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(RESEARCH ARTICLE)

Maternal *listeria monocytogenes* infection induces placental stress and histological alterations in rat embryos

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Abstract

Background: *Listeria monocytogenes* is a gram-positive intracellular pathogen that causes serious pregnancy complications, such as fetal loss, preterm birth, and listeriosis in the neonatal stage. The pathogen has an outstanding placental tropism and exploits the pathways of trophoblast invasion to interfere with the fetal growth.

Hypothesis: The hypothesis was to examine the impact of maternal infection with *L. monocytogenes* on the placental and liver expression of inflammatory cytokines, the oxidative stress parameters, the fetal morphometric outcomes in Wistar rat model.

Methods: Thirty pregnant Wistar rats (n = 10 each) were put into three groups (Group A: control, Group B: low-dose infection: 1×10^3 CFU/mL, and Group C: high-dose infection: 1×10^5 CFU/mL) and inoculated on gestational day 7 intraperitoneally. liver and placental tissues were harvested on day 20. Cytokines (TNF-α, IL-6), oxidative stress (MDA, SOD, CAT, GSH), fetal morphometry, and histological examinations were performed.

Findings: The fetal viability reduced in the high-dose group compared to controls, with increased resorptions. There was a significant decrease in fetal body weight and crown-rump length in the infected groups. Placental and fetal hepatic tissues exhibited a dose-dependent increase in inflammatory cytokines (TNF-α and IL-6) and oxidative stress markers, alongside a significant depletion of antioxidant enzymes (SOD, CAT, and GSH) in both tissues. Furthermore, histological examination of fetal liver confirmed severe structural alterations, including hepatocyte necrosis, vacuolization, and inflammatory cell infiltration

Conclusion: Maternal infection with *L. monocytogenes* triggers dose dependent inflammatory and oxidative cascade in the placenta and liver, resulting in poor fetal development and high embryonic loss. These results bring to the fore the importance of microbiological screening and specific anti-inflammatory interventions in pregnancy.

Keywords: *Listeria monocytogenes*; Placental Inflammation; Oxidative Stress; Fetal Morphometry; TNF-A; IL-6; Rat Model; Reproductive Toxicology

1 Introduction

Listeria monocytogenes is a gram-positive, facultative intracellular bacterium that is distributed ubiquitously in the environment in reservoirs, such as soil, water, and littering. Its ability to endure refrigeration conditions and withstand adverse conditions makes it a consistent spoiler of processed and ready-to-eat food [1]. Pregnancy-related infection is one of the most worrying clinical manifestations of listeriosis, with immunocompromised hosts having much higher risks of developing it than the normal population [2]. It is estimated in epidemiological surveillance that pregnant

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women are about 13-18 times more prone to listeriosis than the general population, and *L. monocytogenes* contributes to an excessive number of adverse perinatal events [3].

The listeriosis pathogenesis during gestation is supported by the complex of virulence factors of the bacterium, which allows the systematic circumvent of the host innate and adaptive immune responses. Epithelial and trophoblastic cell adhesion and invasion are mediated by internalin A and B (InlA and InlB) by receptor-mediated endocytosis and phagosomal escape by listeriolysin O (LLO) [4]. Placenta, due to its immunological tolerance, which does not allow the mother to reject the semi-allogeneic unborn child, in contrast, is a permissive niche in which *listeria* thrives [5]. The inflammation of the labyrinthine zone, rupture of the trophoblast, and premature rupture of the membrane, intrauterine growth restriction, or fetal death are the outcomes of this placental tropism [6].

Molecularly, placental listeriosis initiates a strong pro-inflammatory cytokine storm, which is mainly through tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6). Although these mediators are critical elements of the innate immune response, they have cytotoxic effects on the syncytiotrophoblast, impair trophoblast invasion depth, and disrupt the fine hemostatic regulation in the intervillous space [7]. At the same time, reactive oxygen species (ROS) produced during the respiratory burst of activated macrophages and neutrophils overwhelm the placental antioxidant defenses, causing lipid peroxidation and mitochondrial dysfunction [8]. The resultant oxidative environment increases cytokine-induced damage, which forms a vicious circle of placental damage.

The use of rodent models, especially the Wistar rat, has been extensively used to study the pathophysiological implications of maternal infection because of their similar gestational physiology, experimental conditions easy to control and the availability of both maternal and fetal tissues [9]. Past experimental research on murine listeriosis models has shown that there are dose-related increment in fetal resorption levels, intrauterine growth retardation, and placental histopathological alterations following bacterial inoculation. Nonetheless, quantitative studies, which combine placental cytokine levels, multi-parameter oxidative stress measurements, fetal morphometric measurements, and maternal concurrent hepatic responses are not fully defined in the literature.

The current study is unique in its multi-compartment, concurrent analysis design, as compared to prior studies. Although previous studies have looked at placental and liver cytokines or oxidative stress alone, none have simultaneously measured placenta inflammatory mediators, a complete set of antioxidant enzymes, fetal morphometric indices such as limb or cranial measurement, this combined methodology allows, in the first instance, the direct correlation analysis of molecular abnormalities of the placental and quantitative fetal outcomes, which are mechanistic evidence that goes beyond descriptive pathology. Moreover, the dual-dose design enables characterization of dose-response in all measured endpoints that enhances the inference of causality between bacterial infection intensity and extent of placental-fetal injury.

Based on this, the current research was intended to conduct a systematic analysis of the impact of maternal *L. monocytogenes* infection at two different dosage levels on the inflammatory cytokines of the placenta and liver (TNF-α and IL-6), oxidative stress indicators (MDA, SOD, CAT, GSH), reproductive outcome measures, fetal morphometric measurements, and It is expected that the findings of this study will contribute to mechanistic insights into pregnancy-related listeriosis, and they could be used in prophylaxis and treatment in the future.

2 Material and methods

2.1 Ethical Statement

The ethical considerations of care and use of laboratory animals were strictly followed throughout all the experimental procedures set by the University of Samarra Research Ethics Committee (Approval No. USC-2023-BIO-047). Animals were kept according to the recommendations presented in the National Institutes of Health Guide to the Care and Use of Laboratory Animals. Maximum effort was put in place to ensure the animals were suffering is minimized and to ensure that the animals used are reduced.

2.2 Experimental Animal and Grouping

The Wistar rats (*Rattus norvegicus*) used were 30 adult females aged 8-10 weeks with 180-220 g weight purchased in the Animal house of the College of Veterinary Medicine, University of Tikrit, Iraq. Animals were allowed 2 weeks of acclimation and then experimented under conditions of controlled conditions (23 ± 2 C, 55 ± 5 relative humidity, 12-h light/dark cycle) in the presence of ad libitum standard pellet chow and filtered water. Gestation day 0 (GD 0) was defined as the presence of vaginal plug, which confirmed mating. The experimental groups were randomly created and

comprised of 10 pregnant females each: Group A (negative control, intraperitoneal injection with 0.5 mL sterile phosphate-buffered saline), Group B (low-dose infection, inoculated with 1×10^3 CFU/mL on GD 7), and Group C (high-dose infection, inoculated with 1×10^5 CFU/mL on GD 7 was done on GD 20 in all animals.

2.3 Preparation of Bacterial Strain and Preparation of Inoculum.

One virulent clinical isolate of *Listeria monocytogenes* serotype 4b was recovered in the Microbiology Laboratory of the College of Medicine, University of Tikrit, the isolate was initially identified through conventional biochemical characterization (CAMP test, motility, hemolysis on blood agar), Gram staining, and 16S rRNA gene sequencing. The strain maintained at -80 °C in20% glycerol broth. prior to inoculation, the strain was subculture twice in Brain Heart Infusion (BHI) agar at 37°C. the bacterial suspension was adjusted to 0.5 McFarland standard (approximately 1.5×10^8 CFU/mL), and the subsequent serial dilution in sterile saline were performed to achieve the target concentration of the desired concentrations of1×10^3 CFU/mL and 1.5×10^5 CFU/mL. the final bacterial counts were verified using the viable plate count method on BHI agar.

2.4 Sample Collection

On GD 20, the animals were humanely euthanized using carbon dioxide (CO_2) inhalation. The uterine horns were removed, and the total number of implantation sites, viable fetuses and desorption sites per dam were recorded. Placental units and livers were immediately harvested, and divided: into two portion was fixed was fixed in 10% neutral buffered formalin to continue with the histological processing and the second half was snap-frozen in liquid nitrogen and stored at -80°C for subsequent biochemical analyses.

2.5 Fetal morphometric and external examination

Fetal development was assessed by recording specific parameters, including individual fetal body weight, crown-rump length (CRL), head width, and limb lengths.

2.6 Biochemical Analysis of Placental and liver tissues

All biochemical assays were performed using the supernatants of placental tissue homogenates prepared in ice-cold PBS (1:10 w/v) and centrifuged at 10,000 × g at 4 °C. The placental diameter and weight were measured immediately upon collection. Concentrations of TNF-α and IL-6 were determined using Species-specific enzyme-linked immunosorbent assay (ELISA) kits (Abcam, Cambridge, UK) according to the manufacture's protocols. Malondialdehyde (MDA) levels, a marker of lipid peroxidation, were measured using the thiobarbituric acid reactive substances (TBARS) assay. The activities of superoxide dismutase (SOD), catalase (CAT), as well as the concentration of reduced glutathione (GSH), were measured using commercial spectrophotometric kits (BioVision, Milpitas, CA, USA). protein concentrations were determined using the Bradford method.

2.7 Liver histological Processing

The tissues of the liver were fixed in 10 % neutral buffered formalin 24h, the tissues were then dehydrated through graded series of ethanol, cleared in xylene and embedded in paraffin wax. Section of 4-5 µm thickness were prepared using a rotary microtome and mounted on glass slides and stained with hematoxylin and eosin (H&E). All the histological analyses were done under Leica DM500 light microscope.

2.8 Statistical Analysis

The results were presented in the form of Mean ± Standard Deviation (SD). The one-way analysis of variance (ANOVA) was used to obtain statistical differences between groups and Tukey post hoc test to conduct multiple comparisons. The differences were deemed to be significant at $p < 0.05$. The a priori sample size calculation was based on the results of similar rodent listeriosis infection studies [10,11], with an estimated effect size of placental cytokine results (Cohen f = 0.45) and a type II statistical power of 80 percent and a two-tailed alpha level of 0.05. Calculations of power showed that at least nine animals per group would be needed; ten animals per group were allotted to each group to allow possible attrition. Pearson bivariate correlation analysis was used to evaluate the strength and direction of correlations between the most important parameters of inflammatory and oxidative stress (TNF-α, IL-6, MDA, GSH) and the fetal outcome indices (body weight and viable fetuses per litter) in all individual observations. Each pair was presented with correlation coefficients (r) and p-values. All the analyses were made with the help of IBM SPSS Statistics version 26.0 (IBM Corp., Armonk, NY, USA).

3 Results

3.1 Reproductive Outcomes

Maternal *L. monocytogenes* infection caused dose-related reproductive toxicity (Table 1). While total implantation sites showed a slight decrease ($p > 0.05$), viable fetuses significantly decreased (Group C: 4.8 ± 0.6 vs. Group A: 9.0 ± 0.7; $p < 0.001$), while fetal resorptions increased (Group C: 2.6 ± 0.4 vs. Group A: 0.2 ± 0.1; $p < 0.001$). Additionally, both fetal and placental weights were significantly reduced in infected groups compared to controls ($p < 0.001$; Table 1).

Table 1 Mean ± SD Reproductive outcome parameters in experimental groups (Mean ± SD, n= 10/group).

Parameter	Group A (Control)	Group B (Low Dose)	Group C (High Dose)
Total implantation sites	9.2 ± 0.8	8.1 ± 0.9	7.0 ± 0.7
Viable fetuses/litter	9.0 ± 0.7	7.2 ± 0.8*	4.8 ± 0.6**
Resorptions/litter	0.2 ± 0.1	1.1 ± 0.3*	2.6 ± 0.4**
Fetal body weight (g)	3.42 ± 0.31	2.98 ± 0.28*	2.15 ± 0.22**
Placental weight (g)	0.58 ± 0.05	0.49 ± 0.05*	0.38 ± 0.04**

* $p < 0.05$ vs. Group A; ** $p < 0.001$ vs. Group A (ANOVA with Tukey's post-hoc test).

3.2 Placental Inflammatory Cytokines and Oxidative Stress

The levels of Placental TNF- α and IL-6 were significantly higher in infected animals in a dose-dependent manner (Table 2). TNF-α in Group C (78.3 ± 7.2 pg/mL) was more than four-fold higher than in controls (18.4 ± 1.9 pg/mL; $p < 0.001$). IL-6 followed a comparable trajectory, reaching 103.7 ± 9.6 pg/mL in Group C versus 21.3 ± 2.2 pg/mL in Group A ($p < 0.001$). Oxidative stress indices verified that there were large amounts of lipid peroxidation in infected placentas; MDA levels were 6.42 ± 0.58 nmol/mg protein in Group C and 1.82 ± 0.17 in Group A ($p < 0.001$). Enzyme antioxidants SOD and CAT and the non-enzymatic antioxidant GSH significantly decreased in Groups B and C with Group C showing the most pronounced decrease (SOD: 17.6 ± 1.8 U/mg protein; CAT: 13.9 ± 1.4 U/mg protein; GSH: 9.6 ± 0.9 μmol/mg) .

Table 2 Oxidative stress markers and placental inflammatory cytokines (Mean ± SD, n=10/group).

Parameter	Group A (Control)	Group B (Low Dose)	Group C (High Dose)
TNF-α (pg/mL)	18.4 ± 1.9	42.7 ± 4.1*	78.3 ± 7.2**
IL-6 (pg/mL)	21.3 ± 2.2	58.9 ± 5.4*	103.7 ± 9.6**
MDA (nmol/mg protein)	1.82 ± 0.17	3.74 ± 0.35*	6.42 ± 0.58**
SOD (U/mg protein)	42.8 ± 4.1	28.3 ± 2.7*	17.6 ± 1.8**
CAT (U/mg protein)	38.7 ± 3.6	24.1 ± 2.3*	13.9 ± 1.4**
GSH (μmol/mg protein)	28.4 ± 2.7	17.8 ± 1.7*	9.6 ± 0.9**

MDA: malondialdehyde; SOD: superoxide dismutase; CAT: catalase; GSH: reduced glutathione. * $p < 0.05$ vs. Group A; ** $p < 0.001$ vs. Group A. All correlation are pearson r values $p < 0.001$ for all pairs.

3.3 Fetal Liver Inflammatory Cytokines and Oxidative Stress

Dose-dependent inflammatory and oxidative stress responses were observed in the fetal liver (Table 3). Compared to controls, Group C exhibited significant elevations ($p < 0.001$) in TNF-α (58.7 ± 5.4 vs. 12.4 ± 1.2 pg/mL) and IL-6 (72.4 ± 6.8 vs. 14.8 ± 1.4 pg/mL). Oxidative stress was confirmed by a marked increase in MDA (4.93 ± 0.46 vs. 1.24 ± 0.12 nmol/mg protein), alongside a significant depletion of antioxidants (SOD: 14.8 ± 1.4 vs. 38.4 ± 3.7 U/mg protein; CAT: 11.3 ± 1.1 vs. 33.2 ± 3.1 U/mg protein; GSH: 7.2 ± 0.7 vs. 22.6 ± 2.1 μmol/mg protein; $p < 0.001$).

Table 3: Mean ± SD, n = 10/group fetal liver parameters of inflammatory cytokines and parameters of oxidative stress.

Parameter	Group A (Control)	Group B (Low Dose)	Group C (High Dose)
Fetal TNF-α (pg/mL)	12.4 ± 1.2	31.6 ± 3.1*	58.7 ± 5.4**
Fetal IL-6 (pg/mL)	14.8 ± 1.4	38.2 ± 3.6*	72.4 ± 6.8**
MDA (nmol/mg protein)	1.24 ± 0.12	2.87 ± 0.27*	4.93 ± 0.46**
SOD (U/mg protein)	38.4 ± 3.7	24.6 ± 2.3*	14.8 ± 1.4**
CAT (U/mg protein)	33.2 ± 3.1	19.7 ± 1.9*	11.3 ± 1.1**
GSH (μmol/mg protein)	22.6 ± 2.1	13.4 ± 1.3*	7.2 ± 0.7**

MDA: malondialdehyde; SOD: superoxide dismutase; CAT: catalase; GSH: reduced glutathione IL-6: interleukin-6; TNF-α: tumor necrosis factor-alpha,. * $p < 0.05$ vs. Group A; ** $p < 0.001$ vs. Group A.

3.4 Fetal Morphometric Parameters

Quantitative morphometric showed dose-dependent growth restriction (Table 4). Compared to controls, Group C showed significant decreases ($p < 0.001$) in crown-rump length (19.8 ± 1.9 vs. 28.4 ± 2.6 mm), head width (5.9 ± 0.6 vs. 8.6 ± 0.8 mm), forelimb length (4.2 ± 0.4 vs. 6.8 ± 0.6 mm), and hindlimb length (4.6 ± 0.5 vs. 7.2 ± 0.7 mm).

Table 4 Fetal morphometric parameters (Mean ± SD, n fetuses measured per group ≥ 50).

Parameter	Group A (Control)	Group B (Low Dose)	Group C (High Dose)
Body weight (g)	3.42 ± 0.31	2.98 ± 0.28*	2.15 ± 0.22**
Crown-rump length (mm)	28.4 ± 2.6	24.7 ± 2.3*	19.8 ± 1.9**
Head width (mm)	8.6 ± 0.8	7.4 ± 0.7*	5.9 ± 0.6**
Forelimb length (mm)	6.8 ± 0.6	5.7 ± 0.5*	4.2 ± 0.4**
Hindlimb length (mm)	7.2 ± 0.7	6.1 ± 0.6*	4.6 ± 0.5**

* $p < 0.05$ vs. Group A; ** $p < 0.001$ vs. Group A.

3.5 Histological Examination of Fetal Liver

Hematoxylin and eosin-stained sections of fetal liver tissues at gestational day 20 indicated that there were varied dose-dependent histopathological alterations in the three experimental groups (Figures 1,2,3).

Group A (control) fetal hepatic architecture showed the anticipated developmental characteristics in line with late gestational liver morphology (Figure 1). A conspicuous central vein was found, with growing hepatocytes in the early cord-like structures. The hepatic cords had not yet become completely consolidated, as is typical of fetal stages, but the single hepatocytes had a normal cytoplasmic basophilia and homogeneous nuclear morphology. In any of the control sections examined, no degenerative changes, necrotic foci or inflammatory infiltration was observed.

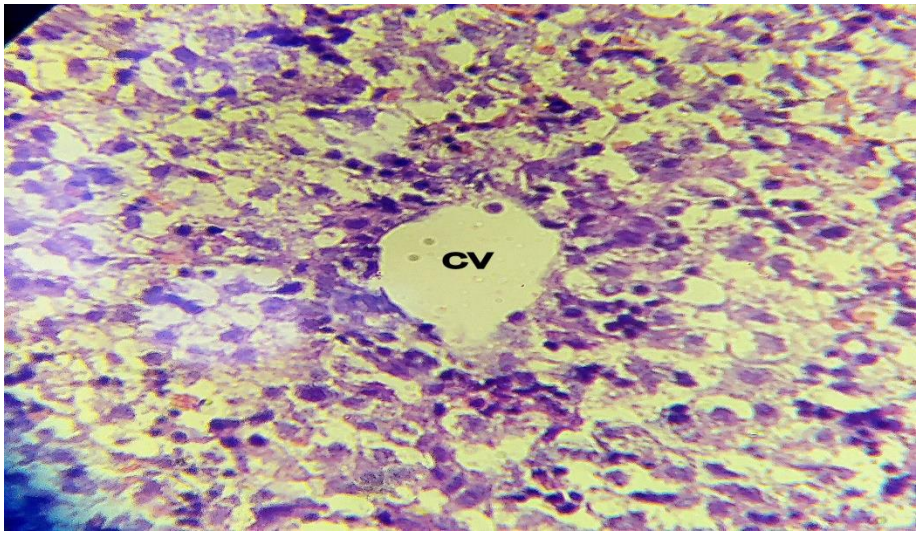

Figure 1 Haematoxylin and eosin-stained section of fetal liver tissues from Group A (control) at gestational day **20**, central vein (cv) (400×).

The fetal liver tissues in Group B (low-dose infection, 1×10^{3} CFU/mL) showed medium histopathological changes (Figure 2). The hepatocytes in the pericentral region were characterized by cytoplasmic Vacuolation and early necrotic alterations and some of them had pyknotic nuclei. Discrete areas of inflammatory cell infiltration in foci were noted in the parenchyma. The total lobular structure was semi-preserved, but there is definite indications of cell stress and initial degeneration compared to controls.

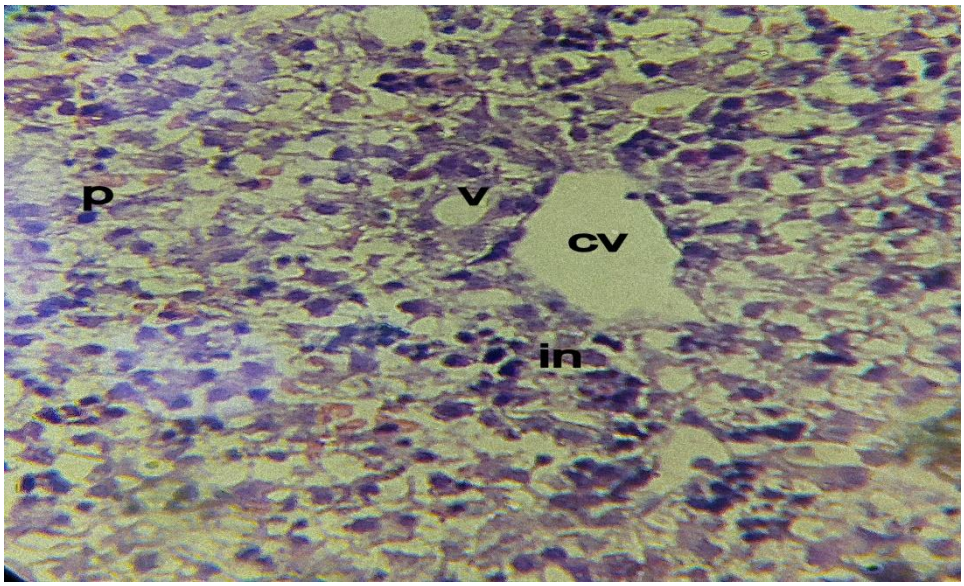

Figure 2 Haematoxylin and eosin-stained section of fetal liver tissues from Group B (low-dose infection) at gestational day **20**, central vein(cv), infiltration(in), vacuolations(v)(400×).

Group C (high-dose infection, 1×10^{5} CFU/mL) had a lot of and widespread histological damage in the fetal liver sections (Figure 3). Most hepatocytes were severely vacuolated with pyknosis of their nucleus, and overt necrosis, predominantly of most parenchymal areas, but not of discrete foci. There was a significant and diffuse inflammatory cell infiltration across the lobule. Sinusoidal spaces were crowded and architecture of central veins was interfered with to some degree. These results point to extensive hepatocellular damage that can be compared to the extent to which the entire mother was infected and supports the biochemical evidence of oxidative stress and cytokines indicated in Section 3.4.

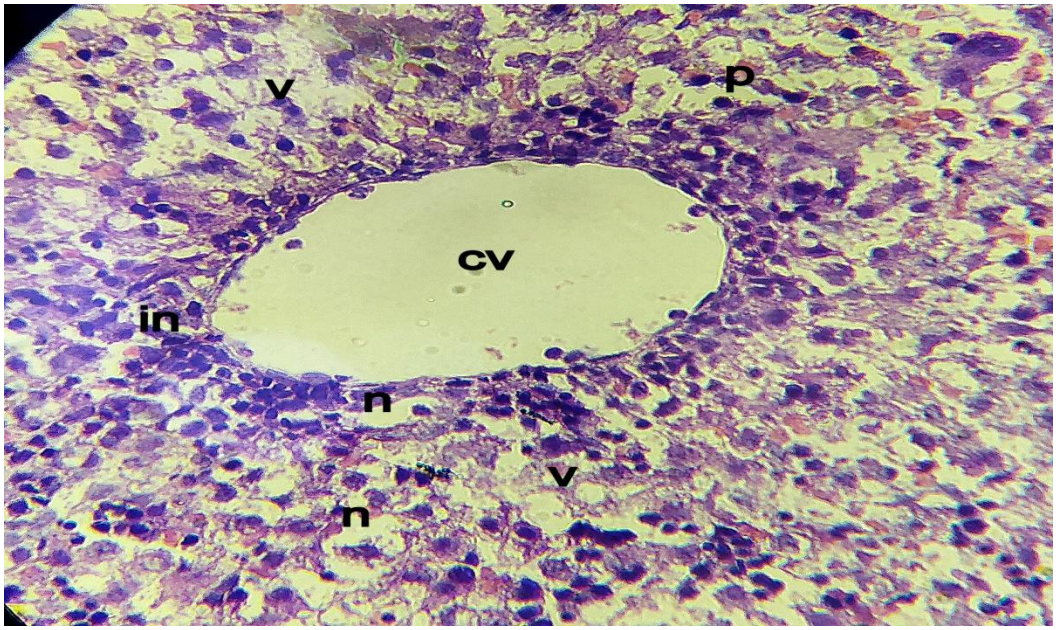

Figure 3 Haematoxylin and eosin-stained section of fetal liver tissues from Group C (high-dose infection) at gestational day 20, central vein(cv), infiltration(in), vacoulation)v), necrosis(n), (400×).

3.6 Correlation Analysis Between Inflammatory/Oxidative Markers and Fetal Outcomes

The Pearson correlation test on all the experimental results showed that there are strong correlations between the placental inflammatory and oxidative stress indicators and fetal outcome parameters (Table 5). A significant negative relationship was found between placental TNF-α level and fetal body weight ($r = -0.847$, $p = 0.001$) and viable fetuses per litter ($r = -0.823$, $p = 0.001$). Similarly, IL-6 was strongly negatively correlated with fetal body weight ($r = -0.831$, $p < 0.001$) and litter viability ($r = -0.809$, $p < 0.001$). Of all the oxidative stress markers, MDA had the most significant negative correlation with fetal body weight ($r = -0.862$, $p < 0.001$) and viable fetuses per litter ($r = -0.841$, $p < 0.001$), indicating lipid peroxidation as a crucial mediator of fetal injury. On the other hand, antioxidant indices GSH and SOD were positively related to both the fetal outcome indices. These statistics give mechanistic quantitative connections between placental molecular damage and subsequent fetal developmental impairment.

Table 5 Pearson correlation coefficient between placental inflammatory/oxidative measures and fetal outcome measures (all experimental measurements were pooled).

Parameter	r (Fetal Weight)	p-value	r (Viable Fetuses)	p-value
TNF-α (pg/mL)	-0.847	< 0.001	-0.823	< 0.001
IL-6 (pg/mL)	-0.831	< 0.001	-0.809	< 0.001
MDA (nmol/mg protein)	-0.862	< 0.001	-0.841	< 0.001
GSH (μmol/mg protein)	+0.793	< 0.001	+0.761	< 0.001
SOD (U/mg protein)	+0.811	< 0.001	+0.778	< 0.001

MDA: malondialdehyde; SOD: superoxide dismutase; GSH: reduced glutathione; IL-6: interleukin-6; TNF-α: tumor necrosis factor-alpha. All correlations are Pearson r values; $p < 0.001$ for all pairs.

4 Discussion

The results of the current study prove that maternal inoculation with *L. monocytogenes* on gestational day 7 causes a dose-dependent cascade of placental and systemic pathological alterations which eventually lead to fetus viability and development in the Wistar rat. These findings align with and build upon findings of earlier studies employing rodent models of listeriosis and present new quantitative data on the mechanistic interactions between bacterial infection, placental immunoinflammatory response, oxidative stress, and fetal morphometric outcomes.

The substantial increase in placental TNF- and IL-6 in infected groups indicates the initiation of pattern recognition receptor (PRR)-mediated innate immune signaling, mainly by Toll-like receptor 2 (TLR2) recognition of the components of *listeria* cell walls and NOD-like activation in response to the presence of intracellular bacteria [12]. When TNF-alpha binds to its respective receptor, TNFR1 on trophoblast cells, it triggers downstream NF-KB and MAPK signaling, which induces apoptosis and inhibits trophoblast invasion [13]. At the same time, IL-6 stimulates acute-phase reaction and enhances local inflammatory exaggeration. Similar increases in these cytokines have been reported in models of Group B streptococcal and Salmonella typhi placental infections [14], and highlight a shared immunopathological response across gram-positive placenta pathogens.

The simultaneous depletion of placental antioxidant enzymes (SOD, CAT) and GSH, and significant increase of MDA, suggest that the inflammatory process is closely intertwined with oxidative stress of L. monocytogenes placental pathology. Activated phagocytes produce superoxide anions, present in the placenta, which can be scavenged by SOD, but which otherwise lead to hydrogen peroxide formation and finally the formation of hydroxyl radicals through Fenton chemistry. Such an oxidative environment encourages lipid peroxidation of trophoblast membranes, protein carbonylation, and mitochondrial dysfunction in the electron transport chain, and all of these damage placental bioenergetic capacity [8;15]. Our results of negative associations between MDA and GSH levels are consistent with the earlier findings of oxidative stress in placental infections with Toxoplasma gondii and Brucella species [16].

The high decline in viable fetuses and the rise in resorption rates of infected groups support the overall association between placental listeriosis and poor gestational outcomes. Failure of the placental barrier, direct infection of the fetus through ascending chorioamnionitis, and cytokine-induced decidual vasoconstriction that decreases fetoplacental blood flow have been identified as some of the factors leading to intrauterine fetal death in listeriosis [3,6]. The impaired fetal growth manifested by shorter crown-rump length, lesser body weight, head and limb measurements in Group C probably indicates placental insufficiency as well as direct cytopathic damage to the fetal tissues. Similar symmetric intrauterine growth restriction is usually seen in situations where the insult is administered at a time when the organogenesis processes are underway, as in the current model where inoculation was done on GD 7, which is at the time when the rat is undergoing early placentation.

Along with histological evidence of hepatocyte necrosis and periportal inflammation, after intraperitoneal inoculation, the bacteria has a hematogenous spread and infects Kupffer cells in the hepatic sinusoids, evoking a granulomatous response. Soon neutrophil recruitment, followed by macrophage activation and subsequent bystander hepatocyte damage through cytokines, has consistently been linked to *listeria* hepatitis models in rodents [17,18]. The size of the hepatic damage in the current research was positively associated with the dose of bacterial inoculation, which supports the dose-response model of *L. monocytogenes* pathogenicity.

One of the most striking contributions of the given study is the formal correlation analysis with fetal outcomes in terms of placental molecular parameters. Such negative correlations of TNF-α with fetal body weight ($r = -0.847$) and MDA with fetal viability ($r = -0.841$) offer a quantitative mechanistic evidence of the fact that inflammatory-oxidative axis of fetal compromise is driven by TNF-α, which is essential and goes beyond the descriptive association that defined most of the previous experimental listeriosis In particular, that MDA was the most strongly inversely correlated with fetal outcome of all the measured markers, emphasizes lipid peroxidation as the closest molecular mediator of placental-fetal injury. It aligns with the vulnerability of the syncytiotrophoblast to oxidative membrane damage due to the large amount of polyunsaturated fatty acids and a relatively small amount of catalytic antioxidant reserves in the inflammatory environment [8;15] The positive associations between the antioxidant markers (GSH: 0.793; SOD: 0.811) and fetal weight also support the GSH depletion as a clinically relevant measure of pregnancy compromise, as has been previously described in translational literature of human preterm birth with maternal erythrocyte GSH levels inversely correlated with negative neonatal outcomes [19,20]. Taken together, these correlation data support the statistical framework which reinforces the mechanistic narrative of the study and can inform the choice of specific biomarkers to monitor placental health in the context of clinical listeriosis treatment.

Although the current study offers a multi-system data on experimental maternal listeriosis, there have been a number of limitations that can be seen. To begin with, the experiments were performed only in Wistar rats; the results might not be completely applicable to other rodent models and human pregnancy since the hemochorial or epitheliochorial placental type, immune tolerance threshold and relative exposure to *listeria* virulence factors differ between species. Second, the quantification of the bacterial loads in placental or fetal tissues was not conducted by counting of colony-forming units or quantitative PCR, which would have produced direct evidence of vertical transmission efficiency as well as allowed correlating the intraplacental bacterial load and concentrations of inflammatory mediators. Third, the molecular signaling pathways that stimulate cytokine production, namely, the activation state of NF- 0 B, MAPK and JAK-STAT pathways in trophoblast and decidual cells, were not targeted; their characterization would greatly enhance

mechanistic explanation of the inflammatory results. Fourth, the markers of apoptosis (cleaved caspase-3, Bax/Bcl-2 ratio, TUNEL-positive cells) were not evaluated in placental or fetal tissues, leaving the proportion between apoptotic and necrotic cell death to fetal injury unsolved. Fifth, immunohistochemistry and fluorescence in situ hybridization did not map bacterial localization in the individual placental compartments (labyrinthine zone, junctional zone, and decidua basalis), which would further clarify the spatial relationship between bacterial colonization and histopathological lesions. Sixth, hormonal variables such as progesterone and estradiol which are important mediators of placental immune homeostasis were not assessed and could have been disturbed by the infection. To construct a more comprehensive mechanistic model of pregnancy-associated listeriosis, future research ought to include quantitative bacteriology, immunofluorescent bacterial mapping, pathway-specific molecular analyses, and hormonal profiling.

5 Conclusion

The present paper shows that maternal infection of the *L. monocytogenes* in pregnant Wistar rats results in a dose-dependent syndrome of fetal pathologic abnormalities associated with high placental and liver levels of pro-inflammatory cytokines, intense oxidative stress with depletion of antioxidants, reduced fetal viability, and impaired fetal growth, The interaction of inflammatory and oxidative processes at the placental interface is at the core of the pathogenesis of adverse fetal outcomes in this model. These results corroborate the essential role of early obstetric screening of listeriosis especially in populations at risk of food insecurity or immunocompromised ones and give a mechanistic foundation to consider using specific anti-inflammatory and antioxidant therapeutic interventions to alleviate the outcomes of pregnancy-related listeriosis.

Compliance with ethical standards

Disclosure of conflict of interest

The author states that there is no conflict of interest.

Statement of ethical approval

The experimental procedures performed on animals were considered and approved by the University of Samarra Research Ethics Committee (Approval No. USC-2023-BIO-047)., Iraq and were followed according to the relevant ethical considerations.

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References

[1] Disson, O., & Lecuit, M. (2012). Targeting of the central nervous system by Listeria monocytogenes. Virulence, 3(2), 213–221. https://doi.org/10.4161/viru.19586

[2] Pouillot, R., Hoelzer, K., Chen, Y., & Dennis, S. B. (2012). Listeria monocytogenes dose response revisited—Incorporating adjustments for variability in strain virulence and host susceptibility. Risk Analysis, 32(1), 133–148. https://doi.org/10.1111/j.1539-6924.2011.01624.x

[3] Madjunkov, M., Chaudhry, S., & Ito, S. (2017). Listeriosis during pregnancy. Archives of Gynecology and Obstetrics, 296(2), 143–152. https://doi.org/10.1007/s00404-017-4401-1

[4] Vázquez-Boland, J. A., Kuhn, M., Berche, P., & Chakraborty, T. (2001). Listeria pathogenesis and molecular virulence determinants. Clinical Microbiology Reviews, 14(3), 584–640. https://doi.org/10.1128/CMR.14.3.584-640.2001

[5] Robbins, J. R., & Bakardjiev, A. I. (2012). Pathogens and the placental fortress. Current Opinion in Microbiology, 15(1), 36–43. https://doi.org/10.1016/j.mib.2011.11.006

[6] Ramos, G. L., & Nascimento, J. S. (2022). Listeriosis in pregnancy: A scoping review. Pathogens, 11(2), 233. https://doi.org/10.3390/pathogens11020233

[7] Yockey, L. J., & Iwasaki, A. (2018). Interferons and proinflammatory cytokines in pregnancy and fetal development. Immunity, 49(3), 397–412. https://doi.org/10.1016/j.immuni.2018.07.017

[8] Burton, G. J., Redman, C. W., Roberts, J. M., & Moffett, A. (2017). Pre-eclampsia: pathophysiology and clinical implications. The BMJ, 358, j3426. https://doi.org/10.1136/bmj.j3426

[9] Bakardjiev, A. I., Stacy, B. A., & Bhatt, D. L. (2004). Listeriosis in the pregnant guinea pig: A model of vertical transmission. Infection and Immunity, 72(1), 489–497. https://doi.org/10.1128/IAI.72.1.489-497.2004

[10] Hamon, M. A., Reglier-Poupet, H., Milon, G., & Cossart, P. (2004). Experimental listeriosis during pregnancy in mice. Microbial Pathogenesis, 37(6), 303–312. https://doi.org/10.1016/j.micpath.2004.09.005

[11] Mohammed, A. J., Salim, H. K., & Khaleel, A. K. (2022). Placental and hepatic oxidative stress responses in female Wistar rats experimentally infected with gram-positive intracellular bacteria. Al-Qadisiyah Journal of Veterinary Medicine Sciences, 21(1), 38–47. https://doi.org/10.29079/vol21iss1art1186

[12] Yoshikawa, M., Uchida, E., & Nakamura, Y. (2018). TLR2-mediated innate immune response to Listeria monocytogenes in placental trophoblast cells: Role of NF-κB activation and downstream cytokine production. Innate Immunity, 24(5), 298–308. https://doi.org/10.1177/1753425918777680

[13] Renaud, S. J., Sullivan, R., & Bhatt, D. L. (2020). TNF-α-mediated disruption of trophoblast invasion: Mechanisms and placentation consequences. Frontiers in Cell and Developmental Biology, 8, 583842. https://doi.org/10.3389/fcell.2020.583842

[14] Ilhan, Z., Güven, A., & Solmaz, H. (2021). Cytokine profiling in experimental placental Salmonella infection: Implications for adverse gestational outcomes. Comparative Immunology, Microbiology and Infectious Diseases, 74, 101574. https://doi.org/10.1016/j.cimid.2020.101574

[15] Sultana, M., Islam, R., Hasan, M. Z., & Rahman, M. S. (2023). Inflammatory cytokines and oxidative stress in placental dysfunction: A comprehensive review. Reproductive Biology and Endocrinology, 21(1), 38. https://doi.org/10.1186/s12958-023-01085-4

[16] Ahmed, M. M., Al-Saffar, A. A., & Hussain, H. T. (2022). Oxidative stress and anti-oxidant enzyme activity in placental tissue of ewes experimentally infected with Toxoplasma gondii. Iraqi Journal of Veterinary Sciences, 36(1), 215–222. https://doi.org/10.33899/ijvs.2021.130259.1827

[17] Cossart, P., & Helenius, A. (2014). Endocytosis of viruses and bacteria. Cold Spring Harbor Perspectives in Biology, 6(8), a016972. https://doi.org/10.1101/cshperspect.a016972

[18] Rai, P., Janardhan, K. S., & Bhatt, J. (2021). Listeria monocytogenes hepatic granuloma formation and the contribution of resident macrophage activation. Infection and Immunity, 89(4), e00749-20. https://doi.org/10.1128/IAI.00749-20

[19] Majeed, Z. H., Hasan, S. A., & Ismail, R. M. (2023). Induction of kidney lesions in rats by Streptococcus pyogenes and evaluate the herbal component activity of Symphytum officinale extract. Annals of Biology 39 (2) : 225-228, 2023

[20] Nazmul, H., Rashid, M., & Rahman, M. A. (2021). Glutathione and the antioxidant system in pregnancy-associated infections: Mechanisms and therapeutic targets. Antioxidants, 10(8), 1239. https://doi.org/10.3390/antiox10081239