular functions, in many body processes. Continuously, the iron accumulates in many tissues such as liver, kidneys [43]. Spleen [44], and uterus [45]. In excess, however, iron is toxic to cells. Free iron ions are extremely toxic and capable of catalyzing many deleterious reactions in cells and tissues [46]. Excess of iron could affect a wide range of mechanisms involved in endometriosis development [47], such as oxidative stress and tissue damage [46], or lesion proliferation [47]. Iron delivered to proliferating cells by transferrin which plays a crucial role in the local regulation of ovarian function and it is important factor for the regulation of granulosa cell differentiation [48].

Cyclin B1 is a marker of proliferation. The expression of cyclin B1 in porcine ovarian granulosa cells is influenced by some metals such as lead, cobalt and iron [46].

Metal-based NPs were reported in many studies to induce changes in reproductive organs histology of laboratory animals and in consequent cause a disruption in reproductive cells production and hormones [49-50]. After 1–5 h of treatment, Au-NPs cause a greater output of estradiol from ovarian granulosa cells, while they decreased the estradiol level after 24 h. TiO₂-NP cause changes in hormone levels and progesterone levels in rats.

In addition to the antioxidants released as a response to the oxidative process, nanoparticles may interact with metal-sequestering proteins and antioxidants (from body fluids and intracellularly), that will likely modify the surface properties of the nanoparticle to some extent, rendering them less toxic [51].

Evaluation of these results should therefore take these variables into account. Moreover, when analyzing the endocrine effects on the female reproductive system, it should be noted that the results varies depending on the size of NPs, duration and concentration of the doses.

REFERENCES

- [1] Noori, A., Momayez, M., Sadeghi, L., Shirani, K. and Babadi, V.Y. The effects of nano titanium dioxide (TiO₂) in spermatogenesis in wistar rat. *European J. of Exper. Bio.*, 3:145-149, 2013.
- [2] Ravishankar, R. V. and Jamuna B. A. Nanoparticles and their potential application as antimicrobials Science against microbial pathogens: communicating current research and technological advances. A. Méndez-Vilas (Ed.): 197-209, 2011.
- [3] [Finney, L.A.](#) and [O'Halloran, T.V.](#) Transition metal speciation in the cell: insights from the chemistry of metal ion receptors. *Science*, 9; 300:931-936, 2003.
- [4] Sharma, V., Shukla, R.K., Saxena, N., Parmar, D., Das, M. and Dhawan, A. DNA damaging potential of zinc oxide nanoparticles in human epidermal cells, *Toxicol. Lett.*, 185, 211-218, 2009.
- [5] YousefiBabadi, L., Najafi, A., Najafi, H., Gholami, M., BeigiZarji, J., Golzadeh, E., Amraie, A. and Shirband, Evaluation of iron oxide nanoparticles effects on tissue and enzymes of liver in rats *J. Pharma. Biomed. Sci.*, 23, 1-4, 2012.
- [6] Huang, Z., Zheng, X., Yan, D., Yin, G., Liao, X., Kang, Y., Yao, Y. and Huang D. Toxicological effect of ZnO nanoparticles based on bacteria, *Hao. BLangmuir*, 24, 4140-4144, 2008.
- [7] Brayner, R., Ferrari-Iliou R., Brivois N., Djediat S., Benedetti M.F. and Fievet F. Toxicological impact studies based on Escherichia coli bacteria in ultrafine ZnO nanoparticles colloidal medium. *Nano. Lett.*;6: 866–870, 2006.
- [8] Zhang L.L., Jiang Y.H., Ding Y.L., Povey M. and York D. Investigation into the antibacterial behavior of suspensions of ZnO nanoparticles (ZnO nanofluids). *J.Nanopart. Res.*; 9:479–489, 2007.
- [9] Nazem, H. and Arefian, Z. Effect of ZnO NPs on tumor marker hormones in

- male rats. *Biomed. Res.*; 26 (1): 82-88, 2015.
- [10] Sun, C., Lee, J.S. and Zhang, M. Magnetic nanoparticles in MR imaging and drug delivery, *Adv. Drug, Deliv. Rev.*, 60, 1252-1265, 2008.
- [11] Park, E.J., Kim, H., Kim, Y., Yi, J., Choi, K. Inflammatory responses may be induced by a single intratracheal instillation of iron nanoparticles in mice., *Toxicol.*, 275, 65- 71, 2010.
- [12] Mirkovic, B., Lah-Turnsek, T. and Kos, J. Nanotechnology in the treatment of cancer, *Zdrav. Vestn*, 79, 146–155, 2010.
- [13] Wahajuddin, A. and Arora, S. Superparamagnetic iron oxide nanoparticles: magnetic nanoplatforms as drug carriers, *Int. J. Nanomedicine*, 7, 3445–3471, 2012.
- [14] Smijs, T.G, and Pavel, S. Titanium dioxide and zinc oxide nanoparticles in sunscreens: focus on their safety and effectiveness. *Nanotechnology, Science and Applications* 2011:4 95–112, 2011.
- [15] Vahid, Y.B., Esmail, A., Hojatollah, S., Leila, S., Najafi Leila, N. and Fazilati, Mohammad, F. Evaluation of Iron Oxide nanoparticles effects on tissue and Enzymes of Thyroid in Rats. *Intern. Res. J. of Biolog. Sci.* ; 2: 67-69, 2013.
- [16] Reza, E. H., Mohammad, F., Leila, S. Vahid Y.B., Somayeh, B. and Esmail, A. Investigation the Zinc Oxide Nanoparticle's Effect on Sex Hormones and Cholesterol in Rat. *Intern. Res. J. of Biolog. Sci.* 2: 54-58, 2013.
- [17] Bancroft, S, and Stevens, A. Enzyme histochemistry. In: *Theory and practice of histological techniques Bancroft and steven, A. (eds), 2nd edition. Churchill living stone, London. PP: 3450. 1982.*
- [18] Junqueira, L.C., Carneiro, J. and Kelley, R.O. *Basic histology. Seventh ed.* Connecticut, USA: Appleton & Lange, 1992.
- [19] SAS. 2010. *Statistical Analysis System, User's Guide. Statistical. Version 9.1th ed.* SAS. Inst. Inc.
- [20] Esmaeillou, M., Moharamnejad, M., Hsankhani, R., Tehrani, A.A. and Maadi, H. Toxicity of ZnO Nanoparticles in Healthy Adult Mice, *Environ. Toxicol. Phar*, 35: 67-71, 2013.
- [21] Espanani, H. R., Shirani, K. Sadeghi, L., Yousefi Babadi, V. and Amraei, E. Investigation of the Zinc Oxide Nanoparticles Effect on Testosterone, Cholesterol and Cortisol in Rats *Res. J. Recent Sci.* 3: 14-19, 2014.
- [22] Puran, B., Kazem, P., Seyed M., Seyed, D. and Alireza, S. Effect of nano-zinc oxide on doxorubicin- induced oxidative stress and sperm disorders in adult male Wistar rats. *Iran J Reprod Med.* 11: 355-364, 2013.
- [23] Zanetti, S.R., Maldonado, E.N. and Aveldaño, M.I. Doxorubicin Affects Testicular Lipids with Long-Chain (C18-C22) and Very Long-Chain (C24-C32) Polyunsaturated Fatty Acids. *Cancer Res.*, 67: 6973-6980, 2007.
- [24] Andrieu, S. Is there a role for organic trace element supplements in transition cow health? *Vet. J.* 176:77-83, 2008.
- [25] Guo, D., Bia, H., Wang D. and Wu Q. Zinc Oxide Nanoparticles Decrease the Expression and Activity of Plasma Membrane Calcium ATPase, Disrupt The Intracellular Calcium homeostasis in Rat Retinal Ganglion Cells, *Int. J. Biochem. Cell B*, 45: 1849– 1859, 2013.
- [26] Han, D., Tian, Y., Zhang, T., Ren, G. and Yang, Z. Nano-Zno Damages Spatial Cognition Capability Via Over-Enhanced Long -Term Potentiation in Hippocampus of Wistar Rats, *Int. J. Nanomedicine*, (6):1453–1461, 2011.

- [27] Monteiro-Riviere, N.A., Wiench. K., Landsiedel. R., Schulte. S., Inman. A.O. and Riviere, J.E. Safety Evaluation of Sunscreen Formulations Containing Titanium Dioxide and ZnO Nanoparticles in UVB Sunburned Skin: An In Vitro and In Vivo Study, *Toxicol. Sci*, 123: 264–280, 2011.
- [28] Landsiedel, R., Ma-Hock, L., Van Ravenzwaay, B, *et al.*, Gene toxicity Studies on Titanium Dioxide and ZnO Nanomaterials Used for UV-Protection in Cosmetic, *Nanotoxicology*, 4, 364–381, 2010.
- [29] Wang, R.L., Liang, J.G., Lu, L., Zhang, L.Y., Li, S.F. and Luo, X.G. Effect of zinc source on performance, zinc status, immune response, and rumen fermentation of lactating cows. *Biol.Trace Elem .Res*. 152: 16-24, 2013.
- [30] Machadoa, V.S., Bicalhoa, M., Pereira, R.V., Caixeta, L.S., Knauer, W.A., Oikonomou, G., Gilbert, R.O. and Bicalho R.C. Effect of an injectable trace mineral supplement containing selenium, copper, zinc, and manganese on the health and production of lactating Holstein cows. *Vet. J*. 197: 451-456, 2013.
- [31] Sales, J.N., Pereira, R.V., Bicalho, R.C. and Baruselli, P.S. Effect of injectable copper, selenium, zinc and manganese on the pregnancy rate of crossbred heifers (*Bos indicus* × *Bos taurus*) synchronized for timed embryo transfer. *Livestock Science*. 142: 59–62, 2011.
- [32] Gottsch, M.L., Murdoch, W.J. and Van Kirk, E.A. Tumour necrosis factor alpha upregulates matrix metalloproteinase-2 activity in preovulatory ovine follicles metamorphic and endocrine implications. *Reprod. Fertil. Develop*. 12: 75-80, 2000.
- [33] Aughey, E; Grant, L; Furman, B.L.; and Dryden, W. F. The effects of oral zinc supplementation in the mouse. *J Comp Pathol* 87:1-14, 1977.
- [34] Straube, E.F, Schuster, N.H. and Sinclair, J.A. Zinc toxicity in the ferret. *J Comp Pathol* 90:355-361, 1980.
- [35] Bombin,S.; LeFebvre,M; r Sherwood,J.; Xu,Y.; Yuping Bao,Y.; and Ramonell ,K.M. Developmental and Reproductive Effects of Iron Oxide Nanoparticles in *Arabidopsis thaliana* .*Int. J. Mol. Sci.*, 16, 24174-24193, 2015.
- [36] Soenen, S.J., Himmelreich, U., Nuytten, N. and De Cuyper, M. Cytotoxic Effects of Iron Oxide Nanoparticles and Implications for Safety in Cell Labelling. *Biomaterials*, 32, 195-205, 2011.
- [37] [Ahamed M.](#); [Alhadlaq, H.A.](#), [Alam J.](#), [Khan, M.A.](#), [Ali, D.](#) and [Alarafi, S.](#) Iron oxide nanoparticle-induced oxidative stress and genotoxicity in human skin epithelial and lung epithelial cell lines. [Curr Pharm Des.](#);19 (37):6681-6690, 2013.
- [38] Weissleder, R.; Stark, D.D.; Engelstad, B.L.; Bacon, B.R.; Compton, C.C.; White, D.L.; Jacobs, P. and Lewis, J. Superparamagnetic iron oxide: Pharmacokinetics and toxicity. *Am.J. Roentgenol*. 152, 167–173, 1989.
- [39] Hussain, M.K.; Mohammed ,H.J.; Al-Ghazali, B.S. and Abdul Hasan, M.T.. Oxidative Stress in Primary Infertility of Women. *Glob. J. of Med. Res*. 1(3):1-8, 2013.
- [40] Gao, G., Ze, Y., Li, B., Zhao, X., Zhang, T., Sheng, L., Hu, R., Gui, S., Sang, X., Sun, Q., *et al.*. Ovarian dysfunction and gene-expressed characteristics of female mice caused by long-term exposure to titanium dioxide nanoparticles. *J. Hazard. Mater.*,243: 19–27, 2012.
- [41] Iavicoli ,I.; Fontana, L.; Leso, V. and Bergamaschi. A. The Effects of Nanomaterials as Endocrine Disruptors *Int. J. Mol. Sci.*, 14 : 16732-16801, 2013.

- [42] Kolesarova ,A., Capcarova, M., Medvedova, M., Sirotkin, A.V. and Kovacik, K. In Vitro Assessment of Iron Effect on Porcine Ovarian Granulosa Cells: Secretory Activity, Markers of Proliferation and Apoptosis. *Physiol. Res.* 60: 503-510, 2011.
- [42] Kojadinovic, J., Potier, M., Le, Corre, M., Cosson, R. P., and Bustamante, P. Bioaccumulation of Trace Elements in Pelagic Fish from the Western Indian Ocean. *Environmental Pollution*, 146,: 548-566, 2007.
- [43] Bires, J., Dianovsky, J., Bartko, P., Juhasova, Z. Effects on enzymes and the genetic apparatus of sheep after administration of samples from industrial emissions. *Bio Metals*, 8:53–58, 1995.
- [44] Ynsa, M.D., Ager, F.J., Millan, J.C.;Gomez-zubelbia, M.A. and Pinheiro, T. Effect of hormone replacement therapy on the elemental contents of uterine tissue. *Biol Trace Elem Res.* 101: 37-46, 2004.
- [45] Reilly, C. *The Nutritional Trace Metals*. Blackwell Publishing, Ltd, Oxford, PP: 1-238, 2004.
- [46] Defrere, S., Lousse J.C., Gonzaleez - Ramos, R., Colette ,S., Donnez, J., VanLangendonck, A. Potential involvement of iron in the pathogenesis of peritoneal endometriosis. *Mol Hum Reprod* 14: 377-385, 2008.
- [47] Durlej,M., Duda, M., Knapczyk, K.,Slomczynska,M. Effects of transferrin on aromatase activity in porcine granulosa cells in vitro. *Folia Histochem. Cytobiol.* 46: 423-428, 2008.
- [48] Kolesarova, A., Roychoudhury, S., Slivkova, L. J., Sirotkin, A., Capcarova, M., Massanyi, P. In vitro study on the effect of lead and mercury on porcine ovarian granulosa cells. *J. Environ Sci. Health A Tox Hazard Subst Environ Eng.*, 45:320-331, 2010a.
- [49] K Kolesarova, A., Capcarova, M., Sirotkin, A., Medvedova ,M., Kovacik, J. Cobalt-induced changes in the IGF-I and progesteron release, expression of proliferation- and apoptosis-related peptides in porcine ovarian granulosa cells *in vitro*. *J. Environ. Sci. Health A Tox Hazard Subst Environ Eng* 45: 810-817, 2010b.
- [50] Risom, L., Moller, P., Loft, S. Oxidative stress-induced DNA damage by particulate air pollution *Mutat. Res.*, 592:119-137, 2005.

ملاحظات :

اختبرت كمية الجرعات المعطاة للفئران اعتمادا على بحوث سابقة وليس اجتهدا وفيها استخدمت وحدة الملغرام لجزيئات اوكسيد الزنك النانوية ووحدة المايكروغرم لجزيئات اوكسيد الحديد النانوية.

15.Vahid, Y.B., Esmail,A. ,Hojatollah, S., Leila,S. ,Najafi Leila, N. and Fazilati, Mohammad, F. Evaluation of Iron Oxide nanoparticles effects on tissue and Enzymes of Thyroid in Rats. *Intern. Res. J. of Biolog. Sci.* ; 2: 67-69, 2013 .

16.Reza,E.H, Mohammad, F., Leila, S.,VahidY.B., Somayeh,B. and Esmail,A.. Investigation the Zinc Oxide Nanoparticle's Effect on Sex Hormones and Cholesterol in Rat .*Intern. Res. J. of Biolog. Sci.* 2: 54-58, 2013.

وربما يعود السبب لكون اوكسيد الحديد اكثر سمية حسب ما تظهره العديد من البحوث علاوة على ان الوزن الجزيئي لاوكسيد الحديد اكثرمن الوزن الجزيئي لاوكسيد الزنك ولهذا يفضل استخدامه بجرعان و اوزان اقل (بالميكروغرام)

Molar mass of Fe₂O₃ = 159.6882 g/mol

Molar mass of ZnO = 81.3794 g/mol