Al-Qadisiyah Journal of Veterinary Medicine Sciences
(P-ISSN 1818-5746/ E-ISSN 2313-4429)
www.qu.edu.iq/journalvm

Influence of Magnetic Iron Oxide Nanoparticles in Reproductive Functions of Female Rats

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Abstract

The current study aims to investigate the dose and time dependently effects of magnetic iron oxide nanoparticles (m-IONPs) on female rat's reproductive functions. Sixty four mature female rats, aged 90 days 164±4.5 g. were divided to control (C) and three treatment groups (16 females each); orally administered with distilled water, and 1, 5, 10 mg/kg/day of IONPs solution (TL, TM, and TH groups), respectively, for 28 days. Eight females from each group were sacrificed after 14 and 28 days of treatment periods. After each period, the females were weighted and sacrificed. Decreased body weight and genital organ weights were shown in TM and TH groups, at both experimental periods, compared with control in a dose-dependent manner. Serum concentration of GnRH, FSH, LH, and estradiol increased in TL group and decreased in TM and TH groups compared with control in a dose-dependent and time-dependent manner. At both periods, the lowest expression levels ($p<0.05$) of pituitary FSHβ and LHβ genes and ovarian FSHR, aromatase, and fecβ genes were recorded in TM and TH groups and the highest levels were expressed in TL group. The ovarian sections of TL females, showed normal architecture, but those from TM and TH groups showed degenerative changes, reduced population of mature follicles. In conclusion, the low dose of mIONPS has improving effect on female reproduction, whereas middle or high doses have pathological effects on reproductive organs.

Keywords: mIONP, Ovaries, Uteri, LH, FSH

Introduction

Nanoparticles are modified materials to the nanoscale with dimensions of about 1- 100 nanometers. Recently, nanoparticles have played a significant role in many medical fields such as medical imaging as a contrast agent, and drug delivery to individual cells. The small size of these nanoparticles is distinguishing them from other materials and gives them advantages in terms of chemical reactivity, energy absorption, and biological mobility [1].One example of well-known nanoparticles is magnetic iron oxide nanoparticles (M-IONPs). M-IONPs have been utilized in vitro as a contrast agent for diagnostic purposes for nearly 50 years [2]. In the last ten years, the process of synthesis of magnetic nanoparticles has developed extensively and has gained roles in many important technical fields [3,4]. Especially, the bio-applications based on magnetic nanomaterials have got an appreciable consideration because nanoparticles have unique properties that make them superior compared to other materials. For example, M-IONPs have some desirable specifications which are chemical and physical stability, biocompatibility, their environmental safety, and they are relatively cheap to produce [5]. Nanomaterials applications in many fields such as environments and public health's been grabbing considerable attention. Experiments on animal models have shown many toxic

Al-Qadisiyah Journal of Veterinary Medicine Sciences
(P-ISSN 1818-5746/ E-ISSN 2313-4429)
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effects like inflammation, decreased growth rate as well as neurobehavioral changes. Great surface-to-volume ratio, chemical composition, size, dosage, and retention in the body represent the major factors that affect nanoparticles toxicity [6]. Recent studies that have been conducted on rodents, cell culture, zebrafish, and chicken have shown a set M-IONPs toxic effects which are cell membrane leakage, abnormal cellular growth, cells death, cellular proliferation, mitochondrial damage, chromosome condensation, and damage to nucleic acid [6].Despite the fact that mIONPs have many beneficial applications in many medical fields, there are only a few studies that investigate their effects on the reproductive system. Therefore, there is a need to uncover their effects on the reproductive system. The aim of present study was to evaluate the effect of mIONPs on the female reproductive system focusing on hormonal secretion, follicles growth, and the activity of ovaries, as well as investigation of their reprotoxicological effects in female rats.

Materials and Methods

Synthesis and characterization of mIONPs: mIONPs were synthesized and its physicochemical properties were characterized according to Salehizadeh et al [7].

Experimental animals: In this experiment, adult female Wister rats, aged 90 days of age and weighted 174±5 g., were obtained from the animal house of the College of Veterinary Medicine, University of Al-Qadisiyah. The experiment was conducted during the period from 15 November, 2020 to 30 March, 2021. The animals were housed in well ventilated wire-plastic cages under controlled conditions of 22 °C temperature. The dark and light time were 12:12 hours. The animals were allowed to acclimatize for one week before experimentation.

Body weights and genital organ weights: The animals were weighted before treatment (0 day) and at the end of each experimental period. Body weight gain after each period was calculated. Relative ovarian weight was calculated according to body weight (g/100 g BW).

Serum Preparation: Abdominal vein blood was collected, left for 20 min. to clot, and centrifuged at 4000 rpm for 10 min. serum was collected and divided into 5 samples in appendroff tubes (0.5 ml each) and kept at -°C until use for assessment of hormones [8].

Experimental design: Sixty four mature female rats were equally divided to control (C) and three treated groups (TL, TM, and TH). Control female rats were drenched with distilled water, whereas treated groups were drenched with 1, 5, 10 mg/kg/day of IONPs solution, respectively, for 28 days. Eight females from each group were weighted and sacrificed after 14 and 28 days of treatment. Relative genital organ weights (ovaries and uteri) were recorded. Blood samples were obtained and serum samples were separated for assessment of serum concentrations of GnRH, FSH, LH, and estradiol using ELISA technique. Ovaries were taken for histopathological examination. Pituitary and ovarian tissue samples were obtained for molecular analysis to evaluate the expression levels of pituitary FSHβ and LHβ and testicular FSHR, aromatase and fecβ genes using qRT-PCR technique.

Histopathological examination: The dissected specimens from ovaries of sacrificial females were prepared and stained with haematoxilin and eosin stains according to Mescher (9).

Molecular analysis (gene expression)

Total RNA extraction and evaluation of its purity: Total pituitary and ovarian RNA were extracted by using TRIzol® reagent kit (Bioneer, Korea). The quality of the extracted

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RNa was quantified using Nano drop spectrophotometer.

cDNA synthesis: Using DNase I enzyme kit, the trace amounts of genomic DNA was removed from the eluted total RNA (accordingly to the procedure described by Promega company, USA). DNase-I treated total RNA samples were utilized in cDNA synthesis by employing AccuPower® RocktScript RT PreMix kit (Bioneer company, Korea).

qRT-PCR master mix preparation and analysis: Master mix was prepared by using AccuPower^{TM} Green Star Real-Time PCR kit depend on SYBER Green dye determination of gene amplification in Real-Time PCR system (Bioneer company, Korea). The levels of the relative quantification gene (fold change) Δ^{CT} Livak approach was used to test the obtained data of qRT-PCR for studied and housekeeping genes [10].

Statistical Analysis

Data was analyzed using one way analysis of variance along with Newman Keuls[11]. The results were showed as mean ± standard deviation. Probability less than 0.05 was considered significant.

Results

Body weight changes and relative organ weight

The result of body weight changes, clarified in table (1), revealed significant decrease ($p<0.05$) of TH group female rats among experimental groups at both periods, whereas TM group female rats recorded higher body weight changes ($p<0.05$) than TH group but it significantly lower ($p<0.05$) than control and TL groups, which showed no significant difference ($p>0.05$) between each other. When compared the two periods, control and TL groups showed significant increase ($p<0.05$) at 28 day period than 14 day period, while no significant difference ($p>0.05$) between the experimental periods was showed in TM group, but TH group recorded significant decline ($p<0.05$) at 28 day compared with 14 day.The ovaries of TH group female rats recorded the lowest relative weight ($p<0.05$), at both periods, among the experimental groups, followed by TM group female rats, then control group female rats, whereas TL group female rats recorded the highest relative weight ($p<0.05$). In comparison between the two periods, control, TL, and TM group female rats recorded no significant differences ($p>0.05$), whereas TH group female rats revealed significant decrease ($p<0.05$) of ovarian weight at 28 day period in comparison with 14 day period (Table 1).In comparison to control, Uteri of TL group female rats recorded higher relative weight ($p<0.05$), whereas those of TM and TH group female rats recorded lower relative weight ($p<0.05$), at both periods. In comparison between the two periods, the relative weight of control group showed no significant difference ($p>0.05$), while the results of TL groups, at 28 day period, recorded significant increase ($p<0.05$) and that of TM and TH group female rats, recorded significant decrease ($p<0.05$) in comparison with the results of 14 day period (Table 1).

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Table (1): Body weight changes and relative genital organ weights in mIONPs treated female rats

	Periods	Groups			
		C	TL	TM	TH
Body weight gain (g.)	14 d	36.51 ± 3.098 Ba	35.11 ± 4.125 Ba	22.87 ± 3.661 Ab	09.34 ± 1.93 Ac
	28 d	69.28 ± 3.326 Aa	73.22 ± 3.877 Aa	23.68 ± 2.890 Ab	- 5.39 ± 2.21 Bc
Ovary (g/100g)	14 d	1.06 ± 0.119 Ab	1.88 ± 0.273 Aa	0.84 ± 0.096 Ac	0.68 ± 0.088 Ad
	28 d	1.08 ± 0.122 Ab	1.92 ± 0.284 Aa	0.87 ± 0.085 Ac	0.60 ± 0.083 Bd
Uterus (g/100g)	14 d	1.533±0.175 Ab	1.787±0.203 Ba	0.842±0.071 Ac	0.821±0.092 Ac
	28 d	1.582±0.187 Ab	1.992±1.841 Aa	0.626±0.068 Bc	0.628±0.088 Bc

Values were presented as M±SE of 10 observations. C group: was drenched with distilled water. TL, TM, and TH groups: were drenched with 1, 5, and 10 mg of Iron Oxide Nanoparticles (mIONPs)/ kg BW/day, dissolved in 0.5 ml of distilled water. Different uppercase letters denote significant difference (p<0.05) between periods for each group. Different lowercase letters denote significant difference (p<0.05) between groups for each period.

Serum concentrations of GnRH, FSH, and LH

At both of the studied periods, the serum concentrations of GnRH, FSH, LH, and estradiol of TM and TH group female rats decreased significantly ($p<0.05$) than control female rats, whereas TL group female rats recorded the highest concentration ($p<0.05$). In comparison between the two studied periods, control and TL female rats recorded no significant differences ($p>0.05$), except GnRH concentration of TL group which showed an increase at 28 day period than 14 day period (Table 2). When comparing the two periods of TM group, GnRH and FSH levels decreased significantly ($p<0.05$) at 28 day period than 14 day period, whereas LH and estradiol showed no significant differences ($p>0.05$) between the two periods. Regarding TH group, GnRH, FSH, LH, and estradiol levels decreased significantly ($p<0.05$) at 28 day period in comparison with 14 day period (Table 2).

Table (2): Serum concentrations of reproductive hormones in mIONPs treated female rats

Serum conc.	Periods	Groups			
		C	TL	TM	TH
GnRH (pg/mL	14 d	78.34 ± 5.347 Ab	86.28 ± 6.732 Ba	52.66 ± 4.309 Ac	38.35 ± 1.716 Ad
	28 d	77.77 ± 4.836 Ab	99.81 ± 6.953 Aa	33.44 ± 3.788 Bc	28.38 ± 1.391 Bd
FSH (IU/L)	14 d	12.85 ± 0.823 Ab	18.87 ± 1.216 Aa	6.335 ± 0.522 Ac	6.016 ± 0.531 Ac
	28 d	13.46 ± 0.918 Ab	18.95 ± 1.419 Aa	4.486 ± 0.484 Bc	5.057 ± 0.641 Bc
LH (IU/L)	14 d	25.45 ± 1.381 Ab	36.29 ± 1.328 Aa	17.44 ± 1.221 Ac	16.34 ± 0.802 Ac
	28 d	24.56 ± 1.486 Ab	37.37 ± 1.475 Aa	17.78 ± 1.089 Ac	14.56 ± 1.015 Bd
Estradiol (ng/mL)	14 d	15.65 ± 1.155 Ab	37.64 ± 2.192 Aa	8.372 ± 0.568 Ac	8.722 ± 0.934 Ac
	28 d	15.42 ± 1.091 Ab	39.15 ± 3.089 Aa	8.194 ± 0.673 Ac	8.365 ± 0.871 Bd

Values were presented as M±SE of 10 observations. C group: was drenched with distilled water. TL, TM, and TH groups: were drenched with 1, 5, and 10 mg of Iron Oxide Nanoparticles (mIONPs)/ kg BW/day, dissolved in 0.5 ml of distilled water. Different uppercase letters denote significant difference (p<0.05) between periods for each group. Different lowercase letters denote significant difference (p<0.05) between groups for each period.

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Molecular analysis

Pituitary FSHβ and LHβ gene expression levels: As illustrated in figure (1), both of the studied periods showed that the lowest expression levels ($p<0.05$) of pituitary FSHβ and LHβ genes were recorded in TM and TH group female rats among the experimental groups, whereas TL group female rats recorded increased expression level ($p<0.05$) than control group female rats. In comparison between the two studied periods, all experimental groups showed no significant differences ($p>0.05$).

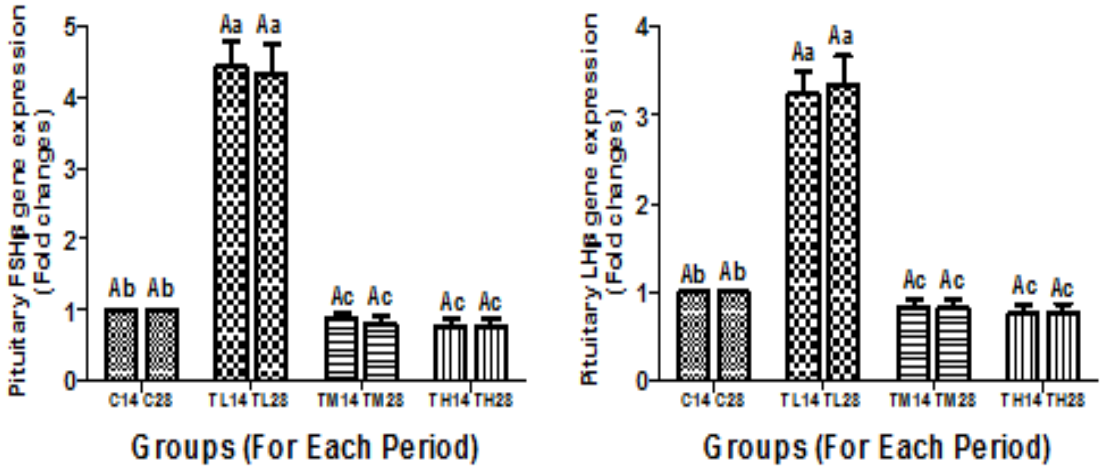

Figure (1): Pituitary FSHβ gene expression level in mIONPs treated female rats

Values were presented as M±SE of 10 observations. C group: was drenched with distilled water. TL, TM, and TH groups: were drenched with 1, 5, and 10 mg of Iron Oxide Nanoparticles (mIONPs)/ kg BW/day, dissolved in 0.5 ml of distilled water. Different uppercase letters denote significant difference ($p<0.05$) between periods for each group. Different lowercase letters denote significant difference ($p<0.05$) between groups for each period.

Ovarian FSHR, aromatase and fecβ gene expression levels: In comparison with control, TL group female rats recorded significant elevation ($p<0.05$) of ovarian FSHR, aromatase, and fecβ gene expression levels, whereas TM and TH group female rats recorded significant decline ($p<0.05$) than control group, at both of the studied periods. In comparison between the two studied periods, all groups showed no significant differences ($p>0.05$), except fecβ gene decreased at 28 day period than 14 day period (Figure 2).

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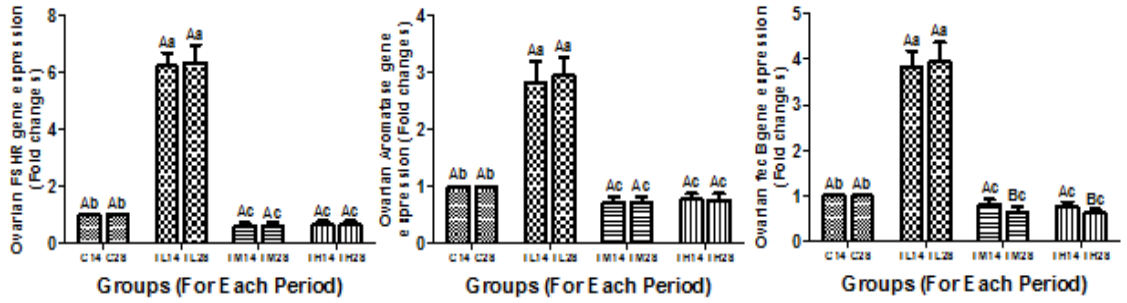

Figure (3): Ovarian FSHR, aromatase, and fecβ gene expression levels in mIONPs treated female rats

Values were presented as M±SE of 10 observations. C group: was drenched with distilled water. TL, TM, and TH groups: were drenched with 1, 5, and 10 mg of Iron Oxide Nanoparticles (mIONPs)/ kg BW/day, dissolved in 0.5 ml of distilled water. Different uppercase letters denote significant difference (p<0.05) between periods for each group. Different lowercase letters denote significant difference (p<0.05) between groups for each period.

Histopathological changes of ovaries

Because same observations were found in both of treatment periods, we choose to illustrate the results of 28 days period. mIONPs treated female rats (TM and TH groups) showed sever hemorrhage and necrosis of the ovarian tissues, particularly in the granulosa cells and secondary follicles, cirrhosis, and ischemia (Figure 3: TM and TH), but some viable and intact oocytes and cortical tissue were observed. TL group female rats (Figure 3: TL) showed normal tissue and normal secondary follicle layers, theca interna, zona granulose, antrum, zona pellucid and oocyte in comparison with control (Figure 3: C). Furthermore, the total number of atretic follicles was hence found to be higher in TH and TM group ovarian tissues as compared with control and TL treated groups.

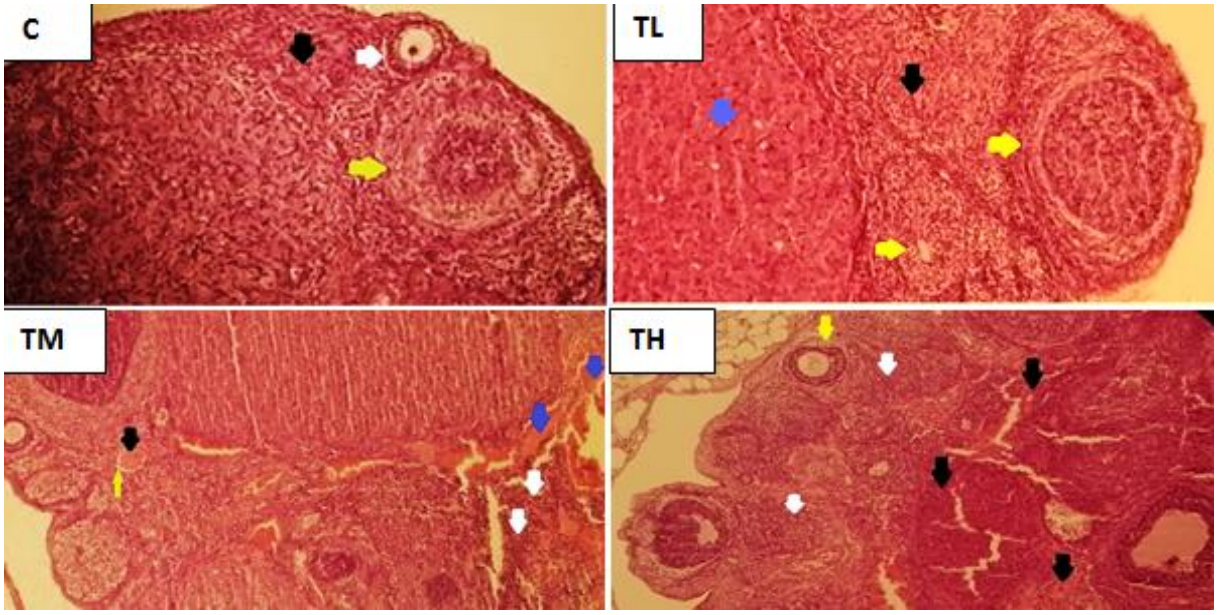

Figure (3): Ovarian sections from Control, TL, TM, and TH female rats.

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Control section shows normal ovarian cortex (black arrow) and viable primary (yellow arrow) and secondary (white arrow) follicles. TL: ovarian section from female rat treated with 1mg of mIONPs/ kg BW shows normal ovarian cortex (black arrow) and medulla (blue arrow), also presence of normal ovarian follicles (yellow arrows) and viable ovarian follicles (white arrows). TM and TH: ovarian sections from female rat treated with 5 and 10 mg of mIONPs/ kg BW, respectively shows severe hemorrhage (blue arrows) and necrosis (white arrows), necrosis of granulosa cells (yellow arrow) of the secondary follicle (black arrow), as well as complete destruction and necrosis of some ovarian follicles (black arrow), as well as wide spread necrosis of medulla (NM) and corpus luteum (CL), mature necrotic and vacculated (white arrows) graafian follicle (GF), and atretic follicle (EF) are obvious in the section surrounding by stroma. H&E, X100.

Discussion

Body weight gain and genital organ's relative weights:

The considerable increase in body weight gain in the TL group could be attributed to the stabilizing effect of mIONPs on cell membranes, which provided protection to the cells and improved various organ functions. It could potentially be related to mIONPs' stimulatory effect on protein synthesis. Increased ribosome production and accelerated protein and DNA biosynthesis may boost the pace of structural and functional protein biosynthesis. Furthermore, mIONPs' stimulatory effect may result in the production of more transporters and enzymes, which may boost the activities of numerous body cells. Iron is a metal ion found in the body that helps in DNA synthesis, mitochondrial respiration, and oxygen transport [12]. Because NP poisoning creates an overabundance of cellular antioxidant defense capacity, reduced body weight gains in female rats in the TM and TH groups could be connected to increased oxidative stress. At this time, reactive oxygen species and cellular amino acid reserves begin to degrade cellular macromolecules like DNA, lipids, and proteins. The amino acid pool will be greatly oxidized as a result of the shift in oxidative status, whereas lipids and proteins will be significantly influenced in conjunction with cytotoxicity. Body weight change in female mice treated with IONPs was dose-dependently reduced, according to Park et al. [13]. While iron is required for many metabolic tasks, excess iron can be hazardous to the body's tissues. As a result, any changes in iron levels could be dangerous to health [14]. In contrast, IONPs have been proven to be safe and even advantageous in several studies [15,16]. The toxicity of mIONPs was found to be dose and time dependent [17]. Oxidative stress was induced by high doses of mIONPs, which altered the mitochondrial enzyme expression, lipid peroxidation, and cell membrane [18]. The low therapeutic index of mIONPs could be related to the mall size of NPs, as small NPs degrade faster than large NPs after cellular absorption. Also smaller NPs have greater reactive surface areas, therefore produce more ROS [19]. Despite the fact that the TL group female rats gained more weight, the relative weights of their genital organs increased dramatically when compared to the control group. At the same time, while the weight growth of female rats in the TM and TH groups was much lower, the relative weights of their genital organs were likewise significantly lower than in the control and TL groups. These findings matched those of the current study's serum reproductive hormones, which demonstrated a substantial increase in LH, FSH, and estradiol concentrations in female rats in the TL group and a significant drop in the TM and TH groups as compared to the control group. This suggests that the increase or decrease is attributable to changes in reproductive hormones, particularly estradiol. The improvement in reproductive organ weights observed in females in the TL group could be related to the pituitary-gonadal axis. Pituitary gonadotropins and ovarian estradiol are increased to increase the potency

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of reproductive organ activity [20]. Pituitary gonadotropins (FSH and LH) and ovarian steroids (namely estradiol) operate in concert to modulate ovarian functions such as oogenesis and steroidogenesis [21]. In this work, high and medium doses of mIONPs acted as an inhibitory effector on the hypothalamus-pituitary-ovarian axis, whereas low levels of mIONPs acted as an enhancing factor.

Reproductive hormonal changes

After 14 and 28 days, the present findings revealed reproductive improvement in female TL rats and reprotoxicity in female TM and TH rats. Hormonal shifts from the pituitary and ovarian sources followed these modifications. In this investigation, the serum levels of FSH, LH, and estradiol, as well as molecular analyses of pituitary FSH, LH, and ovarian LH receptor gene expression levels, were orchestrated. The higher levels of FSH, LH, and estradiol in the TL group female rats were accompanied by increased expression levels of pituitary FSH, LH, and ovarian inh- and LH receptor genes. The results of the TM and TH groups, on the other hand, were diametrically opposed to those of the TL group. Because the weights of the female genitalia are significantly dependent on the degree of estradiol production [22], the reported changes in reproductive organs relative weights are another indicator of mIONPs improvement effect (TL group) and mIONPs reprotoxicity (TM and TH groups) in this study.The administration of 1mg of mIONPs /kg BW (TL group) to female rats markedly enhanced the production of reproductive hormones. As a result, the role of endogenous antioxidants in avoiding mIONP-induced oxidative damage has been emphasized. On the other hand, as indicated by the apparent improvement in the histological structure of the ovaries in the current study, mIONPs may restore ovarian function by boosting oogenic success rate and production of mature oocytes. Reduced oxidative stress and lipid component control were discovered to be critical factors in improving fertility [22].Because it has been proposed that successful female reproduction is the result of a suitable response to reproductive hormonal signals from the anterior pituitary gland, which is controlled by hypothalamic GnRH, any factor that affects this axis could lead to infertility, which could be due to failures at various stages of reproduction [23,24]. On the other hand, numerous types of environmental contaminants may have a negative impact on fertility [25]. According to these findings, high doses of mIONPs may be a risky inducer of female infertility, which might be due to a direct effect on oogenesis or an indirect effect via hormonal signals from the hypothalamus-pituitary-gonadal axis. According to the present findings, it is concluded that low doses of mIONPS have positive effects in improving female rat's reproductive functions, whereas medium and high doses have pathological effects on body functions in general and female reproductive organs in particular.

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