Asian Journal of Health Research
Journal Homepage: https://a-jhr.com
Published by Ikatan Dokter Indonesia Wilayah Jawa Timur

Original Research

Effectiveness of Lipopolysaccharide (LPS) Induction in Wistar Rats as a Low Birth Weight (LBW) Model

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ARTICLE HISTORY
Received: 20 September 2025
Revised: 21 October 2025
Accepted: 23 November 2025

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KEYWORDS
IL-6; Lipopolysaccharide; Low Birth Weight; TNF-α; Wistar Rats

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ABSTRACT

Introduction: Low birth weight (LBW) is linked to stunting, neurodevelopmental delays, and neonatal mortality of up to 80%, with risk factors including maternal infection, poor nutrition, gestational age, obstetric history, and socioeconomic conditions. This study assessed the effectiveness of lipopolysaccharide (LPS) induction in Wistar rats as an LBW model, given LPS's ability to mimic maternal infection and induce a controlled inflammatory response, while addressing the lack of updated data on inflammatory mediators such as VEGF, TNF-α, and IL-6 in LPS-induced LBW.

Material and Methods: Four groups of Wistar rats were used in an in vivo true experimental post-test only design. LPS injection as the independent variable, and the dependent variables include TNF-α and IL-6 levels in the mother's serum and amniotic fluid as determined by ELISA, fetal weight, mortality, crown-rump length, and tail length. Distribution-appropriate statistical tests were used to assess the data, and a p-value of less than 0.05 was considered significant.

Results: Administration of LPS (50–100 μg/kg) significantly reduced fetal weight ($p=0.04$) and increased mortality. The fetal weight decreased by 57.4% in LPS100, and 32 deaths occurred compared to the control group. Crown-rump length (CRL) also significantly decreased at doses of 75 and 100 μg/kg ($p<0.05$), while tail length showed no significant difference ($p>0.01$). Additionally, LPS increased IL-6 and TNF-α levels compared to the control ($p<0.05$), with significant increases in the LPS75 and LPS100 groups compared to LPS50 ($p<0.001$).

Conclusion: LPS induction in Wistar rats decreases birth weight, CRL, and increases fetal mortality, IL-6, and TNF-α, but does not affect fetal tail length.

Cite this as: Patridina G., Prasetyorini N. (2025) Effectiveness of Lipopolysaccharide (LPS) Induction in Wistar Rats as a Low Birth Weight (LBW) Model. *Asian J Heal Res.* 4 (3): 26–35. doi: https://doi.org/10.55561/ajhr.v4i3.278

INTRODUCTION

Up to 80% of cases of stunting, neurodevelopmental problems, and neonatal mortality are caused by low birth weight (LBW) of less than 2,500 g [1]. Every year, nearly 20 million newborns worldwide are affected by low birth weight (LBW) events. LBW has a global prevalence of 15.5%, with roughly 20.6 million kids born each year, 96.5% of whom live in underdeveloped countries. The average rate is 6.2%, per Riskesdas 2018. South-Central Asia has the highest incidence (27.1%), whereas Europe has the lowest (6.4%) [2]. In 2019, Indonesia's newborn mortality rate (NMR) hit 60,000, ranking it in the top 10 nations with the highest NMR globally [3].

Risk factors for adverse pregnancy outcomes include maternal infection, nutritional status, BMI, gestational age, obstetric history, and socioeconomic conditions [4–6]. Inflammation is a natural response to tissue damage, but infection during pregnancy can cause abnormal inflammation, increasing the risk of preterm birth, poor maternal outcomes, and neonatal complications such as low birth weight [7,8]. Gram-negative bacterial cell walls contain a poisonous substance called lipopolysaccharide (LPS), which can cause these consequences. Preterm birth and low birth weight are

more likely to occur in pregnant women exposed to LPS, particularly those who have bacterial vaginosis. Between 25-40% of these instances are caused by intrauterine infections. Late-stage LPS exposure results in preterm birth, death, and fetal growth limitation in animal models. Pro-inflammatory cytokines like TNF-α and IL-6 are induced by LPS and harm fetal development. LPS exposure can cause fetal mortality, growth limitation, or embryonic resorption, depending on when it occurs [9,10].

In 1964, Wigglesworth created the first animal model of low birth weight (LBW) by ligating the uterine arteries of rodents. Since then, LBW models have developed further, most frequently employing mice (10%) and rats (60%), then sheep, pigs, guinea pigs, monkeys, and chicken embryos. The choice of species has a significant impact on study results and model appropriateness. Because of their low cost, ease of upkeep, genetic manipulation, and short gestation, which allows for quicker research, rats and mice are often used [11].

The use of LPS in rat will result in a condition resembling maternal infection. LPS induction causes the production of several pro-inflammatory cytokines, including TNF-α and interleukin-6, which are associated with impaired fetal development [12]. Pregnant rats exposed to LPS during the second trimester may experience fetal mortality and an early delivery, whereas pregnant rats exposed to LPS during the first trimester had embryonic resorption and fetal death. Pregnant rats exposed to LPS during the third trimester experienced early delivery, fetal growth limitation, fetal mortality, and delayed bone formation [13].

Due to its ability to replicate the symptoms of a systemic infection in pregnant women, LPS injection was selected. This ensures that LPS penetrates the bloodstream and causes an inflammatory response that is comparable to those found in natural cases that result in LBW. Additionally, the injection technique makes it possible to consistently regulate dosage and exposure duration to produce animal models with quantifiable and repeatable outcomes [14-16].

Although LPS has been widely used to induce inflammatory conditions during pregnancy, the effectiveness of LPS induction in Wistar rats as a representative model for LBW has not been extensively studied. Research on LPS induction in Wistar rats as a model for low birth weight (LBW) primarily stems from older studies and has not been updated with current data. Additionally, it is yet unclear how inflammatory mediators, including VEGF, TNF-α, and IL-6, contribute to the development of LBW brought on by LPS induction. Our study contributed that using LBW models based on inflammatory induction (LPS), which mimic maternal infection conditions in humans, a mechanism different from nutritional, hypoxia, or uterine ligation models. This model allows for the evaluation of the effects of anti-inflammatory interventions on fetal growth restriction induced by inflammation.

MATERIAL AND METHODS

Research Design

This research uses a true experimental post-test only research design conducted in vivo. The sample population was taken from the Wistar rat population. In this study, four treatment groups were used, consisting of a control group that did not receive LPS induction, and three treatment groups that each received intraperitoneal LPS injections at doses of 50 µg/kg, 75 µg/kg, and 100 µg/kg on gestational days 15, 16, and 17. The dosage determination is based on a human dose of 500 mg, which is then converted to a rat dose using a conversion factor of 0.018, resulting in an equivalent dose of 9 mg [17]. The LPS injection dose was determined based on previous research by Zhao (2013), which showed that daily administration of low-dose LPS (75 µg/kg, i.p.) on days 15 to 17 resulted in IUGR pups. Pregnant dams were given bacterial endotoxin on gestational day 15, which is about equivalent to the second trimester of human pregnancy [16].

Sample Collection

Wistar rats aged 8–10 weeks, with male rats weighing between 30-32 grams and female rats weighing between 26-28 grams, were used in this study. The Federer *formula* $(p-1)(n-1) \geq 15$ was used to calculate the sample size for this investigation, where p is the number of treatment groups, and n is the number of repetitions or experimental animals per group [18]. This formula is used because it is suitable for experimental research with a simple random design and a limited number of subjects, such as in animal studies.

Based on this formula, with four groups, a minimum of 6 rats are needed for each treatment group. Thus, the total number of experimental animals used in a single experiment is 24 rats. The rats were then coded and randomized using stratified randomization techniques. The sample included measures of fetal weight, mortality, crown-rump length, and tail length in addition to blood serum for IL-6 level assessment. LPS injection at different doses is the independent variable, whereas IL-6 levels and fetal development measures are the dependent variables.

For one week prior to the start of the study, experimental rats were kept in a controlled environment with a 12-hour light/dark cycle, a temperature of 20–25°C, and a humidity of 50±5%. They were also allowed

unrestricted access to food and water. In order to facilitate reproduction, two male and six female rats were housed in the same cage beginning at 9:00 PM. At 7:00 AM, the rats were examined for the presence of a vaginal plug, a sign of day 0 of gestation [16]. In this study, there were 24 Wistar rat dams and 149 fetuses overall.

Intervention

Fetal Weight, Mortality, Crown-Rump Length, and Tail Length

In the experiment to determine fetal weight, fetal mortality, crown-rump length, and fetal tail length, rats in treatment group 1 were injected intraperitoneally with LPS at a dose of 100 µg/kg on gestational days 15, 16, and 17, then sacrificed on gestational day 18 to measure these parameters.

IL-6 Levels

IL-6 is a pro-inflammatory cytokine mediator gene expression. IL-6 levels were found to be higher in maternal serum. IL-6 can be detected at increased levels, making it a potential inflammatory marker. In the experiment to determine IL-6 levels, rats were injected intraperitoneally with LPS at a dose of 100 µg/kg on gestational day 15 and sacrificed 6 hours after injection. IL-6 levels were measured in maternal serum and amniotic fluid using an ELISA Kit (Cat. No. MDBR0015; MedikBio, INA). IL-6 concentrations are measured in pg/mL.

TNF-α Levels

TNF-α is the gene expression of the pro-inflammatory cytokine mediator. TNF-α levels were found to be higher in maternal serum. TNF-α can be detected at increased levels, making it a potential inflammatory marker. The same procedure was also applied to the TNF-α level measurement experiment, where rats were intraperitoneally injected with LPS at a dose of 100 µg/kg on gestational day 15 and sacrificed 6 hours later, with TNF-α levels measured using an ELISA Kit (Cat. No. MDBR2856; MedikBio, INA). TNF-α concentrations are measured in pg/mL.

Data Analysis

Data will be expressed in the form of mean ± standard deviation. Binomial data will be analyzed using the Chi-square test or Fisher's test if needed. Normality analysis of numerical data is performed using the Shapiro-Wilk test if the number of data points is below 50, and using the Kolmogorov-Smirnov test if the number of data points is above 50. The Independent T-test will be used if the numerical data has a normal distribution, and the Mann-Whitney test will be used if the numerical data has an abnormal distribution. The result will be considered significant if it reaches a p-value of < 0.05. Statistical analysis will be performed using the IBM SPSS version 24 application.

Ethics

All procedures were carried out in the Physiology Laboratory and the Experimental Animal Laboratory of the Faculty of Medicine, Brawijaya University, in 2024 after being approved by the Ethics Committee of the Faculty of Medicine.

RESULTS

Statistical Analysis

Fetal Weight, Mortality Distribution, Average CRL, and Average Tail Length of Rat

Table 1 presents the data characteristics for the control and LPS-treated groups, including the number of pups (n), average fetal body weight (g), tail length (cm), crown-rump length (cm), and the number of dead pups (n). Given that the distribution of rat fetal weight data was non-normal, the Kruskal-Wallis and Mann-Whitney tests were performed. Fetal weight decreases dose-dependently when LPS is administered. The mean

Table 1. Data Characteristic shown in Mean ± SD

Group	Total Pups (n, Mean ± SD)	Fetal Weight (g, Mean ± SD)	Tail Length (cm, Mean ± SD)	CRL (cm, Mean ± SD)	Dead Pups (n, Mean ± SD)
K-	10.0±0.6	6.8±0.3	2.3±0.4	6.6±0.4	0.0±0.0
LPS50	2.8±1.5	4.0±0.4	2.1±0.6	6.4±0.4	0.5±0.6
LPS75	1.8±1.2	3.1±0.2	2.3±0.2	5.1±0.4	3.3±1.2
LPS100	1.0±0.6	2.9±0.4	1.5±0.3	3.3±0.3	5.3±2.2

Total pups (n), average fetal body weight (g), tail length (cm), crown rump length (cm), and dead pups (n) in control and LPS-treated groups. Data are presented as mean ± SD.
Total sample in negative control group (K-) (n = 6), LPS50 (n = 6), LPS75 (n = 6), LPS100 (n = 6).

Table 2. Statistic Analysis of Low Birth Weight Parameter

Group	Fetal Weight (g, Mean ± SD)	Effect size (Cohen's d)	Interpretation	95% CI (Mean Difference)
K-	6.8±0.3	-	-	6.50 – 7.10
LPS50	4.0±0.4	7.91	Very Large Effect	3.55 – 4.35
LPS75	3.1±0.2	14.51	Very Large Effect	2.87 – 3.33
LPS100	2.9±0.4	11.02	Very Large Effect	2.50 – 3.30
Group	**Crown Rump Length (cm, Mean ± SD)**	**Effect size (Cohen's d)**	**Interpretation**	**95% CI (Mean Difference)**
K-	6.6±0.4	-	-	6.17 – 7.10
LPS50	6.4±0.4	0.50	Moderate Effect	5.97 – 6.80
LPS75	5.1±0.4	3.75	Very Large Effect	4.65 – 5.48
LPS100	3.3±0.3	9.32	Very Large Effect	3.00 – 3.58

Fetal Weight and Crown Rump Length data are presented as mean ± SD.
Effect size was calculated using Cohen's d to assess the magnitude of the difference between groups.
The confidence interval (95% CI) indicates the range of estimated mean difference between groups.

fetal weights in the LPS50, LPS75, and LPS100 groups were 4.0 ± 0.4 g, 3.1 ± 0.2 g, and 2.9 ± 0.4 g, respectively, in contrast to the control group (6.8 ± 0.3 g). In comparison to the control group, these figures indicate a decrease of 41.2%, 54.4%, and 57.4% ($p < 0.01$).

The LPS100 group had the greatest number of deaths (32 rats), indicating that LPS treatment enhanced fetal mortality. There were no deaths in the K-group, while there were three and twenty deaths in the LPS50 and LPS75 groups, respectively. LPS treatment decreased the CRL of rat pups, according to the rat's average CRL and tail length results. One-Way ANOVA and Post Hoc LSD analyses were conducted because the CRL data in this study were normally distributed. All groups had significantly different CRLs, according to the One-Way ANOVA analysis results ($p<0.001$).

When compared to K- and LPS50, administration of LPS75 and LPS100 dramatically decreased CRL ($p<0.05$). Rats administered LPS50 and K- did not differ significantly ($p=0.276$). Rat's CRL was considerably lower when the LPS dosage was increased ($p<0.05$). Since the rat's tail length data distribution was non-normal, the Mann-Whitney and Kruskal-Wallis tests were conducted. There was no discernible difference, according to the Kruskal-Wallis test results ($p>0.01$).

Table 2 shows the results of statistical analysis of effect size (Cohen's d) and confidence interval for fetal weight and CRL length variables. It was found that LPS administration induced a dose-dependent decrease in fetal weight and CRL. The effect size was very large in all experimental groups (Cohen's d = 7.91–14.51), indicating a strong biological impact of maternal inflammation on fetal growth. The 95% confidence interval for the LPS-treated group did not overlap with the control group, confirming a statistically significant difference. In CRL measurements, the LPS50 group showed moderate reduction (d Cohen = 0.5, 95% CI overlap), while the LPS75 and LPS100 groups had very large effect sizes (d = 3.75 and 9.32, respectively) with non-overlapping confidence intervals, indicating statistically and biologically significant fetal growth restriction.

IL-6 Levels Comparison

The average IL-6 values are shown in Fig.1. The figure indicates that giving LPS causes IL-6 levels to rise. Since the IL-6 data in this study were normally distributed, one-way ANOVA and post-hoc LSD analysis were used. The One-Way ANOVA analysis revealed that the levels of IL-6 varied considerably across all groups ($p<0.001$). The LPS treatment significantly increased IL-6 levels ($p<0.05$) in comparison to the control group. Upon increasing the dose, rats in the LPS75 and LPS100 groups exhibited greater levels of IL-6 compared to the LPS50 group ($p<0.001$). IL-6 levels in rats given LPS75 and LPS100 did not differ substantially ($p=0.071$).

TNF-α Levels Comparison

Fig.2 displays the average TNF-α levels. The picture demonstrates how administering LPS raises TNF-α levels. One-Way ANOVA and Post Hoc LSD analysis were used because the TNF-α data in this study were normally distributed. TNF-α levels in each group varied significantly, according to the One-Way ANOVA analysis ($p<0.001$). TNF-α levels were significantly elevated by LPS treatment in comparison to the control group ($p<0.05$). TNF-α levels in the LPS75 and LPS100 groups were higher than those in the LPS50 group when

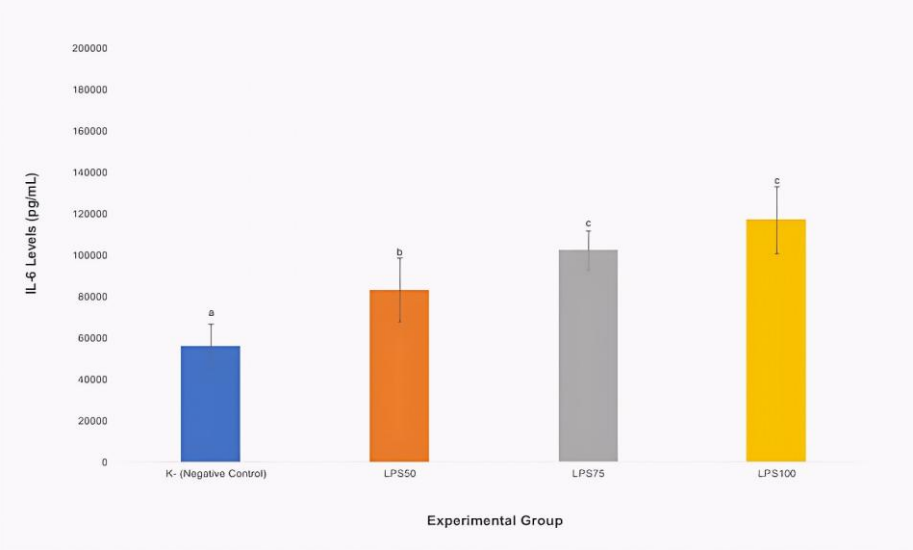


Fig.1. Average IL-6 Levels in Rats

Note: (a,b,c) different notations indicate significant differences.
Total sample in negative control group (K-) (n = 6), LPS50 (n = 6), LPS75 (n = 6), LPS100 (n = 6).

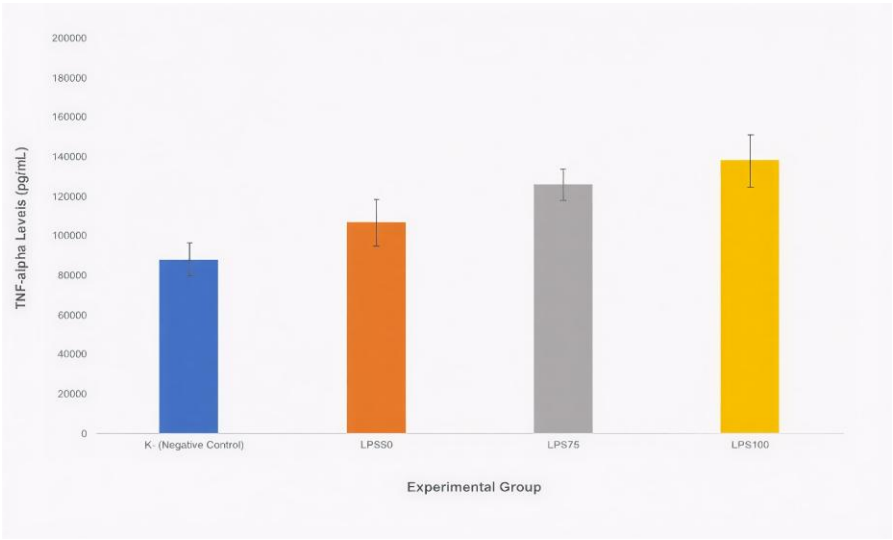


Fig.2. Average TNF-α Levels in Rats

Note: (a,b,c) different notations indicate significant differences.
Total sample in negative control group (K-) (n = 6), LPS50 (n = 6), LPS75 (n = 6), LPS100 (n = 6).

the dose was increased ($p<0.001$). Rats administered LPS75 and LPS100 did not significantly differ in their levels of IL-6 ($p=0.066$).

DISCUSSION

Data Characteristic

This study evaluated fetal weight (FW), fetal length, crown-rump length (CRL), and the number of dead pups in Wistar rats divided into four groups: control (K-), LPS50, LPS75, and LPS100. The results showed that FW, fetal length, and CRL decreased with increasing LPS dose, with the most significant decrease in the LPS100 group. Conversely, the number of dead pups increased in the treatment groups, with the highest number in the LPS100 group. This is in line with other studies that shown that LPS induction can cause Intrauterine Growth Restriction (IUGR) by means of inflammatory mechanisms that impair placental blood

flow and the fetus's ability to receive nutrients, which causes growth abnormalities and raises fetal mortality [19].

The Kolmogorov-Smirnov test was used to ascertain the distribution of the data. According to the analysis's findings, the data is normally distributed because the p-values for the IL-6 and CRL variables are larger than 0.05. The data is not normally distributed, as seen by the p-value for the fetal weight and tail length being less than 0.05. This approach was chosen in accordance with statistical guidelines that suggest non-parametric tests are better suited for small samples with non-normal distributions [20].

Effectiveness of Lipopolysaccharide (LPS) Induction as an Low Birth Weight Model

Lipopolysaccharide (LPS) induction is widely used in research as a model for IUGR. LPS is an endotoxin from gram-negative bacteria that triggers a systemic inflammatory response. LPS exposure during pregnancy boosts the mother's immune system and causes an increase in the cytokine IL-6, which increases inflammation. These cytokines restrict growth and even induce IUGR by disrupting placental blood flow and reducing the fetus's nutritional supply [21]. Beside LPS, several other methods are used to induce IUGR in experimental animals. Dietary restriction reduces maternal nutrient intake, thus mimicking malnutrition during pregnancy, but it is difficult to control due to individual differences in response [22].

Hypoxia exposure reduces the inhaled oxygen levels of the mother rats to simulate hypoxic conditions, but the effects are not limited to the placenta; they can also affect other organ systems [23]. Uterine artery ligation restricts blood flow to the placenta to mimic vascular disruption, but this procedure is invasive and requires high surgical skill [24]. Some studies have shown the effectiveness of LPS in inducing IUGR. Chen et al. (2017) reported that administering LPS to pregnant rats resulted in decreased fetal weight and crown-rump length [25]. Jeong et al. (2023) also found that LPS increased IL-6 levels and caused placental developmental disturbances, contributing to IUGR [26].

The Influence of LPS Induction on the Birth Weight of Rat Pups

This study analyzes the effect of LPS induction on the birth weight of newborn rats as a model for low birth weight (LBW). The Kruskal-Wallis test results showed a significant difference ($p < 0.05$) between groups, with the LPS75 and LPS100 groups experiencing a significant decrease in birth weight compared to the control group ($p < 0.05$). Conversely, the LPS50 group showed no significant difference, indicating that the effect of LPS is dose-dependent [27].

The main mechanism leading to low birth weight is the activation of the maternal immune system, which increases TNF-α and IL-6, causing placental dysfunction and oxidative stress. This hinders the transfer of nutrients and oxygen to the fetus, thus disrupting growth [28]. Additionally, the decrease in VEGF expression due to inflammation impacts placental vascularization, worsening IUGR [29]. These results confirm that LPS is effective as an inflammation-induced LBW model and can be used to understand the mechanisms of fetal growth restriction due to intrauterine infection. However, this model does not fully represent other factors such as nutritional deficiencies or maternal metabolic disorders, so it needs to be combined with other models in LBW studies in humans [24].

The Effect of Lipopolysaccharide (LPS) Induction on Fetal Death

The effect of LPS induction on rat fetal death was analyzed by comparing the mortality rate between treatment groups. The results showed that increasing the LPS dose was directly proportional to the fetal mortality rate, with the LPS75 and LPS100 groups experiencing a significant increase compared to the control group ($p < 0.05$). This indicates that high-dose LPS induction causes more severe fetal developmental disturbances, thereby increasing the likelihood of intrauterine death [24]. The main process via which LPS causes fetal death is linked to the activation of pro-inflammatory cytokines, like TNF-α and IL-6, which are markedly elevated after exposure to LPS. This increase in cytokines can cause placental dysfunction, impaired blood flow to the fetus, and hypoxia, ultimately contributing to fetal death [10]. Other studies also show that LPS exposure triggers oxidative stress and apoptosis of placental cells, further worsening fetal conditions [30].

Additionally, previous research has reported that exposure to LPS during pregnancy not only increases fetal mortality but also inhibits fetal brain development, which can lead to fatal neurological disorders [30]. Therefore, the results of this study confirm that LPS induction as a model for LBW must consider the optimal dose to avoid excessive fetal death, thus still allowing for effective evaluation of fetal growth disorders.

The Influence of LPS Induction on Crown-Rump Length (CRL) and Tail Length

High doses of LPS (LPS75 and LPS100) significantly reduced fetal crown-rump length (CRL) and tail length compared to the control group, while LPS50 showed no significant difference. This indicates that the effect of LPS on fetal growth is dose-dependent,

with higher doses leading to more severe growth disturbances. The main mechanism behind the decrease in CRL and tail length is related to maternal inflammation induced by LPS, which triggers an increase in TNF-α and IL-6. These pro-inflammatory cytokines disrupt placental function, reduce oxygen and nutrient supply to the fetus, and increase oxidative stress, which inhibits fetal linear growth [28].

Although tail length was measured in this study and showed non-significant differences, the literature indicates that this measure is often less sensitive as a marker of stunted growth or low birth weight. For example, in mice with prenatal/postnatal stress exposure, nose-tail length did not differ significantly between groups, although body weight and bone length showed changes [31].

The Effect of LPS Induction on IL-6 and TNF-α Levels

Administration of lipopolysaccharide (LPS) significantly increased interleukin-6 (IL-6) levels compared to the control group, with the magnitude of increase being proportional to the dose administered. This result aligns with previous studies showing that LPS stimulates IL-6 production in various animal models, which is associated with inflammation-mediated fetal developmental disturbances [32]. IL-6 is a key pro-inflammatory cytokine involved in the inflammatory mechanism induced by LPS through the activation of Toll-like receptor 4 (TLR4). Once LPS binds to TLR4 on macrophages and other immune cells, it triggers intracellular signaling pathways, primarily the nuclear factor-κB (NF-κB) and mitogen-activated protein kinase (MAPK) pathways, which promote the transcription of pro-inflammatory cytokine genes, including IL-6 and tumor necrosis factor-alpha (TNF-α). [33].

Elevated IL-6 plays a central role in the inflammatory cascade by promoting further cytokine release and amplifying placental inflammation. Persistent elevation of IL-6 can impair placental function by disrupting nutrient and oxygen exchange between the mother and fetus, resulting in fetal hypoxia, malnutrition, and ultimately low birth weight (LBW) [33]. In addition, excessive IL-6 induced by LPS exposure has been reported to interfere with the expression of placental growth factors, such as vascular endothelial growth factor (VEGF), which is crucial for fetal vascularization and perfusion [24]. The correlation between increased IL-6 levels and the severity of intrauterine growth restriction (IUGR) has been consistently observed, suggesting that prolonged inflammation during pregnancy contributes to impaired fetal growth and organ development. Therefore, IL-6 serves as a useful biomarker for identifying and monitoring inflammation-induced IUGR, enabling earlier and more targeted therapeutic interventions [28].

Consistent with the IL-6 findings, intraperitoneal administration of LPS in Wistar rats also significantly increased TNF-α levels compared to the control group, indicating the activation of a robust systemic inflammatory response. TNF-α is another major pro-inflammatory cytokine that mediates several downstream effects of LPS. Biologically, LPS acts as a primary activator of TLR4, leading to NF-κB translocation into the nucleus following phosphorylation and degradation of its inhibitor, I-κB. NF-κB activation induces the transcription of TNF-α, IL-6, and other inflammatory mediators such as prostaglandins and reactive oxygen species (ROS). This cascade amplifies systemic inflammation and contributes to placental injury [16].

The overproduction of TNF-α further aggravates placental dysfunction by impairing uteroplacental blood flow, increasing oxidative stress, and reducing angiogenic activity. These changes compromise the placental exchange of nutrients and oxygen, leading to fetal growth restriction and increased risk of fetal mortality [34, 35]. Moreover, both TNF-α and ROS can suppress VEGF expression, which is vital for placental angiogenesis and normal vascular development. Disruption of VEGF signaling results in reduced placental perfusion and endothelial dysfunction, thereby exacerbating fetal hypoxia and contributing to LBW [36].

The activation of NF-κB plays a pivotal role in these processes. Under unstimulated conditions, NF-κB remains in the cytoplasm bound to I-κB. Upon LPS exposure, I-κB undergoes phosphorylation and degradation, allowing NF-κB to translocate to the nucleus, where it upregulates the transcription of IL-6, TNF-α, and cyclooxygenase-2 (COX-2) [16]. demonstrated that folic acid (FA) could counteract this mechanism: FA supplementation inhibited LPS-induced NF-κB activation by reducing I-κB phosphorylation and COX-2 expression in the placenta, thereby protecting against preterm birth, fetal death, and IUGR. Immunohistochemical analysis further revealed that NF-κB nuclear translocation occurred primarily in trophoblast giant cells of the labyrinth zone, a key site of maternal-fetal exchange.

Previous research supports these observations. Riddle et al. (2014) reported that LBW in rats was associated with increased systemic and tissue TNF-α expression and activation of the endoplasmic reticulum stress response, potentially disrupting fetal metabolism and development [37]. Similarly, Walker et al. (2010) found that neonatal LPS exposure increased TNF-α and altered long-term neuroendocrine stress responses via pro-inflammatory cytokine mediation [38]. Together, these findings indicate that IL-6 and TNF-α are central mediators in LPS-induced inflammation, initiating

molecular pathways that impair placental function and fetal development.

In summary, LPS induction activates the TLR4–NF-κB signaling pathway, leading to elevated levels of IL-6 and TNF-α and triggering both systemic and placental inflammation. This inflammatory cascade disrupts nutrient and oxygen transfer to the fetus, suppresses angiogenesis through decreased VEGF expression, and ultimately results in fetal growth restriction. Various maternal interventions have been developed to induce FGR in animal models, each targeting different physiological mechanisms. The earliest approaches focused on physically altering uterine circulation to reduce the transport of maternal nutrients and oxygen to the placenta. Procedures such as uterine artery ligation in mice, guinea pigs, and sheep, as well as radial artery diathermy and uterine artery embolization, have been shown to be effective in producing FGR. However, because these interventions disrupt the natural uteroplacental blood flow, they are less suitable for evaluating maternal therapies aimed at improving uterine perfusion or placental barrier function.

In addition to this local intervention, global maternal manipulation can also induce FGR. Malnutrition in mothers, such as severe calorie restriction similar to conditions seen during the Dutch famine, leads to smaller fetuses with asymmetrical growth patterns in species including mice, guinea pigs, rabbits, and sheep. Interestingly, excessive nutrition in the mother, such as overfeeding young pregnant ewes, can also limit fetal growth because the mother's development is prioritized over the needs of the fetus. Similarly, certain nutritional deficiencies, including low-protein or low-sodium diets, have been shown to impair fetal growth without overall calorie deficiency. Environmental factors also play an important role; for example, exposure to heat stress can reduce uterine and umbilical blood flow, leading to the typical brain-sparing effect seen in FGR. Similarly, exposure to hypoxia, whether thru high-altitude pregnancies or hypoxic conditions, can also cause FGR by reducing maternal and placental oxygen availability. The severity and characteristics of the resulting growth restriction depend on the species used and the duration of exposure, with innate adaptations to hypoxia (such as in high-altitude animals) providing some protection thru enhanced antioxidant mechanisms [39].

Since the induction involves lipopolysaccharide (LPS), which specifically stimulates the maternal inflammatory response, the animal model used in this study represents low birth weight (LBW) caused by infection or inflammation. Therefore, the findings should not be generalized to LBW cases resulting from other mechanisms such as intrauterine hypoxia, genetic factors, maternal malnutrition, or non-infectious placental disorders. Interpretation of the results should remain limited to the inflammatory pathway of LBW pathogenesis that this model mimics. Overall, the LPS-induced model effectively reproduces the key pathophysiological processes of inflammation-related LBW in humans and provides a valuable experimental platform for evaluating therapeutic interventions, such as folic acid supplementation, aimed at reducing inflammation-induced fetal damage during pregnancy.

CONCLUSION

According to research, LPS induction in Wistar rats can promote fetal mortality, IL-6, and TNF-α levels, decrease birth weight, and shorten the CRL, but it has no effect on the length of the rat pups' tails. The molecular pathways by which LPS triggers LBW, dose and timing optimization, therapeutic intervention trials, comparison with other LBW models, and investigations on the long-term consequences on post-IUGR offspring are all necessary to support these findings. However, there are limitations in the use of the LPS model. Some studies show that this model may not fully reflect the complexity of LBW in humans, and high doses of LPS can cause toxicity and death in experimental animals [40]. Therefore, further research is needed to optimize LPS dosage and administration protocols, as well as combine this model with other approaches to improve its relevance to clinical conditions in humans.

ACKNOWLEDGMENT

No particular funding from any public, private, or nonprofit organization was obtained for this study.

CONFLICT OF INTEREST

In the interest of transparency, the authors declare that they have no conflicts of interest with regard to the publishing of this work.

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