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Evaluation of placental bed uterine in L-NAME-induced early-onset preeclampsia (EO-PE) like the rat model

L-NAME ile indüklenen erken başlangıçlı preeklampsi (EO-PE) sıçan modelinde uterin plasental yatağın değerlendirilmesi

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Abstract

Objective: Preeclampsia (PE) is the leading cause of maternal death worldwide and is associated with long-term morbidity in both mothers and newborns. Animal modeling is considered a functional source for understanding PE pathogenesis, diagnostic standards, and therapeutic approaches.

Materials and Methods: This study aimed to demonstrate and evaluate the use of N-nitro-L-arginine methyl ester (L-NAME) in a Wistar rat model under conditions similar to PE. A total of 12 rats were divided into 4 groups, each consisting of 3 members, including the pregnant control group and treatment groups administered low-dose (PE 25 mg/kg L-NAME/day), medium-dose (PE 50 mg/kg L-NAME/day), and high-dose L-NAME (PE 75 mg/kg L-NAME/day) L-NAME from gestational day 4 to 19. Measurements included blood pressure, creatinine, and proteinuria levels, placental histological changes, and placental tissue hypoxia-inducible factor 1-alpha, and plasma endothelial nitric oxide synthase levels.

Results: The results showed that intervention with L-NAME at 75 mg/kg body weight/day (PE3) induced PE earlier than that with 50 mg/kg body weight/day L-NAME.

Conclusion: The model conditions also support further research into PE pathogenesis.

Keywords: eNOS, HIF1α, L-NAME, MAP, preeclampsia, proteinuria, spiral arteries

Öz

Amaç: Preeklampsi (PE) dünya çapında anne ölümünün önde gelen nedenidir ve hem annelerde hem de yenidoğanlarda uzun süreli morbidite ile ilişkilidir. Hayvan modelleri, PE patogenezini, tanı standartlarını ve tedavi yaklaşımlarını anlamak için işlevsel bir kaynak olarak kabul edilir.

Gereç ve Yöntemler: Bu çalışmada, N-nitro-L-arginin metil esterin (L-NAME) Wistar sıçan modelinde PE'ye benzer koşullar altında kullanımının gösterilmesi ve değerlendirilmesi amaçlandı. Gebeliğin 4-14 günlerinde olan toplam 12 sıçan, gebe kontrol grubu ve düşük doz L-NAME (PE 25 mg/kg L-NAME/gün), orta doz L-NAME (PE 50 mg/kg L-NAME/gün) ve yüksek doz L-NAME (PE 75 mg/kg L-NAME/gün) uygulanan tedavi grupları dahil olmak üzere her biri 3 üyeden oluşan 4 gruba ayrıldı. Ölçümler kan basıncını, kreatinin ve proteinüri düzeylerini, plasental histolojik verileri ve plasental doku hipoksisi ile indüklenen faktör 1-alfa ve plazma endotelyal nitrik oksit sentaz seviyelerini içeriyordu.

Bulgular: Sonuçlar, 75 mg/kg vücut ağırlığı/gün (PE3) L-NAME müdahalesinin, 50 mg/kg vücut ağırlığı/gün L-NAME müdahalesinden daha erken PE'yi tetiklediğini gösterdi.

Sonuç: Model koşulları aynı zamanda PE patogenezine yönelik daha ileri araştırmaları da desteklemektedir.

Anahtar Kelimeler: eNOS, HIF1α, L-NAME, MAP, preeklampsi, proteinüri, spiral arterler

PRECIS: Treatment of rats with L-NAME at a dose of 75 mg/kg/d results in inadequate spiral artery remodeling, HBP, proteinuria, and IUGR, thus clearly mimicking the syndrome of EO-PE.

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Introduction

Preeclampsia (PE) is a leading cause of maternal mortality worldwide and is associated with long-term morbidity in both mothers and newborns[1]. It is a hypertensive condition characterized by a diastolic[2] blood pressure (DBP) of 90 mmHg and a systolic blood pressure (SBP) of 140 mmHg, accompanied by proteinuria (0.3 g/24 hours) appearing after the 20th week of pregnancy[3]. The current classification is based on the onset and severity of symptoms, but it does not accurately reflect the underlying pathophysiological processes. The different PE classes include early onset (EO) <34 weeks, late onset (LO) ≥34 weeks, or preterm (<37 weeks), and term (≥37 weeks) PE[4]. EO or premature PE is more often complicated by fetal growth restriction and more severe symptoms than LO or term PE[5]. The main pathological feature of EO-PE is incomplete transformation of spiral arteries[6]. This condition manifest as a multisystem syndrome with maternal and neonatal morbidities exceeding those of normal pregnancies[7]. The pathogenesis of EO-PE is diverse and is associated with factors such as excessive inflammation, oxidative stress, metabolic disturbances, and apoptosis[7,8]. According to current knowledge, the disease develops through preclinical and clinical stages[10]. Important pathological modifications, including inadequate trophoblast invasion and remodeling of spiral arteries at the placental base, are recognized as critical factors in the preclinical phase[11]. The term placental bed describes the maternal-fetal interface or the area where the placenta attaches to the uterus, which requires adequate vascularization for fetal development[12].

Experimental animal models are valuable for examining PE pathogenesis, diagnosis, and treatment options[13]. In reproductive research, rats have significant anatomical and behavioral advantages over mice[14]. Many models have been projected to meet or at least resemble the above criteria, including reductions in uterine perfusion pressure, nitric oxide synthase (NOS) knockout rats [parallel to the N-nitro-L-arginine methyl ester (L-NAME) model], transgenic, sFlt-1 infusion, and alpha tumor necrosis factor infusion models[15]. Physiologically, these models represent hypoxia, nitric oxide (NO) dysregulation, renin-angiotensin deviation, angiogenesis disturbances, and disproportionate maternal immune responses. The importance of NO in endothelial cells lining the arteries in controlling vascular tone has been previously reported[16]. Heart rate, blood volume, and cardiac output all increase during pregnancy, although blood pressure usually remains at or slightly below pre-pregnancy levels[17]. NOS inhibition and L-NAME have been shown to reduce hypertension in pregnant rats while maintaining normal blood pressure levels until delivery. Additionally, during pregnancy, NO is crucial for controlling the cardiovascular system[18]. Considering that PE is associated with vascular endothelial dysfunction and significant inflammation, it is crucial to create animal models that replicate the pathology of the circulatory system to gain a better understanding of the onset and progression of PE[19].

Previous research has shown a potential relationship between PE onset and placental development, particularly dysfunction during early pregnancy[5]. PE syndrome in mothers is believed to be caused by vascular dysfunction, oxidative stress, and metabolic abnormalities, although the exact mechanisms remain unclear[20]. However, there has been no evaluation of uteroplacental ischemia as the onset of the problem, which is the onset of EO-PE. Hypoxia due to reduced blood flow can trigger the production of several vasoactive chemicals, potentially disrupting the location of the placenta in the uterus[21]. Many PE models show high blood pressure during pregnancy, but specific characteristics and features are still debated. Each disease has advantages and limitations, with insufficient symptoms or an inability to indicate further symptom development, but none of these characteristics reflect human conditions at the placental level. Therefore, this study aimed to evaluate vascular defects, kidney injury, and uterine damage at the placental base in an L-NAME-induced PE model.

Materials and Methods

Materials

The reagent used in this study, L-NAME, was purchased from Sigma-Aldrich St. Louis, Missouri, USA (Cas. No: 51298-62-5). Proteinuria (Cat. No. FY-RA 4983) and creatinine (Cat. No. FY-EU14140) were obtained from Eiyue Biological Company, China, while hypoxia-inducible factor 1α (HIF1α) (Cat. No. RK 03528) was acquired from ABclonal, Company Inc., UK. Female Wistar rats (Rattus norvegicus) were provided by the Laboratory of Integrated Research and Testing (LPPT) at Gadjah Mada University (UGM), Jogjakarta. On November 1, 2022, the Research Ethics Committee of the Faculty of Medicine, Universitas Sebelas Maret (UNS), with number 301/UNS/27.06.9.1/TU.00/2022, approved the research protocol.

Animal Experiments

This research used an *in vivo* experimental method with a pre-posttest control group design, except for assessing HIF1α and endothelial nitric oxide synthase (eNOS) levels. The samples were 30 female Wistar rats weighing 180-200 g and 6 males weighing 250-350 g, all obtained from the LPPT 4 Animal Experiment Center, with the females being 8 and 12 weeks old. In a 12-hour light-dark cycle, the rats were kept in a pathogen-free atmosphere with a humidity of 55±15% and a temperature of 22±2 °C. Access to food and water was provided ad libitum, and testing was performed with a 5:1 ratio between females and males. There were 12 confirmed pregnant rats identified using vaginal swabs, which were then randomly divided into 4 groups. The groups received the following treatments: (1) Pregnant control (PC) (n=3); (2) PE1 (n=3) administered with L-NAME 25 mg/kg/day; (3) PE2 (n=3) administered with 50 mg/kg/day; and (4) PE3 (n=3) administered with 75 mg/kg/day. Pregnant rats were given continuous doses of L-NAME mixed with drinking water on the 4th or 19th day of pregnancy through gavage and were administered until GD19.

Blood Pressure Measurement

Blood pressure was measured on GD0, GD5, GD10, GD14, and GD18 using a CODA non-invasive blood pressure monitoring technique (BP-2010A, Softron, Beijing, China). For 5 min, rats were placed in a heated jacket at 40 °C to increase blood flow to tails. Once the blood pressure had stabilized, the detector was placed near the base of the tail, and measurements were performed. The 5 repetitions were performed, and the average value was calculated.

Tissue Preparation for Measurement of Spiral Artery Diameter, Neutrophil Count, Percentage of Placental Uterine Layer Necrosis, and Placental HIF1α Levels

The uterine tissue was fixed as part of the endometrium where the placenta is located. Tissue processing stages included grossing and fixation (8-48 hours), dehydration, and clearing embedding (24 hours at 58 °C), whereas paraffin blocks were prepared by blocking, cutting, identification, and incubation processes. Hematoxylin-eosin (H&E) staining included deparaffinization, hematoxylin staining, mounting, and histopathological observation of uterine tissue under a light microscope (Olympus CX32; Olympus, Tokyo, Japan). Histopathological preparations were performed at the UGM PA Lab to assess HIF1α levels in placental tissue. To collect the supernatant, 0.1 g was combined with 0.9 mL of PBS solution and centrifuged for 5 min at 5000 rpm and 4 °C.

Measurement of eNOS Levels in Blood

A 1-cc blood sample was centrifuged for 10 min at a speed of 1500 rpm to extract plasma eNOS levels for serum formation. This was followed by the preparation of the Abclonal Kit reagent (Cat. No. RK 03528).

Urine Analysis Measurements

The estimated urine output consisted of GD4, GD10, and GD17, and rats were kept in individual metabolic cages (Techniplast, Italy). Subsequently, 24-h urine protein and creatinine levels were measured in each group using kits from Eiyue Biological Company, China.

Statistical Analysis

Results are reported as mean ± standard deviation. In addition, One-Way ANOVA and Tukey's post-hoc test with a 95% confidence interval were applied to statistical analysis. The analysis was performed using GraphPad Prism version 9.1.1 software.

Results

Changes in Systolic, Diastolic, and Mean Arterial Pressure in Pregnant Rats

Figures 1a, b, and c show the variations in SBP, DBP, and mean arterial pressure (MAP) at each time point during pregnancy. The 4 groups of rats did not differ significantly in SBP, DBP, or MAP before L-NAME administration. After L-NAME treatment, these parameters indicated progressive increases, with SBP, DBP, and MAP in the PE2 and PE3 groups slightly increasing toward the end of pregnancy (Figure 1). Specifically, SBP, DBP, and MAP improved significantly in the PE3 group compared with the control ($p<0.05$) and the L-NAME-treated group ($p<0.05$).

Renal Function Modifications in Rats Receiving L-NAME Injections

Urine parameters were measured to characterize kidney filtration and excretion, which may be affected by injury and

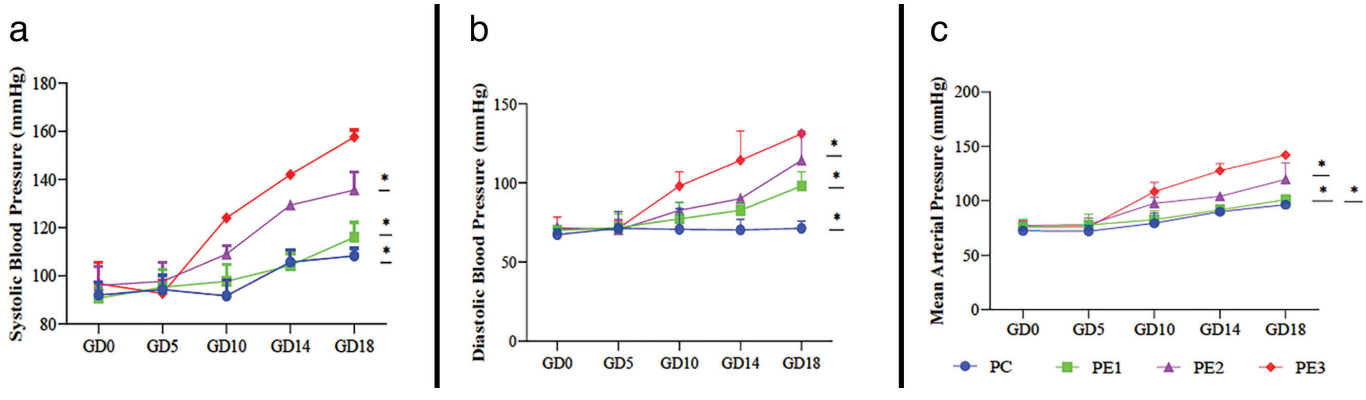

Figure 1. The CODA non-invasive blood pressure method (BP-2010A, Softron, Beijing, China) was used to determine the blood pressure of pregnant rats non-invasively. Trends of deviation in (a) SBP, (b) DBP, and (c) MAP of each group during pregnancy. *: Using repeated measures analysis of variance with $p<0.05$, significance was assessed in relation to the control group. The values are shown using a mean ±95% confidence interval. PC rats comprise the PC group. Three doses of L-NAME were administered to the rats: 25 mg/kg for PE1, 50 mg/kg for PE2, and 75 mg/kg for PE3

PC: Pregnant control, SBP: Systolic blood pressure, DBP: Diastolic blood pressure, MAP: Mean arterial pressure, L-NAME: N-nitro-L-arginine methyl ester, PE: Preeclampsia

affect reabsorption. The results showed that before treatment, the urine protein levels of the 4 groups were similar for GD4 and GD10, and the creatinine levels were similar only for GD4, and there were no differences in the levels of proteinuria and creatinine in the GD4. Additionally, the urine protein and creatinine levels of pregnant rats treated with L-NAME were significantly higher than those of the PC group ($p<0.05$; Figure 2a and b).

Changes in the Structure and Function of the Placental Layer in Pregnant Rats Injected with L-NAME

Increased neutrophil activation, necrosis percentage, and dilation of spiral artery diameter were observed in the placental base and structure of uterine spiral arteries in pregnant rats (GD19). Histopathological images of placental myometrium and uterine spiral arteries in pregnant rat groups PC, PE1, PE2, and PE3 (H&E; magnification, x400; scale bar, 25 µm for neutrophil count and uterine spiral artery diameter; magnification, x100 for neutrophil count). Values are presented as mean ± standard deviation.

Figure 3 shows that the number of neutrophils in the L-NAME-treated groups was higher than that in the PC group (3.26±0.69), and significant differences were found in the PE2 (17.29±1.67), and PE3 group (22.26±0.13) ($p<0.05$).

Figures 4a and b show that the percentage of necrosis was lower in the L-NAME-treated groups than in the PC group (5.16±2.69). There were significant differences between PE1 (10.08±0.98 %), PE2 (10.29±1.67), and PE3 groups (15.26±2.13) ($p<0.05$).

Figures 5a and b show that the diameter of the uterine spiral artery in the L-NAME-treated group was smaller than that in the PC group (162.54±0.025). There were significant differences between PE2 (162.42±0.021) and PE3 (162.34±0.023) ($p<0.05$) (Figure 6).

Vasoconstriction Reactions in Pregnant Rats Treated with L-NAME

Generally, the pathogenesis of PE is associated with vasoconstriction factors. In this study, tissue EL tests and plasma assays were used to detect the levels of HIF1α and eNOS in the placental plasma of the PC, PE1, PE2, and PE3 groups (Figures 7a and b). Significant differences in HIF1α levels were recorded in the PE3 group (2.50±0.51 pg/mg) $p<0.05$ compared to PC (1.75±0.11 pg/mg), as shown in Figure 7a. The effects of different L-NAME treatment regimens on eNOS levels in the circulation of pregnant rats in each group were determined by plasma analysis toward the end of pregnancy (GD19). The eNOS levels in each L-NAME-treated group were significantly decreased compared with PC (84.12±0.53 ng/dL; $p<0.05$).

Adverse Pregnancy Outcomes in the L-NAME-treatment Groups

The study groups experienced adverse pregnancy outcomes, primarily due to inadequate remodeling of spiral arteries and inflammation in the uterus and placenta. Rats administered high-dose L-NAME (75 mg/kg) had significantly smaller placentas than controls. There was also a significant decrease in fetal weight ($p<0.05$) and shorter crown-rump length. Furthermore, the PE2 and PE3 groups showed significant differences in fetal weight, crown-rump length, placental diameter, and weight compared with the PC group (Figures 7a-d).

Discussion

The administration of high-dose L-NAME (75 mg/kg) evaluated on gestational days 10, 14, and 18 increased blood pressure (SBP, DBP, and MAP), elevated proteinuria on GD17, and creatinine levels from GD10 to 17. The treatment also increased the number of neutrophils, increased the occurrence of necrosis

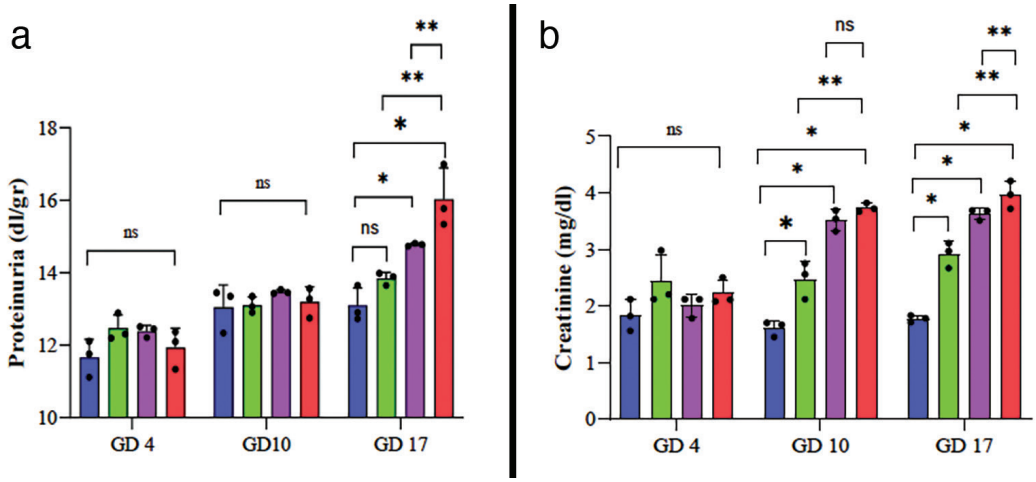

Figure 2. Urine Parameters during PE. The urine proteinuria (a) and creatinine (b) levels are displayed per day. All parameters increased on day 10 of PE pregnancy compared with the control. Urine creatinine levels increased on day 10 of PE. Statistical analysis was performed using repeated-measures ANOVA. Mean ± standard deviation is shown (n=3-4). P-values are displayed *: $p<0.05$ and ns >0.05. **: Bonferroni test $p<0.05$ vs. PE3

PE: Preeclampsia

at the placental base, reduced the diameter of uterine spiral arteries, elevated placental HIF1α levels, decreased plasma eNOS levels, and heightened the incidence of intrauterine fetal death and intrauterine growth restriction.

The specific identification parameters for EO-PE are SBP 140 mmHg and DBP 90 mmHg[1]. In this investigation, GD4 or GD18 were selected as the optimal starting points for optimizing L-NAME administration. A comprehensive literature review validated the use of L-NAME at doses of 25, 50, and 75 mg/kg per day to induce PE in pregnant rats. The results showed increased SBP, DBP, and MAP in hypertensive rats after L-NAME administration, with pressure differences of up to 15 mmHg observed in the PE2 and PE3 groups from GD10. These blood pressure differences persisted until birth on GD19, with the PE3 group showing the greatest variation. All rat groups, except for PE3, demonstrated a typical pregnancy trend, including decreases in MAP, DBP, and SBP toward late pregnancy. Meanwhile, rats in the PE3 group showed a continuous increase in blood pressure throughout pregnancy rather than a decrease. These results were inconsistent with[22], where 40 SD rats (Sprague-Dawley rats) treated with PE 50 mg/mL had significantly higher SBP than the control[23]. Differences in the results may be due to variations in the types of rats used. According to research, the normal blood pressure of SD rats is higher than that of Wistar[24]. Low to medium doses or late administration failed to produce the average blood pressure characteristic of PE pregnancy and did not significantly affect SBP, DBP, or MAP.

Proteinuria parameters were measured to characterize kidney filtration and excretion. Research has proven that proteinuria and creatinine levels significantly decrease during and after PE compared with the control[25]. Both parameters were measured for GD4, 10, and 17 in all groups. There were no significant changes in proteinuria across all groups on GD4 and 10. On day 17, proteinuria significantly increased in the early PE2 and PE3 groups compared with the control. These results were consistent with[26], where 4 groups given 50 mg/kg PE had significantly higher proteinuria than the relatively PC group. Differences in the results may be due to variations in the types of rats used. There were no significant changes in

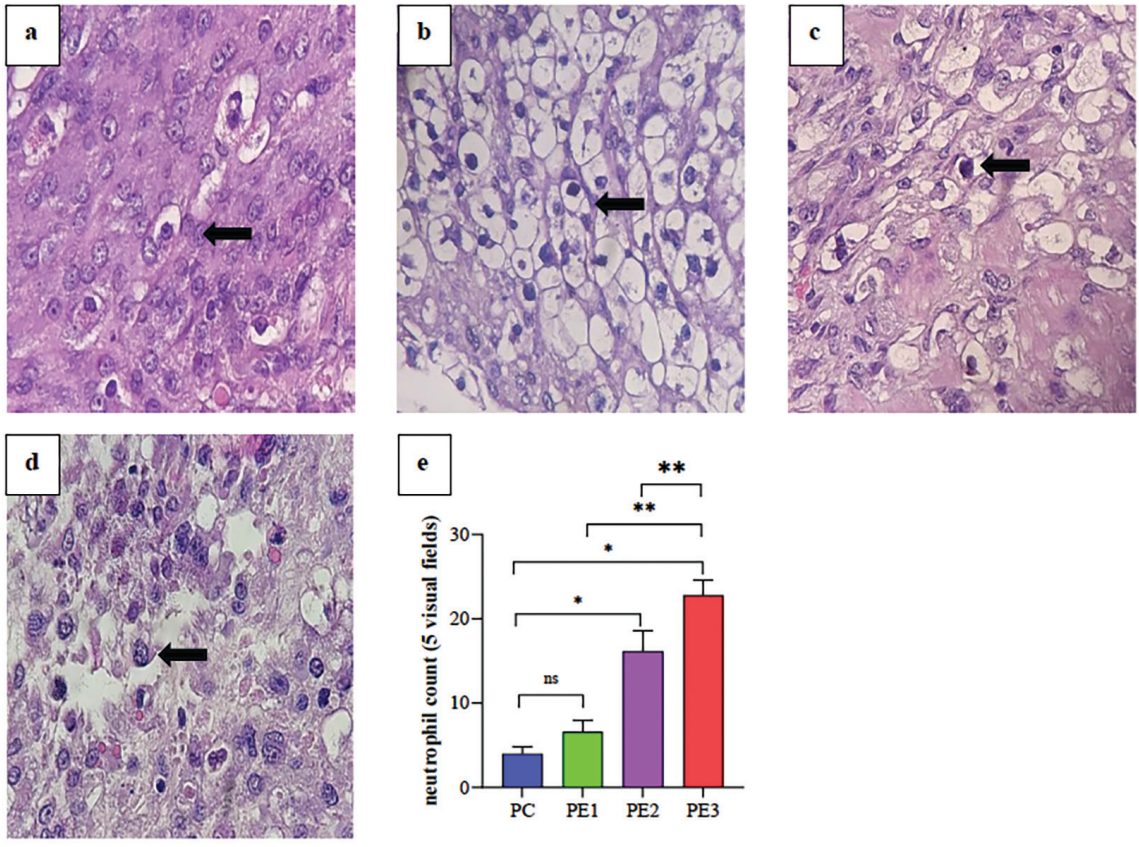

Figure 3. Changes in the design and process of placental bed oxidative stress in pregnant rats (GD19). (a) Histopathological images of the myometrial layer in pregnant rats (PC) control group, (PE1) group treated with 25 mg/kg body weight per day, (PE2) group treated with 50 mg/kg body weight per day, and (PE3) group treated with 75 mg/kg body weight per day (H&E; magnification, x400; scale bar, 25 µm). Additionally, black indicators indicate neutrophils in 5 fields of view. (b) The number of neutrophils in pregnant rats on GD19. *: Tukey post-hoc test significant p≤0.05 vs. PC; ns p>0.05 vs. PC. **: p<0.05 vs. PE3

PE: Preeclampsia, H&E: Hematoxylin-eosin

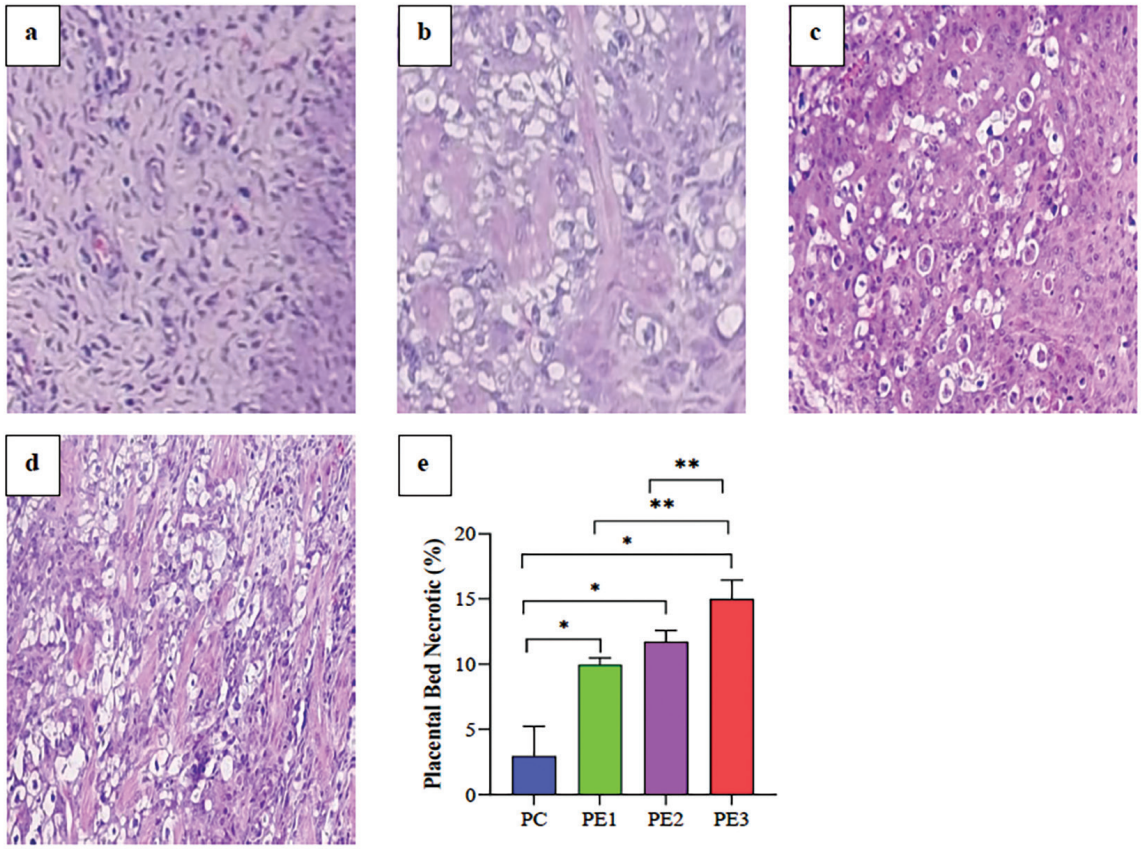

Figure 4. Variation in the shape and function of the placental base in pregnant rats, along with the percentage of inflammatory necrosis (GD19). (a) Histopathological images of the myometrial layer in (PC), control, (PE1), group treated with 25 mg/kg body weight per day, (PE2), and (PE3) groups treated with 75 mg/kg body weight per day in pregnant rat groups (H&E; magnification, x100; scale bar, 25 µm). The percentage of necrosis in 5 fields of view is indicated by black boxes. (b) Proportion of pregnant rats on GD19 experiencing necrosis. *: Tukey post-hoc test significant p≤0.05 vs. PC; ns p>0.05 vs. PC. **: p<0.05 vs. PE3

PE: Preeclampsia, PC: Pregnant control, H&E: Hematoxylin-eosin

creatinine levels across all groups on GD4, but a significant increase was observed on days 10 and 17. These results may be attributed to L-NAME, which induces glomerular damage by increasing glomerular pressure. The increase in pressure leads to thickening of the blood vessel walls in the kidneys[27] and also creates stress, which results in the loss of protein in the urine, resulting in proteinuria[28].

The term "placental bed" describes the interface between the mother and fetus, namely, the area inside the uterus where the placenta attaches to the uterine wall. Administration of 75 mg/kg body weight per day L-NAME successfully induced the highest increase in neutrophil counts compared with the PE, PE1, and PE2 groups. This result was consistent with the research conducted by[29] using 35 Sprague-Dawley rats induced with RUPP. The results showed that there was a higher neutrophil count in rats with PE[29]. Generally, PE is characterized by reduced placental perfusion accompanied by ischemia and hypertension during pregnancy. Women with this condition also show increased inflammation and a higher number of neutrophils in their blood vessels compared with healthy women. The main pathological feature of EO-PE is the imperfect transformation of spiral arteries, resulting in placental hypoperfusion and decreased fetal nutrient supply. Based on the results, the administration of L-NAME at 75 mg/kg body weight per day reduced the diameter of the uterine spiral arteries compared with the PE, PE1, and PE2 groups. This was supported by[11], where 16 placental beds from Cesarean hysterectomy specimen collections showed a difference in diameter between the Normotensive (500 micrometers) and PE (200 micrometers) groups. The administration of L-NAME at a dose of 75 mg/kg body weight per day as an NO inhibitor can trigger placental hypoxia and ischemia, as evidenced by the narrowing of the average diameter of spiral arteries, which is a significant characteristic of EO-PE.

The vasoconstriction reaction in pregnant rats treated with L-NAME was also evidenced by placental HIF1α levels and eNOS levels in the blood. HIF1α levels decreased with the use of 75 mg per kilogram body weight per day and showed a significant difference compared with the PC group. This

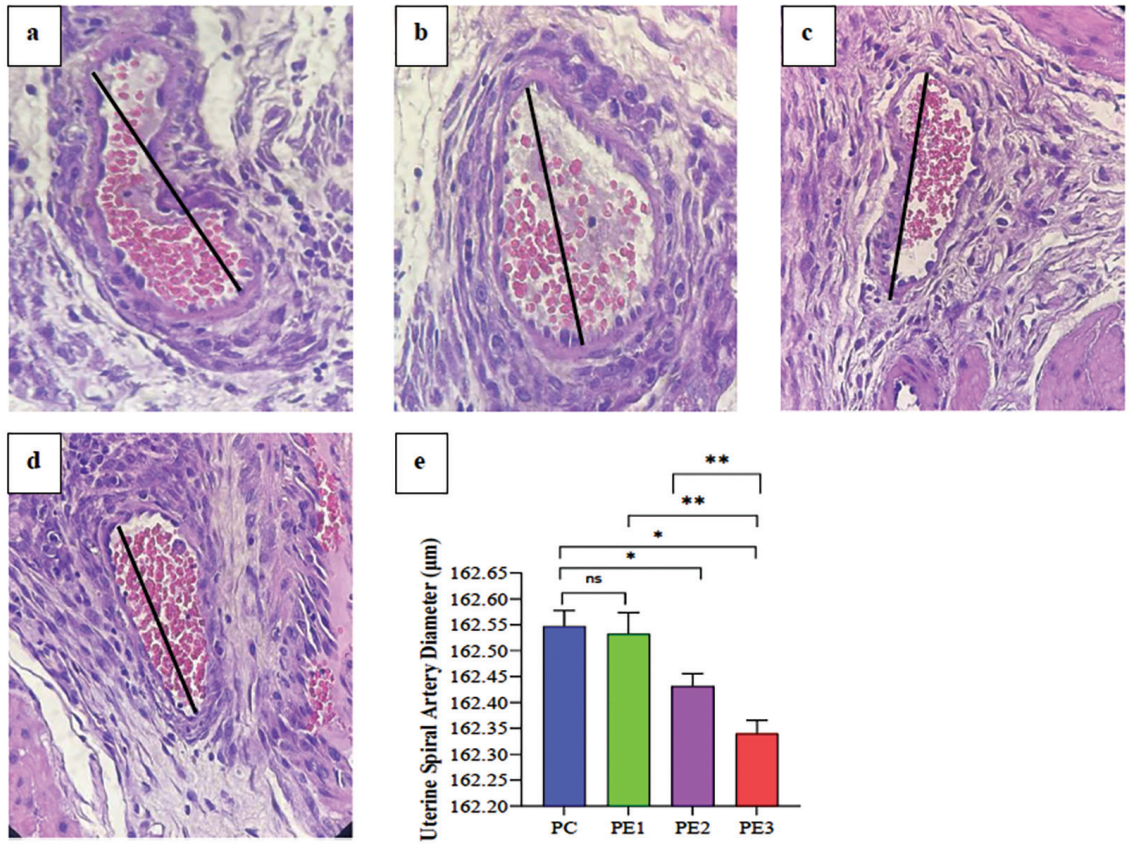

Figure 5. Modifications in the anatomy and function of uterine spiral artery remodeling in pregnant rats (GD19). (a) Histopathological images of the diameter of the uterine spiral artery in pregnant rats (PC), control group, (PE1) group treated with 25 mg/kg body weight per day, (PE2) 50 mg per kg body weight per day, and (PE3) 75 mg/kg body weight per day (H&E; magnification, x400; scale bar, 25 µm). The diameter of the uterine spiral artery is indicated by black lines spanning from outer to inner. (b) Pregnant rats on GD19 with the uterine spiral artery diameter. *: Significant p≤0.05 vs. PC in Tukey's post-hoc test; ns) p>0.05 vs. PC; **: p<0.05 vs. PE3

PE: Preeclampsia, PC: Pregnant control, H&E: Hematoxylin-eosin

result was consistent with a previous study in which 15 rats were administered L-NAME, with 2 (13.4%) and 13 (86.6%) showing light and medium immunohistochemical staining for HIF1α expression in placental tissue, respectively[30]. Abnormalities in early placental development, starting from inadequate trophoblast invasion into spiral arteries, lead to decreased uteroplacental perfusion and hypoxia[4]. The decreased eNOS levels in the PE3 group were consistent with the findings in 84 pregnant women at the Department of Obstetrics and Gynecology, University Hospital at FMRP-USP. A significant decrease in plasma eNOS concentration was observed in PE compared with HP[31]. The reduction in eNOS levels is associated with hemodynamic decline caused by systemic vascular dilatation, which affects the level of organ hypoperfusion and contributes to the development of PE[32].

The administration of L-NAME also affected pregnancy outcomes, with significant differences observed between the PE3 and PC groups regarding placental diameter and weight, as well as fetal weight and length. Similarly, the authors found that 9 SD rats administered L-NAME had lower body weights compared to those treated with aspirin or quercetin alone[22]. Endothelial dysfunction caused by L-NAME leads to narrowing of the uteroplacental spiral arteries, which hinders the flow of nutrients from the mother to the fetus. The model examined in this research is easy to create and has potential utility in evaluating the pathophysiological indicators of PE, pregnancy outcomes, and complexity and identifying therapeutic targets for prevention. However, placental histopathological lesions in PE were not analyzed in this study. Further studies are needed to confirm the effect of histopathological features on the placenta of L-NAME-induced PE rats.

Conclusion

In conclusion, the administration of L-NAME on 75 mg/kg BW/day (PE3) induced EO-PE, consistent with the rat model. This method is reliable for investigating the pathogenesis of PE. The model facilitates the investigation of changes in blood pressure,

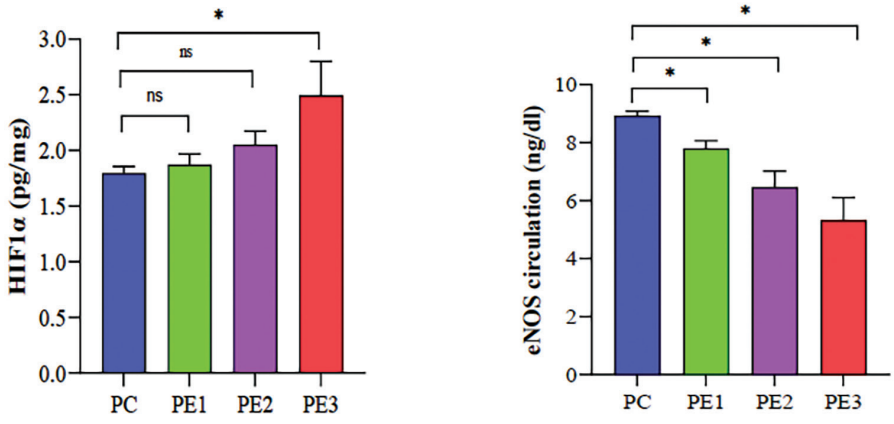

Figure 6. Using Elisa assays, placental hypoxia levels in each group of pregnant rats at the end of pregnancy (GD19) were measured and presented as means ± standard deviation. Legend: From GD4, PC, PE1 rats were given low-dose L-NAME (25 mg/kg body weight/day); PE2 rats were given medium-dose L-NAME (50 mg/kg); and PE3 rats were given high-dose L-NAME (75 mg/kg body weight/day) on GD4. *: Significance by Tukey post-hoc test compared to PC group ($p<0.05$), Tukey Post-hoc test not different compared to PC group (ns)

PE: Preeclampsia, PC: Pregnant control, L-NAME: N-nitro-L-arginine methyl ester

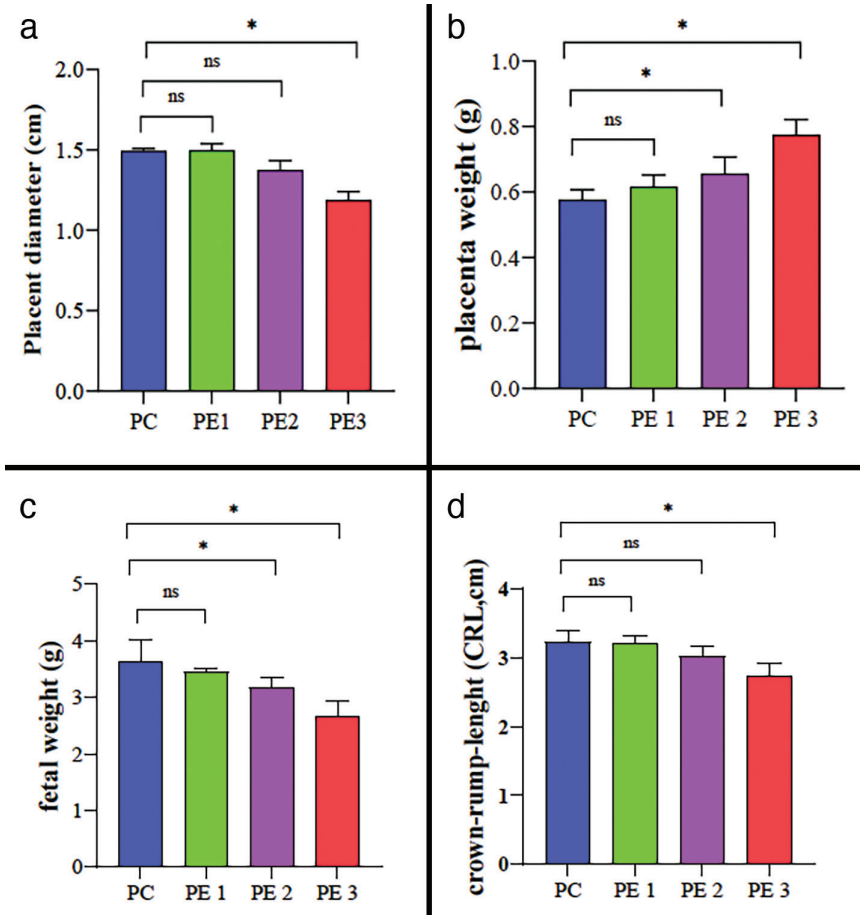

Figure 7. Impact of Pregnancy and Trophoblast Placental Structure Modifications in Rats During Pregnancy (GD19). (a) Placental diameter for each group separately. (b) Placental weight according to status. (c) Fetal significance in each group. (d) Height in each group from crown to rump. (D). Values are presented as mean ± standard deviation. The PC group underwent normal pregnancy; PE1, PE2, and PE3 rats underwent treatment with low-dose L-NAME (25 mg/kg body weight/day), medium-dose (50 mg/kg), and high-dose (75 mg/kg) starting from GD4. GD (Gestation Day); L-NAME *: Significant $p\leq0.05$ vs. PC; ns $p>0.05$ vs. PC (Tukey post-hoc test)

PE: Preeclampsia, PC: Pregnant control, L-NAME: N-nitro-L-arginine methyl ester

kidneys, blood vessels, and the uterine placental layer over time, as well as the discovery of new biomarkers in the early stages of pregnancy.

Ethics

Ethics Committee Approval: On November 1, 2022, the Research Ethics Committee of the Faculty of Medicine, Universitas Sebelas Maret (UNS), with number 301/UNS/27.06.9.1/TU.00/2022, approved the research protocol.
Informed Consent: Written informed consent was obtained from the patients.

Authorship Contributions

Surgical and Medical Practices: F.F., D.I., Concept: F.F., D.I., S.S., S.So., Design: F.F., D.I., S.S., S.So., Data Collection or Processing: F.F., D.I., Analysis or Interpretation: F.F., D.I., Literature Search: F.F., D.I., S.S., S.So., Writing: F.F.
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