Published Online First: March, 2024
https://doi.org/10.21123/bsj.2024.8218
P-ISSN: 2078-8665 - E-ISSN: 2411-7986
Baghdad Science Journal

Effect of ZnO NPs on Ovarian Histological Structure and Function in Adult Female Rats

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Received 12/12/2022, Revised 21/10/2023, Accepted 23/10/2023, Published Online First 20/03/2024

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Abstract

Nanotechnology is one of the most important techniques that is widely used in many fields by changing the physical and chemical properties of materials to form new scale substances called nanoparticles to make them more effective. ZnO NPs is one of these particles that is used in multiple applications, especially in cosmetic and sunscreen products. Thus, the uses of these particles in great quantities make them in constant contact with the body and can enter the circulating blood in a different ways. The aim of this study was to find out the effect of ZnO NPs on the histological structure and functions of the ovary, as well as to determine the levels of gonadotropin hormones (FSH and LH). Therefore, 54 adult female rats were randomly divided into three primary groups according to the duration of exposure (1, 2, and 4 weeks). These groups were then further divided into three subgroups, each of which consisted of six rats, one of which served as a control group and was injected with 1 ml of distilled water, while the others served as treated groups and were injected with 1 ml of ZnO NPs at low and high concentrations 50 and 200 mg/kg respectively, an average of three intra-peritoneal injections per week. The results showed different histological changes in the ovarian tissue sections of treatment rats at different doses and durations, as well as significant decrease ($p \leq 0.01$) in the levels of estrogen, progesterone, LH, and FSH hormones in treated animals at different doses and durations. It can be concluded that the ZnO NPs have ability to damage the ovarian tissue, and disruption of its cellular functions that caused an imbalance in reproductive hormones levels (P and E2, LH and FSH).

Keywords: Gonadotropin hormones, histopathological, ovary, sexual hormones, ZnO NPs.

Introduction

Nanotechnology is generally considered as a new and rapidly emerging field that includes the manufacturing, processing, and application of structures and systems by controlling their shape and size on the nanometer scale. In comparison to materials on a micrometer scale the size of these particles ranges between 1 to 100 nm, and they have at least one dimension as well as contain specific surface area [1]. Because of these unique properties, these molecules have been used in many fields of human activities [2]. ZnO NPs are one of the widest nanomaterials used in an increasing amount of various industrial products, and during the last two decades, ZnO NPs became the common metal oxide nanoparticles applied in biological application [3]. Nanoparticles are used in food manufacture, food

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packaging, and various lifestyles [4]. Cosmetics and sunscreen products incorporate zinc oxide nanoparticles in their composition owing of their capacity to absorb UV light[5]. Therefore, the widespread usage of ZnO NPs makes people in direct contact with them, making them more vulnerable to ZnO NPs exposure and their harmful effects [6].

The ovary is the female gonad, consisting of a pair of intraperitoneal endocrine organs normally found in the lower left and right quadrants of the abdomen, respectively. It plays essential role in reproduction as well as hormones production [7]. Many adult ovarian events are controlled by two hormones: luteinizing hormone (LH) and follicle-stimulating hormone (FSH), which are synthesized and secreted by the anterior pituitary gland and are regulated by gonadotropin-releasing hormone (GnRH) which is secreted by the hypothalamus, these hormones work on the ovary and play a key role in gonadal functions and follicular development and growth [8]. It is responsible for the developing and releasing of oocytes as well as the production of a variety of steroid hormones, especially estrogen and progesterone [9]. The skin, lungs, and the digestive system of humans are in direct contact with the environment, thus, because of their small size, the nanoparticles can pass through these entry gates into the circulatory and lymphatic system, and then aggregate in different tissues and organs of the body [10]. The effect of zinc oxide on biological functions depends on its shape, particle size, exposure time, concentration, pH, and biocompatibility [11]. The toxic effects of zinc oxide nanoparticles are attributable to their solubility, since these particles dissolve in the extracellular region, increasing the cell level of Zn^{+2} ions [12]. Elevation in Zn^{+2} levels can activate numerous signaling pathways, which can result in mitochondrial membrane potentials breakdown, caspase activation, gene regulation, and apoptosis [13]. Some studies on reproductive function indicate that exposure to some nanoparticles (NPs) may disrupt endocrine functions such as regulation levels of sexual hormones [14]. Dose and time-dependent effects revealed disruption in thyroid function, necrosis and damage to different body organs[15]. This study aims to reveal the effect of ZnO nanoparticles on the ovarian function represented by sexual hormones and the changes that may occur in the histological structure of the ovary.

Materials and Methods

Preparation of ZnO NPs

ZnO NPs utilized in this study were obtained from skyspring nanomaterials. Two concentrations of ZnO NPs solution (50 and 200 mg/kg) were prepared by dissolving the powder of zinc oxide nanoparticles in distilled water, which was then mixed by vortex for 10 minutes.

Animals Housing

Fifty-four adult female Sprague-Dawley albino rats weighing 220-240 g at the age of 8-10 weeks were purchased from the National Center for Drug Control and Research (NCDCR), Iraqi Ministry of Health. These animals were transferred to the animal house laboratory in the College of Science, Mustansyriah University. They were kept for 10 days to allow for adaptation before starting the treatment under the controlled temperature conditions of 25 °C. Animals were provided with rat pellets and tap water for feeding and drinking.

Experiment Design

A total of 54 healthy adult female rats were assigned randomly to three main groups according to the periods of exposure, which they were 1, 2 and 4 weeks. Then, each group was subdivided into three subgroups of equal number of animals (6 rats), one of which served as the control group and the others were used as treatment groups. The treatment groups received two different dosage of ZnO NPs (50 and 200 mg/kg), while, the control group received distilled water, through intraperitoneal route at an average of three doses times each week.

Collection of Blood Samples and Tissue Specimen

After the experiment ended, the rats were anesthetized with the diethyl ether solution for several minutes. Four ml of blood samples were collected, and the serum was separated by centrifugation at 3000 rpm for 15 min. The rats were dissected, and their ovaries were removed and

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washed with normal saline (0.9% NaCl), before being preserved in a 10% formalin solution.

Hormone Analysis

Ovarian hormones (E2 and P) as well as gonadotropin hormones (FSH and LH) were evaluated in collected serum using a Cobas e411 automated analyzer.

Histological Study

Microscopic examination of tissues requires preparation of very thin and high-quality sections that are mounted on glass slides and suitably stained to clarify the normal and abnormal structures [16].

Statistical Analysis

The Statistical Analysis System application (SAS-2012) was used to find out the effects of different factors on some parameters that were used in this study, and the least significant difference-LSD test (ANOVA) was used to significantly compare the means.

Results and Discussion

Reproductive Hormones Analysis

Statistical analysis results of the presented study in Table 1 showed a high significant decrease ($p \leq 0.01$) in the levels of estrogen (E2) in all animals exposed to doses (50 and 200 mg/kg) of ZnO NPs at different periods 1, 2 and 4 weeks, when compared to the control group. While, the results of progesterone (P) showed a non-significant decrease ($p \leq 0.05$) in groups exposed to doses (50 and 200 mg/kg) of ZnO NPs in the short term (1 week), but a significant high decrease ($p \leq 0.01$) observed in groups exposed to doses (50 and 200 mg/kg) of ZnO NPS during 2 and 4 weeks when compared to the control group.

In contrast, the results in Table 2 showed a non-significant decrease ($p \leq 0.05$) in the levels of LH and FSH hormones in rats exposed to ZnO NPs at different doses (50 and 200 mg/kg) for 1 week, whereas, the levels of LH and FSH hormones in rats exposed to both doses of ZnO NPs for (2 and 4) weeks demonstrated a high significant decrease ($p \leq 0.01$) when compared to the control group.

Histological Changes of Ovary

The microscopic examination of the ovarian tissue sections for the control groups showed that they had normal appearance, primordial and primary follicles, and corpus luteum formation (Fig.1 A).

In contrast, the experimental treatment groups with ZnO NPs at different doses and times showed different histological changes in their ovarian tissues. The ovarian sections of treated animals with ZnO NPs for 1 week showed the early formation of a corpus luteal cyst surrounded by fibrous tissue reactions. After 2 weeks of treatment, an ovarian tissue section showed necrosis of the corpus luteum lining cells with the formation of the cyst lumen. Whereas the section of the ovarian tissue for animal groups that were exposed to ZnO NPs for 4 weeks showed noticeable advance necrosis of luteinizing follicular cells surrounded by abundant fibrous tissue reaction, as shown in Fig. 1.

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P-ISSN: 2078-8665 - E-ISSN: 2411-7986
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Table 1. Effect of ZnO NPs on levels of progesterone and estrogen hormones during different periods of exposure in female albino rats.

Hormones	Groups	Mean ± SE			LSD value
		1st Week	2nd Week	4th Week	
Progesterone (ng/ml)	Control	12.28±0.13 A a	12.62±0.13 A a	12.52±0.09 A a	0.362 NS
	ZnoNPs: 50 mg/kg	12.36±0.12 A a	11.67±0.14 B b	11.23±0.16 C b	0.423 **
	ZnoNPs: 200 mg/kg	11.96±0.14 A a	11.46±0.14 B b	8.96±0.15 C c	0.436 **
	LSD value	0.394 NS	0.419 **	0.411**	---
Estrogen (Pg/ml)	Control	51.20 ±0.20 A a	52.63 ±0.21 A a	51.87 ±0.36 A a	0.807 NS
	ZnoNPs: 50 mg/kg	49.48 ±0.19 A b	34.59 ±0.39 B b	26.65 ±0.22 C b	0.858 **
	ZnoNPs: 200 mg/kg	47.18 ±0.26 A c	29.99 ±0.28 B c	19.39 ±0.18 C c	0.741 **
	LSD value	0.674 **	0.918 **	0.80 **	---

(A, B, C) Represents a significant difference among means in the same row.
(a, b, c) Represent a significant difference among means in the same column.
(**) Means high significant difference between means p≤ 0.01.
(NS) Means non-significant difference between means.

Table 2. Effect of ZnO NPs on levels of LH and FSH hormones during different periods of exposure in female albino rats.

Hormones	Groups	Mean ± SE			LSD value
		1st Week	2nd Week	4th Week	
LH (mIU/ml)	Control	0.312 ±0.002 A a	0.307 ±0.002 A a	0.314 ±0.003 A a	0.0078 NS
	ZnoNPs: 50 mg/kg	0.308 ±0.003 A a	0.197 ±0.002 B b	0.139 ±0.003 C b	0.0079 **
	ZnoNPs: 200 mg/kg	0.306 ±0.002 A a	0.172 ±0.002 B c	0.099 ±0.002 C c	0.0066**
	LSD value	0.0074 NS	0.0071 **	0.0079**	---
FSH (mIU/ml)	Control	0.209 ±0.001 A a	0.215 ±0.002 A a	0.211 ±0.003 A a	0.0062 NS
	ZnoNPs: 50 mg/kg	0.208 ±0.002 A a	0.173 ±0.002 B b	0.130 ±0.002 C b	0.0064 **
	ZnoNPs: 200 mg/kg	0.205 ±0.001 A a	0.143 ±0.002 B c	0.100 ±0.002 C c	0.0053 **
	LSD value	0.0044 NS	0.0061 **	0.0071 **	---

(A, B, C) Represent a significant difference among means in the same row.
(a, b, c) Represents a significant difference among means in the same column.
(**) means high significantly difference between means p≤ 0.01.
(NS) means non-significantly difference between means.

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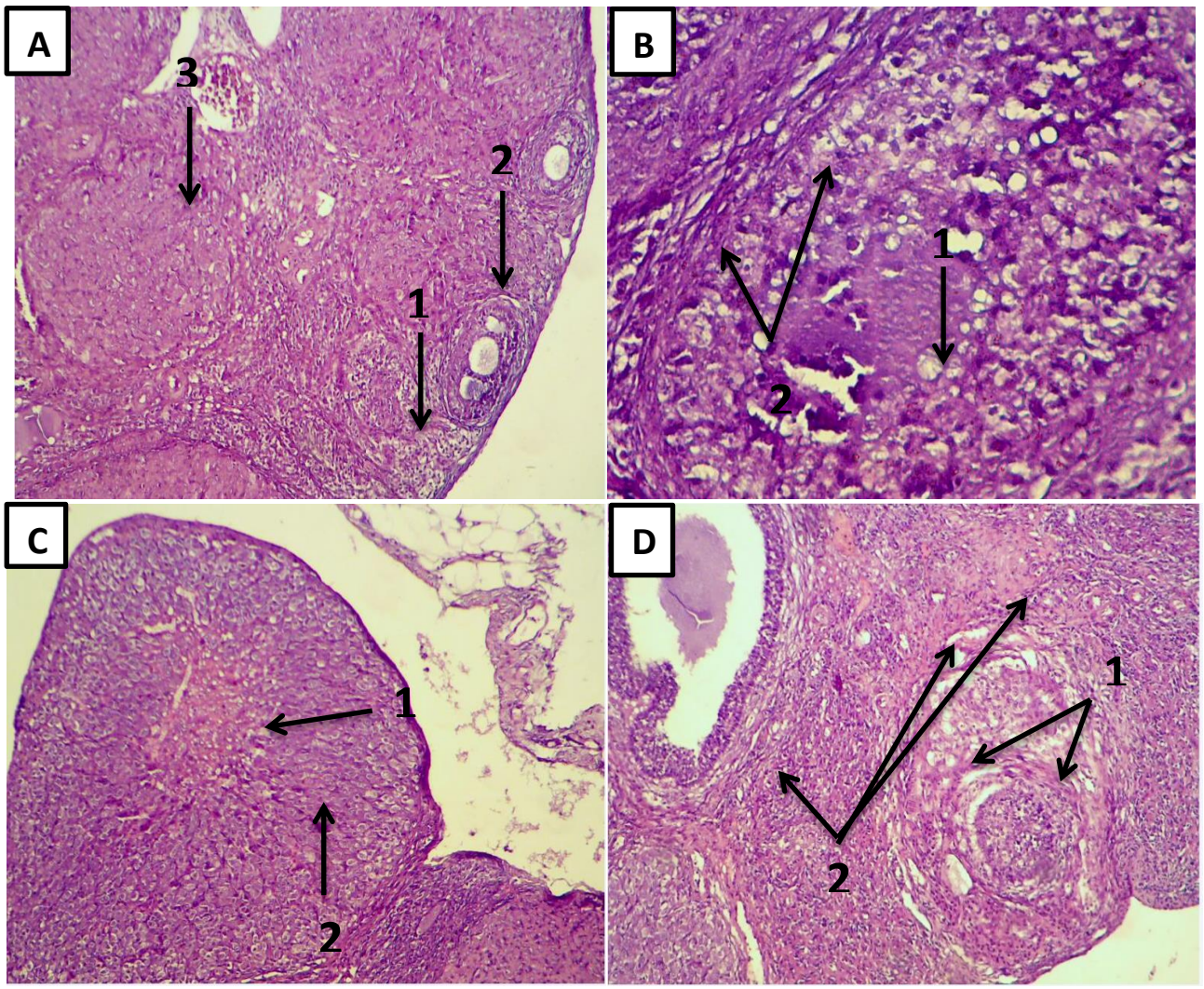

Figure 1. The histopathological changes of rat ovaries treated with ZnO NPs through different periods, (H&E) 40x.

(A) Section of ovary for control group showing normal appearance, 1. primordial follicles, 2. primary follicles, 3. corpus luteal.

(B) Section of ovary that was treated with 200 mg/kg for 1 week, showing 1. beginning formation of corpus luteal cyst surrounded by 2. fibrous tissue reaction.

(C) Section of ovary that treated with 200 mg/kg for 2 weeks, showing 1. necrosis of lining cells of corpus luteum with 2. formation of the lumen of cyst.

(D) Section of ovary that treated with 200 mg/kg for 4 weeks showing 1. advance necrosis of luteinizing follicular cells surrounded with 2. abundant of fibrous tissue reaction (fibrosis).

Discussion

The present study showed that ZnO NPs had an influence on the levels of ovarian hormones. A previous study reported changes in the sex hormone levels, after exposure to some NPs [14]. Other studies [17] demonstrated a significant decrease in estrogen and progesterone hormones levels of the female rats that were injected intraperitoneally with different doses of ZnO NPs (100 and 200) mg/kg, when compared to the control group. Others reported a significant decrement ($p<0.05$) in estrogen hormone levels in serum of *O. niloticus juvenile* female after injection with high and low doses (2.05 and 1.23 µ/l)

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of ZnO NP during 4 days when compared to the control group [18]. In addition, Xu and his colleagues [19] reported a study on adult female mice in which they exposed them to ZnO NPs for 7 days, and found that the estradiol and progesterone hormone levels in serum declined gradually with increasing the dose of ZnO NPs compared to the control group, considerable, significant differences were found in the levels of estradiol and progesterone hormones between the treated groups with (100 or 200) mg/kg of ZnO NPs and the (400) mg/kg ZnO NPs group.

Previous study suggested that the exposure to the nanoparticles (NPs) might alter the sex hormone levels in the blood via an indirect effect on the hypothalamic-pituitary-gonadal axis, or a direct effect on the stimulation of secretory cells such as granule cells, theca cells, follicular cells and the corpus luteum [20].

On the other hand, the secretion of gonadotropin hormones (LH and FSH) from the anterior pituitary gland in women is under ovarian control by negative and positive feedback mechanisms that are secretory or inhibitory hormones [8]. So, the defect in the ovarian functions, or a disruption in the hypothalamic-pituitary-gonadal axis alters in GnRH, LH, and FSH levels [21]. For that, the statistical analysis results of this study followed the same path as the previous study [22], revealing that rats exposed to (40 mg/kg) of ZnO NPs for 21 days exhibited a significant decrease in FSH levels when compared with the control group. While they found no changes in the level of LH hormone, they suggested that the nanoparticles might inhibit endocrine system function by blocking the pituitary hypothalamus axis; which could be due to a reduction in GnRH levels.

In summary, the effects of nanoparticles on ovarian hormone secretion and the hypothalamic-pituitary-gonadal axis can occur in two ways; the first occurs through the passage of nanoparticles (NPs) through the blood-brain barrier into the hypothalamus gland, and secretory cells of the pituitary gland, which may alter secretion of GnRH, LH, and FSH, therefore dysfunction the normal mechanism of positive and negative feedback of the hypothalamic-pituitary-gonadal axis which causes the abnormal secretion of estrogen and progesterone hormones. Second, the nanoparticles may reach the ovaries via the circulatory system and accumulate in granulosa cells and theca cells, which affect steroid genesis [23].

The ovarian histological changes observed in this study confirmed the results obtained regarding the changes in the levels of ovarian hormones (E2 and P). The small size of the nanoparticles (NPs) enables them to enter most of the body organs by penetrating the physiological barriers by traveling through circulatory system [24]. Destructive effects on various organs and tissues can be caused by exposure over short and long terms to heavy metals such as zinc, it was observed that after injecting female rats with ZnO NPs intraperitoneally, the light microscopic section showed changes in the tissue of the ovary [17]. In the same path, Hou and Zhu [23] who observed that zinc oxide nanoparticles accumulated in several organs, including the ovary, and caused a disturbance in their tissues, resulting in ovarian damage and generate oxidative stress after repeated application of ZnO NPs in female rats, the nanoparticles can disturb the development of the oocyte by damaging the protective barrier of granulosa cells, theca cell, and zona pellucida, which led to the effects on the levels of sex hormone through the hypothalamic pituitary-gonadal-axis or by a direct effect on the secretory ovarian cells.

Another study [25] observed that ZnO-NPs caused a significant reduction in the number of mature oocytes and inhibited the growth of ovarian granulosa cells after 30 days of treatment. Mahdi and Al-Nakeeb [26], indicated some histopathological changes in animals exposed to nanoparticles, including shrinkage in the oocyte in growing follicles, fat degeneration, and pyknosis in all follicle cells in the granulosa layer of growing follicles, furthermore there was precipitation of amyloid protein in follicle cells. The accumulation of NPs may affect organ tissues along with the multiplication of many defensive cells such as phagocytic cells [27], as well as the formation of ROS and antioxidant defenses, which makes these organs targets to oxidative stress [28]. Furthermore, accumulation of ZnO has been shown to induce oxidative stress, toxicity, and genotoxicity in ovary

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https://doi.org/10.21123/bsj.2024.8218
P-ISSN: 2078-8665 - E-ISSN: 2411-7986
Baghdad Science Journal

tissues, decrease follicle atresia, and follicular development, and increase ovary damage by apoptosis, and apoptotic signaling activation [29]. Animals treated with 5 mg/kg−1 BW ZnO NPs revealed an effect on female rat reproductive health, revealing altered expression of some proteins that may contribute to a reduction in body weight, ovary size, follicle count, and follicular cyst development, as well as the interleukin TNF-α and IL-1β levels which lead to reduced fertility and ovarian damage [30].

Conclusion

This study showed clear histological changes in the ovarian tissue sections in all animals exposed to ZnO NPS at different times, implying that the damage in ovarian tissues caused disruption in their cellular functions, resulting in a significant reduction in ovarian hormones (P and E2). Likewise, these particles have affected the hypothalamus-pituitary-gonadal-axis which caused a decrement in the levels of LH and FSH hormones, the reasons for these alterations are due to the toxic and harmful effects of the ZnO NPs on theca and granulosa cells inside the follicles of the ovary in addition to the effects on the ovarian functions which have effects on E2 and P which in turn alter the synthesis and release of gonadotropin hormones (LH and FSH).

Acknowledgment

Gratitude and appreciation are submitted to Mustansiriyah University Baghdad/Iraq, for its support to achieve this work.

Authors' Declaration

- Conflicts of Interest: None.
- We hereby confirm that all the Figures and Tables in the manuscript are ours. Furthermore, any Figures and images, that are not ours, have been included with the necessary permission for re-publication, which is attached to the manuscript.
- Authors sign on ethical consideration's approval Ref.No: BCSMU/0922/0015Z. Sep/01/2022.
- Ethical Clearance: The project was approved by the local ethical committee in University of Mustansiriyah.

Authors' Contribution Statement

N.M. conceived and designed the analysis. N.M and F.G. collected the data; performed the analysis. H.J. contributed data and analysis tools. F.G. wrote the paper.

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Published Online First: March, 2024
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Published Online First: March, 2024
https://doi.org/10.21123/bsj.2024.8218
P-ISSN: 2078-8665 - E-ISSN: 2411-7986
Baghdad Science Journal

تأثير جزيئات أوكسيد الزنك النانوي على التركيب النسيجي والوظيفي للمبيض في إناث الجرذان البالغة

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[2]كلية الزراعة، جامعة ميسان، ميسان، العراق.

الخلاصة

تعد تقنية النانو واحدة من أهم التقنيات المستخدمة على نطاق واسع في العديد من المجالات من خلال تغيير خصائص المواد الفيزيائية والكيميائية لتكوين مواد جديدة تسمى الجسيمات النانوية لجعلها أكثر فعالية. تعتبرجسيمات أوكسيد الزنك النانوية هي واحدة من هذه الجزيئات التي تستخدم في تطبيقات متعددة ، خاصة في مستحضرات التجميل ومنتجات الوقاية من اشعة الشمس. ولهذا فإن استخدام هذه الجسيمات بكميات كبيرة يجعلها بتماس دائم مع الجسم والتي من الممكن أن تدخل الدورة الدموية بطرق مختلفة. كان الهدف من هذه الدراسة هو التحري عن تأثير جسيمات أوكسيد الزنك النانوي على التركيب النسجي والوظيفي للمبايض وكذلك تقييم مستوى هرمونات الغدد التناسلية (FSH و LH). ولاجل ذلك تم استخدام 54 من إناث الجرذان البالغة وقسمت بشكل عشوائي إلى ثلاث مجاميع رئيسية وفقًا الى فترات التجريع (1 ، 2 ، 4) أسابيع ، بعدها قسمت كل مجموعة رئيسية إلى ثلاث مجاميع فرعية ، كل مجموعة منها تتكون من 6 جرذان ، احدى هذه المجاميع تم استخدامها كمجموعة سيطرة وحقنت بـ 1 مل من الماء المقطر ، بينما المجموعتين الأخرى استخدمت كمجاميع معالجة وحقنت بـ 1 مل من محلول اوكسيد الزنك النانوي (ZnO NPs) بتركيزين مختلفين منخفض وعالي (50 و 200) مغم / كغم على التوالي ، بمعدل ثلاث جرعات في الأسبوع عن طريق الحقن داخل الصفاق. أظهرت النتائج تغيرات نسجية مختلفة في مقاطع أنسجة المبايض لدى الجرذان المجرعة بتراكيز وفترات زمنية مختلفة ، وكذلك أظهرت النتائج ايضا" انخفاض معنوي ($p \leq 0.01$) في مستويات هرمون الاستروجين والبروجسترون و LH و FSH ، لدى الحيوانات المعالجة بتراكيز وفترات زمنية مختلفة. وقد تم الأستنتاج بأن لجزيئات دقائق الزنك النانوية القدرة على إتلاف أنسجة المبيض ، وتعطيل وظائفه الخلوية مما أدى الى خلل في مستويات الهرمونات التناسلية (P و E2 و LH و FSH).

الكلمات المفتاحية: هرمونات تناسلية ، امراض نسيجية ، مبيض ، هرمونات جنسية ، جسيمات أوكسيد الزنك النانوية.