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Lipopolysaccharide Affects the Oxidative Stress Markers and Angiogenic and IGF1 mRNA Expression in the Uterus and Ovaries of Wistar Albino Rats

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Abstract

Mammals' immune systems react strongly to lipopolysaccharide, an endotoxin present in the outer membrane of gram-negative bacteria. Previous data show that the lipopolysaccharide challenge has a significant impact on reproductive function. This study aimed to explore the toxic effects of lipopolysaccharide on the uterine and ovarian tissues of female Wistar albino rats. Additionally, we investigated the effects of lipopolysaccharide on the expression of angiogenic and insulin growth factor 1 protein as well as oxidative stress markers in the uterus and ovaries. The experimental rats were allocated into two groups: (1) control group ($n=8$): received intraperitoneal injection of pyrogen-free 0.9% NaCl and (2) treatment group ($n=12$): single intraperitoneal injection of a non-lethal dose of lipopolysaccharide. Rats were sacrificed, and uterus and ovaries were collected at 6- and 72-hour time points after lipopolysaccharide infusion. The exposure of the female rats to lipopolysaccharide induced a significant change in the antioxidant activity in both ovarian and uterine tissues especially after 72 hours of exposure. The exposure of nonpregnant rats to lipopolysaccharide leads to the degeneration of the ovarian follicles and superficial epithelium layer of the uterus. Also, the exposure of female rats to lipopolysaccharide leads to the upregulation of angiogenic proteins in both the ovaries and uterus and decreased expression of insulin growth factor 1 and insulin growth factor R1, especially after 72 hours. In conclusion, lipopolysaccharide is considered as one of the most potent bacterial virulence factors that causes endometrial inflammation and reproductive disorders.

Keywords: Angiogenic, insulin growth factor 1, lipopolysaccharide, ovaries, oxidative stress, uterus

Introduction

Lipopolysaccharide (LPS), also referred to as an endotoxin, is a big molecule made up of a lipid and a polysaccharide. Mammals' immune systems react strongly to LPS, which is present in the outer membrane of gram-negative bacteria (Miao & Cui, 2022). Multiple pieces of data show that the LPS challenge has a significant impact on reproductive function.

Lipopolysaccharide influences granulosa cells and theca-interstitial cells' in vitro release of sex steroid hormones in female rats (Taylor & Terranova, 1996; Yoo & Lee, 2016). The number of atretic follicles in proestrus ovaries rose significantly after receiving a systemic dose of LPS and prolactin, along with a rise in apoptotic cells and macrophages (Besnard et al., 2001; Taylor & Terranova, 1996).

The day of vaginal opening was also substantially postponed in female rats following prenatal or neonatal LPS exposure (Izvolskaia et al., 2016; Ozgocer et al., 2015). According to these findings, LPS may have an impact on ovarian steroidogenesis and folliculogenesis. The precise process underlying LPS's harmful impact on female reproduction is still unclear (Yoo & Lee, 2016).

Short-term exposure to LPS can cause oxidative stress as early as 6 hours after exposure, and it can last for up to 72 hours. This is due to a substantial decrease in the antioxidant defense enzymes

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as well as an increase in lipid peroxidation and nitric oxide (NO) levels (Ozgocer et al., 2015). By activating caspase-3, the LPS-induced oxidative stress may be followed by apoptosis, impairing testicular functions (Halawa et al., 2018).

In addition, prenatal or neonatal LPS exposure significantly delayed the day of vaginal opening in female rats (Izvolskaia et al., 2016). These studies suggest that LPS might affect ovarian steroidogenesis and folliculogenesis. However, the exact mechanism underlying the adverse effect of LPS on female reproduction is not clearly understood yet.

An imbalance between free radical generation and antioxidant defense is known as oxidative stress. Hydroxyl radicals (•OH), superoxide radicals ($O_2^{\cdot-}$), and nitric oxide (•NO) are the most frequent reactive oxygen species. They can be formed as a result of dopamine metabolism catalyzed by monoamine oxidase, the Fenton reaction in the presence of iron, lipid peroxidation, and defective mitochondrial complex I activity (Noworyta-Sokołowska et al., 2013). Under normal conditions, antioxidant enzymes such as glutathione peroxidase, catalase, superoxide dismutase (SOD), and low-molecular-weight antioxidants (ascorbate, tocopherol, and carotenoids) as well as plant flavonoids deactivate free radicals rapidly (Flieger et al., 2021). There are many literatures that highlight the significant effects of angiogenic proteins on the reproductive performance of pregnant and non-pregnant females (Corrêa et al., 2012; Ravikumar et al., 2020). The outcome of the pregnancies depends on the placental function during the different gestational ages. During pregnancy, it alters as the fetus grows and as the demands for energy substrate transfer and gas exchange increase. The placental function depends mainly on the expression of angiogenic proteins (Vaswani et al., 2013). Moreover, the exposure of non-pregnant females has a great influence on the reproductive system structure and functions in females, resulting in subsequent decrease in reproductive performance (Ignatiuk et al., 2019).

Lipopolysaccharide can induce oxidative stress after a short period of exposure as early as 6 hours and continue up to 72 hours, resulting in a significant decrease in antioxidant defense enzymes and an increase in lipid peroxidation and NO levels. Lipopolysaccharide-induced oxidative stress could be accompanied by apoptosis through the activation of caspase-3, resulting in the impairment of testicular functions. This effect is crucial for the reproductive performance and future fertility of males due to the LPS effect on the germinal epithelium of testes (Halawa et al., 2018). In this study, we postulated that the female Wistar albino rats' exposure to LPS treatment might have an impact on the expression of angiogenic and IGF1 mRNA as well as oxidative stress markers in the uterus and ovaries that subsequently affected reproductive performance.

Materials and Methods

Chemicals

Lipopolysaccharide (*E. coli*, serotype 0111:B4; Sigma-Aldrich) was dissolved in distilled water. Rats in the control group were administered intraperitoneal pyrogen-free 0.9% NaCl.

Animals

The Animal Care and Use Committee of the Faculty of Veterinary Medicine, Mansoura University, approved all procedures performed on the rats (VM.R23.04.63). Twenty female Albino rats (age: 8 weeks old; weight: 250–300 g) were purchased from the Faculty of Medicine, Mansoura University, Mansoura, Egypt. The animals were allowed to be accommodated for 2 weeks under the laboratory conditions (12 hours of light/dark cycles) before the commencement of the experiment. Animals will receive feed and water ad libitum. Animals received human care in compliance with the guidelines for animal care of the National Institutes of Health.

The experimental rats were allocated into two groups:

1. Control group (G1: $n=8$): received intraperitoneal injection of pyrogen-free 0.9% NaCl. According to Nezić et al. (2009).
2. Treatment group ($n=12$): a single intraperitoneal injection of a non-lethal dose of LPS administered at a dose of 4 mg/kg body weight (BW) (0.18 of the LD50 22.15 mg/kg) (Nezić et al., 2009) and subdivided into two groups. The first one was sacrificed after 6 hours (G2: $n=6$), and the second one was scarified after 72 hours of exposure to LPS (G3: $n=6$).

Rats were sacrificed, and the uterus and ovaries were collected at 6- and 72-hour time points after LPS injection. At the end of the experimental period, rats were sacrificed after being anesthetized with ether. Blood, ovaries, and uterus samples were collected from all experimental animals.

Biochemical Analyses

Tissue homogenate was centrifuged at 4000 rpm for 20 minutes at 4 °C and stored at −80°C, according to Fernandez-Botran et al. (2002), until antioxidant and oxidative stress markers were performed. Oxidative stress markers such as malondialdehyde (MDA), SOD, glutathione (GSH), and catalase (CAT) were measured using Biodiagnostic kits (Egypt) according to previous studies (Halawa et al., 2022; T. Karabulut et al., 2016; T. S. F. Toydemir Karabulut, 2018).

Real Time Polymerase Chain Reaction Analyses

The translation of mRNA for angiogenic proteins vascular endothelial growth factor, vascular endothelial growth factor 1, endothelial growth factor synthase (VEGF, VEGFR1, eNOS3, respectively), IGF1, and IGF1R were measured via real-time polymerase chain reaction (PCR) technique according to Samy et al. (2020) and Halawa et al. (2022).

Ribonucleic acid (RNA) extraction from ovarian and uterine tissues was performed using Trizol reagent (Thermofischer Scientific, Stafford House, Boundary Way Hemel Hempstead, UK) following the manufacturer's instructions. One microgram of extracted RNA was transferred into cDNA using HisenscriptTM cDNA synthesis kit (Intronbio, South Korea). The newly formed cDNA strand is used as a template for gene expression analysis using primer pairs listed in Table 1. Polymerase chain reaction was conducted in the Pikoreal real-time PCR system (Thermoscientific, Lituania). Polymerase chain reaction cycling conditions were as follows: initial denaturation at 94°C for 9 minutes followed by 35 cycles of denaturation at 94°C for 1 minute, annealing at 55 °C for 40 seconds, and elongation at 72 °C for 1 minute. Polymerase chain reaction was terminated by a final elongation step at 72 °C for 9 minutes. Gene expression analysis was conducted against b-actin as a housekeeping gene following the method of Livak and Schmittgen (2001), applying the $2^{-\Delta\Delta C_T}$ method for the calculation of fold expression relative to control group.

Table 1.
Sequences of forward and reverse primers of angiogenic proteins (VEGF, VEGFR1, eNOS3) IGF1 and IGF1R used for qRT-PCR quantitation

Gene	Forward Primer	Reverse Primer	Accession Numbers
VEGF	ACCATGCCAAGTGGTGAAGT	ACTCCCTAATCTTCCGGGCT	NM_001287114.1
VEGFR1	ATGTGCCAAACGGCTTTCAC	GGCTCGGCACCTATAGACAC	NM_019306.2
eNOS3	TAGACTGGGAGGGAGTCAGC	AAGCATACGAAGAGGGCAGC	NM_021838.2
IGF1	GACCCGGGACGTACCAAAAT	GAACTGAAGAGCGTCCACCA	NM_178866.4
IGF1R	GGTCATCTGGTGACACTGCT	TTACTGTGGCGAGTGGGTTG	XM_039102225.1
B-actin	CCCGCGAGTACAACCTTCTT	AACACAGCCTGGATGGCTAC	NM_031144.3

Histopathological Examination
Fixation of different tissue samples in neutral buffered formalin was done for the histological hematoxylin–eosin stain (H&E) and analyses. One gram of each ovary and uterine tissues was washed with phosphate buffer solution (PBS) (pH 7.4) and then homogenized in nine volumes of PBS. Fixed tissues were serially dehydrated in graded ethanol and xylene. Specimens were embedded in the paraffin block. The tissue blocks were cut at 6 μm using a microtome (HM350S, MICROM, Germany). Sections were stained with H&E and examined under a light microscope (BX51, Olympus, 5128 Nishimachi, Ina-shi, Nagano 396-0026, Japan).

Statistical Analyses
The SAS® program (version 9.2, SAS Institute, Cary, NC, USA) was used for data analysis. The distribution of all variables was examined for normalcy using the Shapiro–Wilk test. Analysis of variance of replicate data was applied to assessments of oxidative stress markers and fold changes for the angiogenic and IGF genes. The post hoc test was Turkey's. For all analyses, $p \leq .05$ will be defined as significant.

Results

The Effects of Lipopolysaccharide on Oxidative Stress and Antioxidant Status Markers in Ovaries
The SOD (Figure 1A) showed a significant decrease in the ovaries of rats in the second group (6 hours after LPS treatment) when compared to the control rats (172.51 ± 7.52 vs. 214.67 ± 14.03, $p < .05$). Meanwhile, the SOD levels in the ovaries were not significantly different in group 3 (after 72 hours of LPS treatment) when compared to both control (G1) and G2 ($p > .05$).

The analysis of the NO (Figure 1B) in the ovarian tissue revealed a significant increase in G2 (6 hours after LPS) when compared with the control and G3 (43.72 ± 6.39 vs. 20.728 ± 0.75 and 34.64 ± 0.81, respectively, $p < .05$).

The levels of malondialdehyde as a potential oxidative stress marker resulting from lipid peroxidation were measured in the ovarian tissue after the treatment of rats with LPS. We investigated a significant increase in the MDA in G3-treated rats when compared to control and G2-treated rats (20.14 ± 4.45 vs. 5.43 ± 0.69 and 7.73 ± 0.57, respectively, $p < .05$) (Figure 1C). The glutathione transferase enzyme (Figure 1d) showed a significant increase in the ovarian tissue after 6 hours (1.51 ± 0.13) and 72 hours (1.38 ± 0.08) compared to the control-treated rats (0.83 ± 0.026, $p < .05$).

Moreover, the concentration of glutathione antioxidant in the ovarian tissue was decreased (G2 $p < 0.05$) when compared with the control and G3 treatments (22.36 ± 1.76 and 20.1 ± 1.63, respectively) (Figure 1E).

The ovarian total antioxidant capacity evaluates oxidative stress after exposure to LPS and provides an assessment of the reductive potential disorders of the ovaries. The TAC showed more than double-fold decrease after 72 hours of exposure of rats to LPS when compared to the control and G2-treated rats (Figure 1F). In the same manner were the levels of catalase enzyme in the ovarian tissues of the rats. The CAT was 0.18 ± 0.04 when compared with control (0.28 ± 0.01) and G2 (0.12 ± 0.06) (Figure 1G).

The Effects of Lipopolysaccharide on Oxidative Stress and Antioxidant Status Markers in Uterus
Regarding the SOD and NO as well as TAC and CAT in the uterine tissues, there was no significant difference ($p > .05$) between the control-treated rats and the other two time points after LPS administration (Figure 2A and B). On the other hand, MDA showed a significant difference between control (14.51 ± 2.42), after 6 hours (31.58 ± .91), and after 72 hours (22.7±1.49) (Figure 2C). Glutathione S transferase (GST) showed a significant decrease in the control group (0.97 ± 0.02) than group 1 (1.34 ± 0.05) and group 2 (1.36 ± 0.06) (Figure 2D). Reduced glutathione (GSH) showed a significant increase in the control group (33.59 ± 0.6) than after 6 hours (18.83 ± 3.24) and after 72 hours (16.74 ± .98, Figure 2E). Additionally, the TAC and CAT levels in the tissue of the uterus showed no significant difference between the control-treated rats and other two time points after LPS administration ($p > .05$, Figure 2F and G).

The Effects of Lipopolysaccharide on Angiogenic Proteins (VEGF, VEGFR1, eNOS3) IGF1 and IGF1R
The expression of IGF 1 was significantly decreased after 72 hours in the ovarian tissue than the control and G1 rats (1, 0.7, 0.41 for control, G1, and G2, respectively, $p < .05$). On the other side, IGFR1 was significantly downregulated in the ovarian tissues after both 6 and 72 hours (Figure 3A and B).

The expression of eNOS3, VEGF, and VEGFR1 were significantly increased ($p < .05$) in the ovarian tissue when compared to the control-treated rats. The upregulation of eNOS3 and VEGFR1 were more than double-fold especially after 72 hours. Meanwhile, the VEGF was increased by about threefold than the control and other treated rats after 72 hours (Figure 3C, D, and E).

The same time, the expression of both IGF 1 and IGF R1 mRNA were significantly downregulated, especially after 72 hours of LPS exposure (Figure 4A and B).

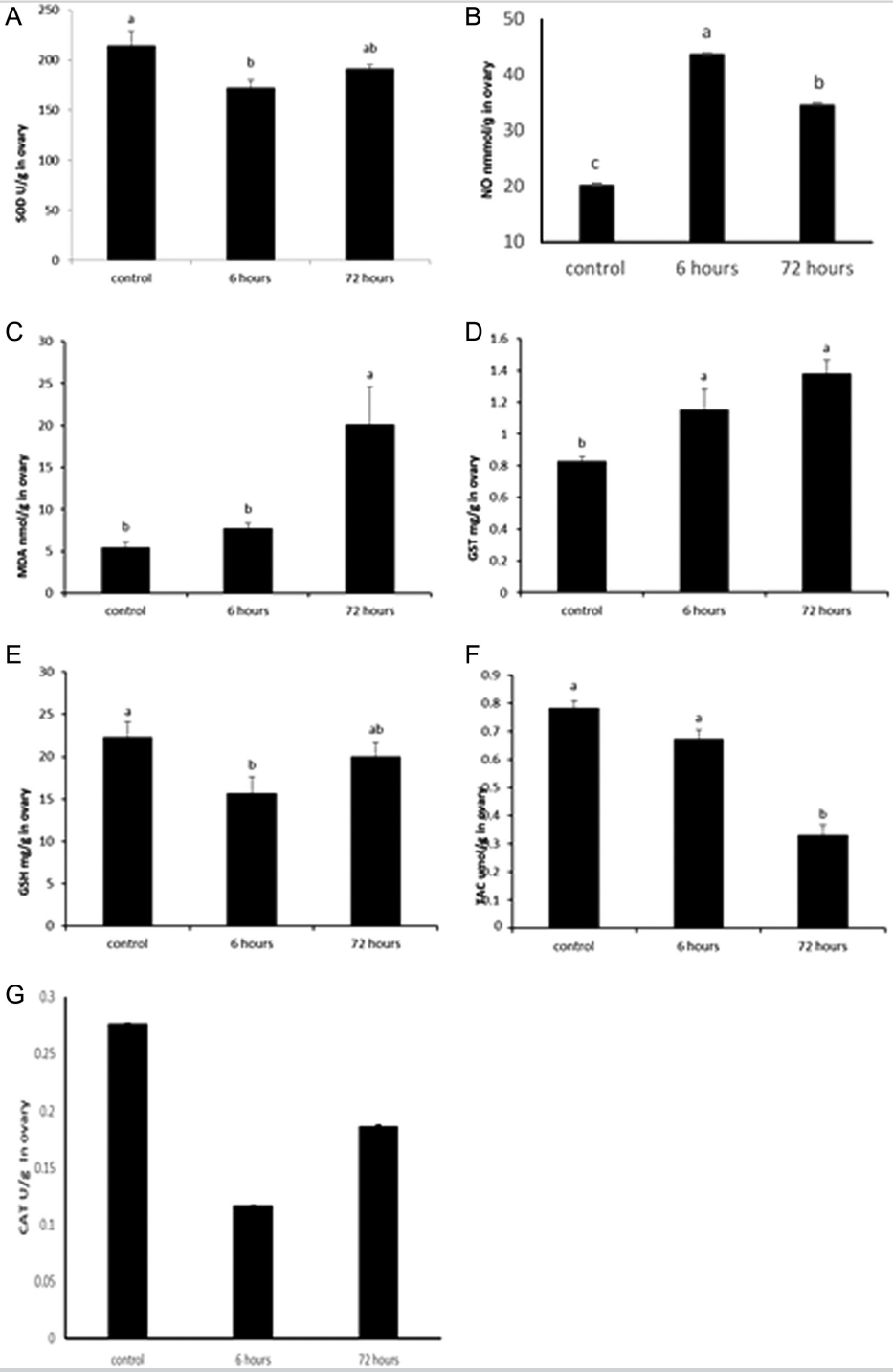

Figure 1.
*Effects of LPS on ovarian oxidative/antioxidant markers. Data are presented as means ± SEM. Means with different superscripts are significantly different (*p < *.05). (A): SOD (superoxide dismutase), (B)NO (nitric oxide), (C) MDA (malondialdehyde), (D) GST (glutathione S transferase), (E) GSH (reduced glutathione), (F) TAC (total antioxidant capacity), and (G) CAT (catalase). Control, 6 hours, and 72 hours indicate control, G2, and G3, respectively.*

The expression of angiogenic proteins in the uterine tissue of experimental rats after exposure to LPS showed that, there was a significant increase of NOS3, VEGF, and VEGFR1 ($p < .05$), especially 72 hours post-exposure (Figure 4C, D, and E).

The Effects of Lipopolysaccharide Infusion on the Uterine and Ovarian Tissue Histopathology

Photomicrographs of the uterine sections of female albino rats (Figure 5: A: normal, B: G2, and C: G3). The normal uterus sections

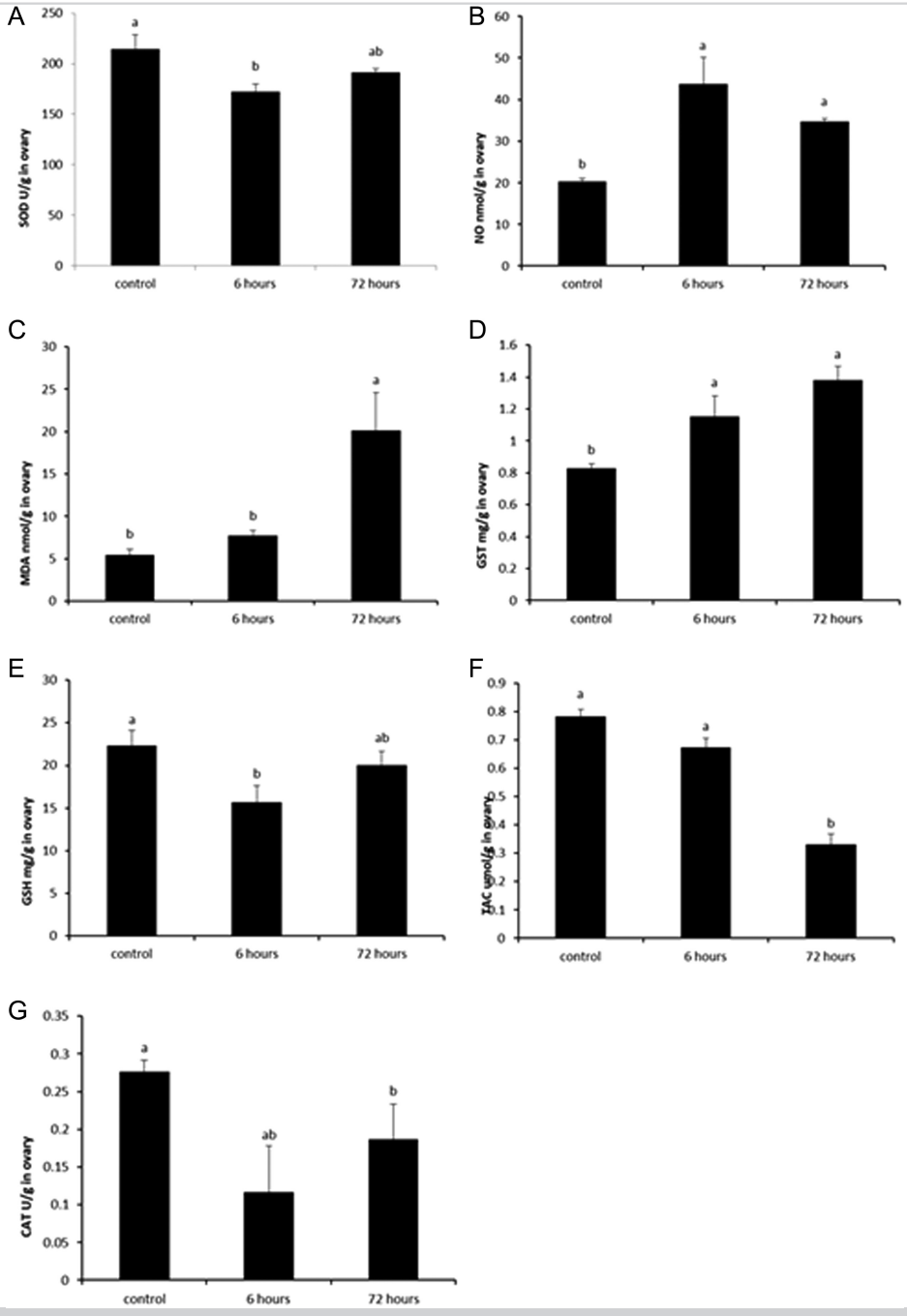

Figure 2.
Effects of LPS on uterine oxidative/antioxidant markers. Data are presented as means ± SEM. Data are expressed as mean ± SEM. Means with different superscripts are significantly different (p < .05). (A) SOD (superoxide dismutase), (B) NO (nitric oxide), (C) MDA (malondialdehyde), (D) GST (glutathione S transferase), (E) GSH (reduced glutathione), (F) TAC (total antioxidant capacity) and (G)CAT (catalase). Control, 6 hours, and 72 hours indicate control, G2, and G3, respectively).

showed (Figure 5A) demonstrating the typical endometrial layer, proper folding, and normal collagen fiber distribution. The sections of the treated uteri showed a typical endometrial layer, proper folding, and normal collagen fiber distribution (Figure 5B), and enhanced endometrial collagen deposition. Figure 5C demonstrates a section of the uterus with glandular dilatation and inflammatory cell infiltration.

Photomicrographs (D: normal, E: G2, and F: G3) show sections from the ovaries of female albino rats. Figure 5D illustrates the normal distribution of ovarian stromal components, developing follicles, and corpora lutae. With vacculation, the ovarian alterations reached their peak, with a reduction in the number of developing follicles and the amount of inter-follicular tissue (Figure 5E), and no mature Graafian follicles were visible. The distribution of both stromal and vascular

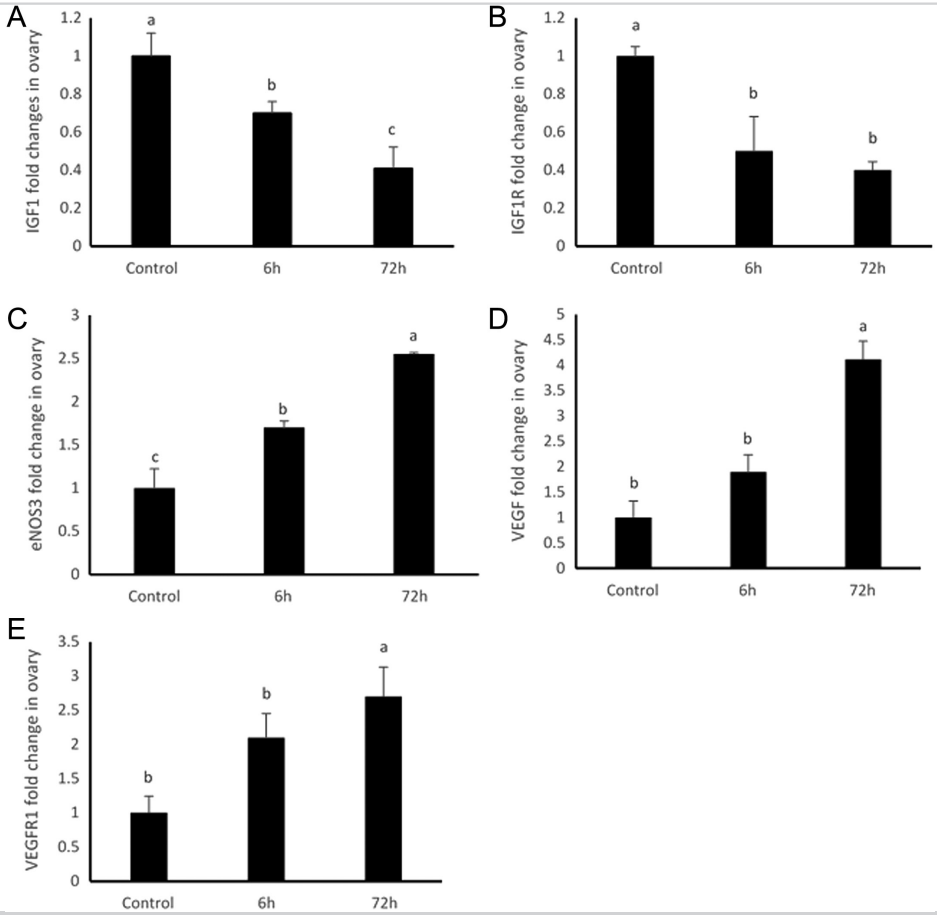

Figure 3.
Effects of LPS on the expression of insulin growth factor (A) IGF1, (B) IGF1R and angiogenic, (C) NOS3, (D) VEGF, and (E) VEGFR1 mRNAs in the ovaries. Data are presented as means ± SEM. The different letters (a, b, c, d) indicated significant difference ($p < .05$*) between experimental groups. Control, 6 hours and 72 hours indicates (control, G2, and G3, respectively).*

elements, as well as the ovarian follicles in the ovaries, returned to the normal condition (Figure 5F).

Discussion

Lipopolysaccharide, a major component of gram-negative bacteria's outer membrane, is a bacterial endotoxin that causes inflammation by increasing the production of cytokines like leukemia inhibitory factor, monocyte chemotactic protein, and interleukin 6. It has been demonstrated that immunological stress brought on by prenatal LPS exposure causes a variety of developmental disturbances, including an increase in embryonic mortality, a decrease in birth weight, and problems in the development of the central nervous system (Ignatiuk et al., 2019; Izvolskaya et al., 2016). Oxidative stress is described as a balance between free radical generation and antioxidant defense. Hydroxyl radical, superoxide radical, and NO are the most frequent reactive oxygen species. They can be formed as a result of dopamine metabolism catalyzed by monoamine oxidase, the Fenton reaction in the presence of iron, lipid peroxidation, and defective mitochondrial complex I activity (Luduvico et al., 2022; Noworyta-Sokołowska et al., 2013). In the current study, the exposure of the female rats to LPS induced a significant change in the oxidant and antioxidant activity in both ovarian and uterine tissues, especially after 72 hours of exposure. The significant changes in the ovarian tissue were regarding the MDA, GST, and NO. However, the CAT and SOD were recognized to decrease after 6 hours of LPS exposure. But the decrease in CAT was not significant. Previous studies proved that LPS induces detrimental effects on the ovarian and uterine tissues via changes in the oxidant and antioxidant capacity. The results in the current study were in the same way as those published before regarding the effects of LPS on the oxidant and antioxidant variation in the male testis (Halawa et al., 2018). Lipopolysaccharide may cause oxidative stress in rats due to disruption of the germ cell layer in the seminiferous tubules, which enhances the production of proinflammatory cytokines and the creation of ROS (Zhao et al., 2008).

In the current study, the exposure of non-pregnant rats to LPS leads to significant changes in the ovarian and uterine histology. The significant changes were the degeneration of the ovarian follicles and the superficial epithelium layer of the uterus. Previous studies proved that LPS exposure affects steroidogenesis and folliculogenesis, consequently resulting in delayed puberty onset (Ignatiuk et al., 2019). Previous studies have proven the significant impact of LPS on reproductive function (Gram & Kowalewski, 2022; Seo et al., 2022; Yamamoto et al., 2023). Lipopolysaccharide intraperitoneal administration produced oxidative stress in male rats, leading to functional

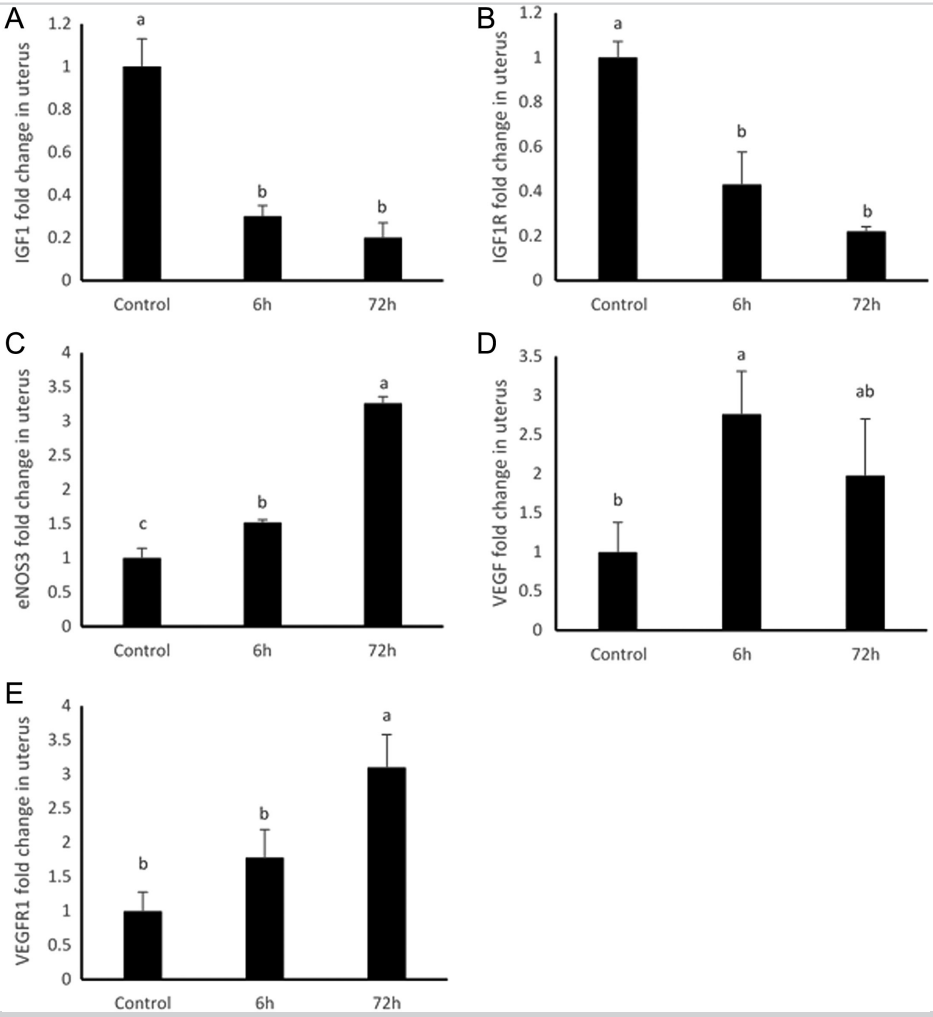

Figure 4.
Effects of LPS on the expression of insulin growth factor (A) IGF1, (B) IGF1R and angiogenic, (C) NOS3, (D) VEGF, and (E) VEGFR1 mRNAs in the uterus. Data are presented as means ± SEM. The different letters (a, b, c, d) indicated significant difference ($p < .05$*) between experimental groups. Control, 6 hours and 72 hours indicates (control, G2, and G3, respectively.*

damage in the testicular mitochondria and, subsequently, spermatogenesis dysfunction (Yoo & Lee, 2016). Furthermore, the LPS treatment increased the number of atretic follicles in proestrus ovaries as well as the quantity of apoptotic cells and macrophages (Adetunji et al., 2020; Gindri et al., 2019; Zhao et al., 2022). Additionally, exposure to LPS during pregnancy or in the uterine cavity dramatically delays the day of vaginal opening in female rats (Izvolskaia et al., 2016). In cows, it was proven that the LPS infusion inhibits the follicular activity regardless of the developmental stage of the big follicles. Furthermore, depending on the concentration of LPS in the follicular fluid, the transcriptional levels of steroidogenesis-related genes alter dramatically (Magata, 2020). Ovarian status influences every stage of life's health and is one of the primary factors in female life span (Yan et al., 2022; Yang et al., 2020).

Lipopolysaccharide, an endotoxin, is most likely responsible for *Escherichia coli*'s inflammatory effects (Beutler et al., 2003). By triggering the innate immune system, LPS, which makes up the majority of gram-negative bacteria's cell membranes, causes a localized or systemic inflammatory response. When cows have subacute rumen acidosis, mastitis, or uterine inflammation, their plasma LPS concentrations rise (Blum et al., 2000; Dosogne et al., 2002).

Similar to the changes in the ovarian tissue in the present study, the oxidant/antioxidant status of the uterus also showed a significant variation between treated and non-treated rats, with significant effects of time of sample collection. The most significant changes were recognized regarding the MDA, GSH, and GST. Similar to these results were that published by Halawa et al. (2018) concerning the MDA, GSH, and GST in rat testis. The changes in the oxidative stress biomarkers were attributed to the imbalance between the production and destruction of ROS, causes a direct damages to the uterine environment. (Vašková et al., 2023; Wan et al., 2020; Wang et al., 2022). Basically, infertility may be caused by the pathological excess of oxidative stress markers compared to antioxidants, which also cause other female reproductive abnormalities (Liu et al., 2023; Tossetta et al., 2023). Antioxidants are therefore essential for healthy female reproduction. They contribute to the oocyte metabolism, the maturation of the endometrium through the activation of the antioxidant signaling pathways Nrf2 and NF-B, and the hormonal control

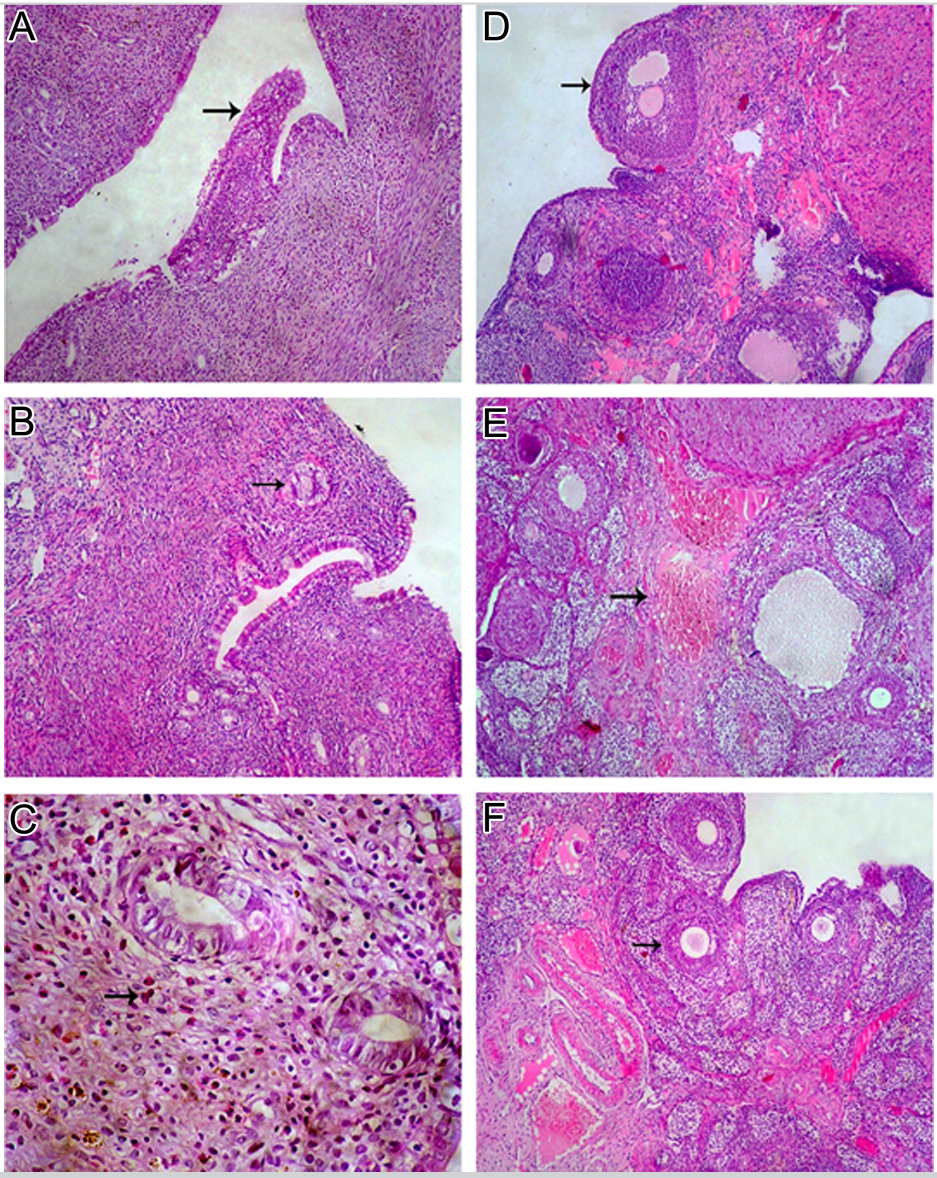

Figure 5.
Photomicrographs of sections in the uteri (A: normal, B: G2 and C: G3) of female albino rats. Section of control uterus(A) showing the normal endometrial layer, normal folding (arrow) and normal distribution of collagen fiber in the endometrium. (B) Section of treated uterus showing luminal folding, cellular vacculation (arrow), increased collagen deposition in the endometrium. (C) Section of uterus showing glandular dilation and inflammatory cell infiltration (arrow); Photomicrographs (D: normal, E: G2 and F: G3) of sections in the ovary of female albino rats.(D) showing normal distribution of ovarian stromal elements, growing follicles (arrow) and corpora lutae. (E) The ovarian changes reached maximal severity showing reduction in the number of growing follicles and the amount of interfollicular tissue with vacculation (arrow) and no mature Graafian follicle is seen. (F) The ovarian tissues were returned to normal structure and the distribution of both stromal and vascular elements as well as the ovarian follicles (arrow).

of vascular function (Vašková et al., 2023). Antioxidants can directly neutralize free radicals, serve as cofactors for crucial enzymes involved in cell differentiation and growth, or boost the activity of antioxidant enzymes. Accordingly, supplementation with antioxidants to make up for low levels can increase fertility (Gao et al., 2023; Vašková et al., 2023).

In the current study, the exposure of female rats to LPS leads to the upregulation of angiogenic proteins in both the ovaries and uterus and decreased the expression of IGF 1 and IGF R1, especially after 72 hours. In cows, the infected uteri in postpartum are frequently linked to *E. coli* (LPS) (Ordell et al., 2016). One of the most potent bacterial virulence factors that causes endometrial inflammation is LPS, which is found on the outer membrane of *E. coli* and other gram-negative bacteria (Sheldon & Bromfield, 2011; Sheldon et al., 2017). Additionally, LPS has been linked to pregnancy losses and implantation failure in the human species (Dubuc et al., 2011; Gilbert, 2011). Oxidative stress and glycolysis have also been linked to enhanced

proliferation in many cell types, including epithelial cells from various species (Chanrot et al., 2017).

A well-known angiogenic factor is essential to numerous physiological and pathological processes. By promoting embryo development, increasing endometrial receptivity, and enabling interactions between the growing embryo and the endometrium, VEGF also aids in the process of embryo implantation (Guo et al., 2021). The VEGF is a potent angiogenic and vasopermeability factor produced by a variety of cells, including fibroblasts, vascular smooth muscle cells, endothelial cells, and macrophages (Dulak et al., 2004; Li et al., 1995). Reproductive failure, including recurrent implantation failure and recurrent miscarriage, is associated with a change in angiogenic protein expression (Binder et al., 2014; Guo et al., 2021; Hoozemans et al., 2004). Similar to the current results regarding the stimulatory effects of LPS on the expression of VEGF and NOS3, this indicates that LPS increased VEGF mRNA expression in a concentration-d ependent manner, peaking 2 hours after stimulation in pericyte (Kim et al., 2008). It was proven that vascular endothelial growth factor protein levels peaked between 24 and 48 hours after increasing LPS levels were first detected in conditioned media and cell lysate. Within 6 hours, LPS also markedly increased the expression of inducible NO synthase in lung pericytes (Kim et al., 2008). The previous authors also proved that within 6 hours, LPS markedly increased the expression of iNOS in lung pericytes. The stimulatory effects of LPS on the expression of angiogenic proteins in the ovarian and uterine tissues may be attributed to the effects of LPS on the activation of pro-inflammatory mechanisms (Jhamat et al., 2020). Also, the upregulatory effects of LPS on angiogenic proteins may be partly mediated by the p38 MAP kinase pathway (Kim et al., 2008).

In the present study, the expression of IGF 1 and IGF R1 was found to be decreased in response to LPS either after 6 and/or 72 hours. The current results were similar to those investigated before regarding the fact LPS has inhibitory effects on the mRNA expression of IGF1. The IGF1 and IGF2 signal through the IGF1R, leading to growth and metabolic effects via the downstream PI3K/Akt pathway (Suh et al., 2013). Altogether, the obtained results indicated that LPS has a detrimental effect on both ovarian and uterine tissues via its effect on the alteration of biochemical markers as well as the expression of angiogenic and IGF 1 proteins. Furthermore, LPS and oxidative stress parameters should be considered with urinary tract and uterine infections (*E. coli*) in women and animals.

Ethics Committee Approval: Ethical committee approval was received from the Ethics Committee of Mansoura University (Approval number: VM.R23.04.63, Date: 13/04/2023).

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