Progesterone Inhibits LPS-Induced Oxidative Stress Through Nrf2/Keap1 Pathway in Bovine Endometrial Epithelial Cells

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Research Article

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Abstract

Background

Postpartum uterine infection can lead to endometrial inflammation and oxidative damage. Progesterone makes the animal more susceptible to uterine infection. Progesterone has been proved to play an anti-inflammatory role in inhibiting uterine innate immunity, and to reduce tissue oxidative damage. But the effect of progesterone on the oxidative damage of bovine endometrium has not been reported. The purpose of this study was to explore the effect and mechanism of progesterone (1, 3, and 5 ng/mL) on oxidative damage in primary bovine endometrial epithelial cells (BEEC) induced by lipopolysaccharide (LPS) from *Escherichia coli*.

Results

Compared with the LPS group, there were decreases ($P < 0.05$) in the levels of reactive oxygen and malondialdehyde, and increases ($P < 0.05$) in the activities of superoxide dismutase and catalase, and total antioxidant capacity in the cotreatment groups of progesterone and LPS. The cotreatment of LPS and P4 upregulated ($P < 0.05$) the mRNA abundance of antioxidant genes and the key protein levels in Nrf2/Keap1 pathway, and promoted the Nrf2 protein to enter the nucleus. The use of progesterone receptor antagonist mifepristone reversed the antioxidative effect of progesterone.

Conclusions

Progesterone protects BEEC from LPS-induced oxidative damage by activating Nrf2/Keap1 pathway through progesterone receptor.

1 Background

In the modern dairy industry, endometritis is one of the common postpartum diseases of dairy cows, and can lead to infertility and even death [1]. The disease is mainly caused by the invasion of pathogens such as *Escherichia coli* into the endometrium [2]. Lipopolysaccharide (LPS) is a component of the outer membrane of Gram-negative bacteria, and can induce inflammatory response. It can be used as a non-specific immune stimulant to produce inflammatory mediators and cytokines [3]. The release of some cytokines such as nitric oxide is involved in the pathogenesis of endometritis, resulting in oxidative damage [4].

Oxidative stress refers to the imbalance between oxidation and antioxidation in bodies. When the cells cannot remove the excess free radicals themselves in time, the accumulation of a large number of oxidation intermediates aggravates the damage,which has negative effects on animal production and health [5]. Reactive oxygen species (ROS) is a marker product of cell oxidative damage. Zhao et al.[6]

found that ROS caused oxidative damage of Marc-145 cells by increasing inducible nitric oxide synthase and upregulating apoptotic genes. ROS can attack the phospholipid layer of biofilm to produce malondialdehyde (MDA)[5, 7] and inhibit the activity of antioxidant enzymes such as glutathione peroxidase (GSH- PX)[8]. It has been found that 1 μg/mL LPS increased ROS level and reduced GSH activity in bovine mammary epithelial cells [9].

Progesterone (P4) has the functions of maintaining pregnancy, thickening the endometrium, and inhibiting the maternal immune rejection to the fetus [10]. It plays an important role in maintaining the sexual cycle and pregnancy [11]. The pathogenesis of endometritis can be related to progesterone. In the presence of persistent corpus luteum, the postpartum progesterone concentration can exceed 5 ng/mL for more than 20 days, and persistent high concentrations of progesterone makes the uterus more susceptible to infection [12]. Studies have confirmed that progesterone may play a neuroprotective role on the developing brain by reducing lipid peroxidation and fighting free radicals [13]. Wu et al.[14] found that progesterone reduced oxidative damage and apoptosis of nerve cells induced by Aβ (25-35) through progesterone receptor membrane component 1 in mice. P4 also plays a regulatory role in uterine innate immunity [15], and has been shown to inhibit LPS-induced inflammatory factors and oxidative damage in human endometrium [16].

Nuclear factor erythroid 2 related factor 2 (Nrf2) is a new cellular antioxidant regulator [17]. Nrf2 is activated through Keap1 dependent disinhibition signaling mechanism [18, 19]. Activated Nrf2 mediates the expression of a series of antioxidant enzymes and signal proteins and plays an important role in regulating drug metabolism, antioxidant defense and oxidant signal [20–22]. Lin et al.[23, 24] found that curcumin can alleviate oxidative damage of RAW264.7 cells by activating Nrf2 pathway, and that Nrf2 knockout mice were more susceptible to diseases related to oxidative pathology. It has been found that 1 μg/mL LPS enhanced the mRNA abundance of inflammatory factors and induced activation of Nrf2 in bovine endometrial epithelial cells [25, 26]. Similarly, LPS can cause apoptosis of bovine mammary epithelium and activation of Nrf2 pathway [21]. Studies have shown that Nrf2/Keap1 pathway may participate in the multi-effect neuroprotective effect of P4 on traumatic brain injury, so as to reduce brain edema, apoptosis and inflammatory response after traumatic brain injury [27, 28]. As mentioned above, progesterone can regulate uterine immunity and alleviate oxidative damage of nerve cells. However, the mechanism of P4 on LPS-induced oxidative damage of bovine endometrium is not clear.

The purpose of this study was to explore the effect of progesterone on the oxidative damage of primary bovine endometrial epithelial cells (BEEC). BEEC was treated with LPS to induce the oxidative damage. The changes in oxidative damage markers, antioxidative enzyme activities, the mRNA transcriptions, and key proteins of Nrf2/Keap1 pathway were determined. Progesterone receptor antagonist mifepristone (RU486) was used to explore the potential mechanism.

2 Results

2.1 Effects of Progesterone on ROS Level

As shown in Figure 1, the level of ROS increased ($P < 0.01$) after LPS challenge. Compared with the LPS group, the addition of 3 ng/mL P4 decreased ($P < 0.05$) the ROS level in BEEC.

2.2 Effects of Progesterone on Levels of MDA, SOD, and T-AOC

As shown in Figure 2, LPS stimulation caused increased ($P < 0.01$) content of MDA, and decreased ($P < 0.05$) activities of SOD, CAT, and T-AOC. Compared with the LPS group, the content of MDA decreased ($P < 0.01$), and the activities of SOD, CAT, and T-AOC increased ($P < 0.05$) in the cotreatment groups of LPS and P4 (1 and 3 ng/mL). No difference ($P > 0.05$) was observed between the LPS group and the cotreatment group of LPS and 5 ng/mL P4.

2.3 Effects of Progesterone on the mRNA Expressions of Antioxidative Genes

As depicted in Figure3, the cells treated with LPS showed decreased ($P < 0.05$) mRNA abundance of *NFE2L2*, *HMOX1*, *NQO1*, *GCLC*, and *GPX2*, and increased ($P < 0.05$) mRNA abundance of *KEAP1* as compared with the blank control. The changes in *GSTP1* was not obvious ($P > 0.05$). Compared with the cells treated with LPS, the gene expressions of *NFE2L2*, *HMOX1*, *NQO1*, *GCLC*, and *GPX2* were generally higher ($P < 0.05$) in cells cotreated with LPS and P4 (1 and 3 ng/mL), but not the cells cotreated with LPS and 5 ng/mL P4 ($P > 0.05$).

2.4 Effects of Progesterone on the Key Protein Levels of Nrf2/Keap1 Pathway

The effects of progesterone on the protein levels of Nrf2, Keap1, NQO1, and HO-1 were detected using western blot (Figure 4). LPS caused decreases ($P < 0.05$) in the expressions of total Nrf2 protein and downstream HO-1 and NQO1 protein, and an increase ($P < 0.05$) in the content of Keap1 protein in BEEC as compared with the blank control. The Nrf2 protein in the nucleus in LPS group was higher ($P < 0.05$) than that in the control group. The protein expressions of total Nrf2, Nrf2 in nucleus, NQO1, and HO-1 was higher ($P < 0.05$) in LPS and P4 cotreatment group than those in the LPS group.

2.5 Effects of Progesterone on Nrf2 Translocation in BEEC

As shown in Figure 9, LPS treatment increased the number of Nrf2 in the nucleus. Co-treatment of progesterone with LPS showed higher level of Nrf2 in the nucleus as compared with the LPS group.

2.6 Effects of RU486 on Nrf2 Translocation in BEEC

As depicted in Figure 5 and 6, the cotreatment of LPS, P4 (3 ng/mL), and RU486 (35 ng/mL) caused increased ($P < 0.05$) levels of ROS and MDA, and decreased ($P < 0.05$) activities of SOD, CAT, and T-AOC

as compared with the cells cotreated with LPS and P4 (3 ng/mL). There were decreases (P< 0.05) in the relative abundances of antioxidative genes in Nrf2 pathway after the addition of RU486 (Figure 7). As shown in Figure 8, the amount of total Nrf2, Nrf2 in nuclear, NQO1, and HO-1 were less (P < 0.05) in cells cotreated with LPS, P4 (3 ng/mL), and RU486 (35ng/mL) than those cotreated with LPS and P4. Similarly, RU486 reduced the level Nrf2 in the nucleus in cells cotreated with LPS and P4 (Figure 9).

3 Discussion

In the present study, we reported that progesterone inhibited the production of ROS, and promoted the activity of antioxidant enzymes to protect BEEC from LPS-induced oxidative damage. Progesterone activated the Nrf2/Keap1 pathway, and promoted the entry of Nrf2 into the nucleus. This antioxidant effect was most prominent in P4 of 1 and 3 ng/mL, not 5 ng/mL. The addition of progesterone receptor antagonist RU486 inhibited the antioxidant effect of P4.

The excessive accumulation of ROS is one of the important signs of oxidative injury. The antioxidant enzyme system is the first line of defense against damage [29]. It has been found that LPS stimulated ROS production and inhibited the activities of antioxidant enzymes in BEEC and bovine mammary epithelial cells [30, 31]. Our data were consistent with these studies, indicating the oxidative damage of BEEC induced by LPS. We found that the production of ROS and MDA decreased after P4 treatment in LPS-stimulated BEEC. This was consistent with the results of previous reports *in vitro* that progesterone can eliminate MDA and protect against oxidative damage in mouse nerve cells [31]. In order to study the relationship between ROS clearance and P4 antioxidant capacity, we detected the activities of some enzymes in the antioxidant enzyme system. The results showed that the activities of SOD, CAT, and T-AOC increased after the addition of P4. Therefore, we speculated that progesterone may relieve injury by enhancing the activity of antioxidant enzyme system and reducing the level of ROS.

Nrf2/Keap1 signaling pathway is a key pathway regulating the expression of antioxidant enzymes and protect cells from oxidation induced cytotoxicity [32, 33]. Under normal physiological conditions, Nrf2 is anchored in the cytoplasm through Keap1 dependent ubiquitination proteasome degradation to maintain antioxidant and cytoprotective enzymes at basal level and to keep cellular homeostasis [34, 35]. The modification of key cysteine thiols and/or the phosphorylation of electrophiles and oxidants leads to the activation of Nrf2, and prompts Keap1 to release Nrf2, and finally Nrf2 enters the nucleus [36]. Nrf2 regulates the antioxidant defense system through a variety of mechanisms, including the regeneration of oxidative cofactors and proteins, the synthesis of reducing factors, the increase in redox transports, and the induction of stress response proteins. As a stress response protein, HO-1 can enhance antioxidant activity and maintain redox homeostasis. The role of HO-1 in redox transport, such as cystine/glutamate transport, is mainly supported by cystine transporter [37, 38]. HO-1 has the ability to regulate cellular redox defense to maintain redox homeostasis [39, 40]. NQO1 is an important reductant that regulates transcription through anti-oxidative response element (ARE)[41–44]. The combination of the activated Nrf2 and ARE promotes the expression of antioxidant genes such as NQO1 and HO-1, and further enhances the activity of antioxidant enzymes [41]. In this study, we detected the decreased levels of HO-1,

NQO1, and total Nrf2, the increased Keap1 level and translocation of nuclear Nrf2 in BEEC after LPS stimulation. The downregulation of the downstream antioxidant genes of Nrf2 pathway, including *NFE2L2*, *HMOX1, NQO1*, *GPX2*, *GCLC*, and *GSTP1*, was consistent with the Western blot result. *HMOX1* and *NQO1* have significant effects on maintaining the redox balance of BEEC. The activities of *HMOX1* and *NQO1* are regulated by *NFE2L2* [45]. It has been reported that Nrf2 is an important factor to promote the gene transcription of biosynthesis key enzymes (GSH -synthase and SLC7A11), GSH reductase and GPx4, and protect mouse nerve cells from ferroptosis caused by oxidative stress [46, 47]. Our results suggested the LPS-induced oxidative stress and the activation of Nrf2 pathway in BEEC. Similarly, it has been reported that LPS induced inflammation and oxidative stress in bovine endothelial cell line [36].

We observed that the addition of 1 and 3 ng/mL P4 promoted the activation Nrf2 pathway and the expressions of the downstream antioxidant genes and proteins in BEEC with oxidative stress response, which was in agreement with the report that progesterone reduced oxidative injury in experimental colitis in rats [48]. These results revealed that 1 and 3 ng/mL P4 inhibited LPS-induced oxidative stress in BEEC. Progesterone plays its role through progesterone receptor (PR). Mifepristone (RU486) is a specific inhibitor of PR. RU486 was used to verify the effect of progesterone in BEEC. We observed that RU486 reversed the antioxidant effect of progesterone, indicating that the antioxidant effect of P4(1 and 3 ng/mL) was mediated by PR.

In our study, the addition of 5 ng/mL progesterone had no obvious antioxidative effect in BEEC. In the study of vascular endothelial progenitor cells, it has been found that low concentration of progesterone (3.2 ng/mL) enhanced the expression of progesterone receptors A and B, whereas high concentration of progesterone (5.68 ng/mL) inhibited the progesterone receptor expressions [49–51]. Kempisty et al.[52] suggested that early rise in P4 concentration (> 5 ng/mL) has a negative effect on embryo development because serum progesterone concentration over 5 ng/mL increases the risk of bacterial infection. Hill et al. [53–55] sets the progesterone threshold at 1.53~4.36 ng/mL, and found that the progesterone concentration over 4.36 ng/mL has a significant negative effect on DNA hypermethylation on endometrium and induced apoptosis and oxidative stress in endometrial cell. Therefore, it is possible that 5 ng/mL P4 reduces the expression of progesterone receptor as compared with 1 and 3 ng/mL P4. In addition, 5 ng/ml P4 makes the uterus more susceptible to infection and induces apoptosis in BEEC and finally aggravates oxidative damage. Further research is needed to clarify the underlying mechanism.

In conclusion, 1 and 3 ng/mL P4 inhibited the LPS-induced oxidative damage in primary bovine endometrial epithelial cells through activating Nrf2/Keap1 signaling pathway. This antioxidative effect of P4 was probably mediated by PR.

4 Conclusion

This study shown that 1 and 3 ng/mL P4 inhibited the LPS-induced oxidative damage in primary bovine endometrial epithelial cells through activating Nrf2/Keap1 signaling pathway. This antioxidative effect was probably mediated by PR.

5 Methods

5.1 Cell Culture

The primary bovine endometrial epithelial cells were isolated as described from Dong et al.[56]. The uterus of healthy nonpregnant cows was collected from the slaughterhouse using aseptic operation. It was necessary to ensure that the uterus was free of infection and disease. The uterine horns of healthy cows were collected and placed in an ice box at 4°C and were brought back to the laboratory. The uterine surface was disinfected with iodophor and 75% alcohol. Under sterile conditions, the uterine horn was cut into small pieces of 3~4 cm, and was rinsed repeatedly with phosphate-buffered saline (PBS, pH values from 7.2 to 7.4) containing 500 U/mL penicillin and 500 U/mL streptomycin. The uterine horn was cut longitudinally to expose the endometrial tissue. The endometrial tissue was put into 0.1% streptoproteinase (P5147, sigma, USA) and then diluted in DMEM/F-12 (D8900, sigma, USA) at 4°C for 18 h. We use a sterile scalpel to scrape the surface of endometrium to collect cells in the super clean bench. The obtained cell suspension was centrifuged at 100×*g* for 5 min and was washed with PBS for 3 times. The cells were resuspended in DMEM/F-12 containing 15% fetal bovine serum (Gibco, USA) and were cultured at 37°C with 5% CO_2. The medium was changed every 24 h for routine culture.

5.2 Experiment Design and Treatments

LPS (L6529) was from *E. coli* O55:B5. Progesterone (P0130) and RU486 (M8046) were purchased from Sigma-Aldrich. LPS lyophilized powder was dissolved in DMEM/F12 at a concentration of 1 mg/mL as stock solutions at -20°C. LPS was further diluted to 1 μg/mL by DMEM/F12 during experiment. Progesterone was configured to a storage concentration of 100 μg/mL using DMEM/F12. Under physiological conditions, the concentration of progesterone in cow serum ranges from 1.03 ng/mL to 5.1 ng/mL [57], so the final concentration of progesterone we selected was 1, 3 and 5 ng/mL. RU486 is an antagonist of progesterone receptor (PR). The 50% inhibition concentration of RU486 as a progesterone receptor inhibitor was 0.2 nM [58]. RU486 was dissolved in ethanol and was stored at -20°C with the concentration of 118.5 μg/mL. RU486 was further diluted to 35 ng/mL with DMEM/F12 during the experiment. To determine the effect of P4 on the LPS-induced oxidative damage of BEEC, the cells were treated with LPS (1 μg/mL), or cotreated with LPS and P4 (1, 3, and 5 ng/mL). RU486 was used to further explore the potential mechanism of P4 on the oxidative damage. The cells were treated with RU486 (35ng/mL) or cotreated with LPS, P4 (3 ng/mL), and RU486 (35 ng/mL). To detect the oxidative markers, the cells were collected 12 h after treatment. For the detection of the gene expressions, the cells were collected at 6, 12, and 24 h. To detect the key protein levels of the Nrf2/Keap1 signaling pathway, the cells were collected at 90 min. The selection of time point was based on the pre-experimental results.

5.3 Reactive Oxygen Species Evaluation

The level of ROS was detected using fluorescent probe DCFH-DA combined with flow cytometry (FACS Calibur, BD Biosciences). According to the instruction of the kit (Reactive Oxygen Species Assay Kit, Beyotime, Beijing, China), the BEEC were inoculated in six-well plates and were treated according to the

experiment design. Then the cells were collected and washed with PBS for three times, followed by the addition of the probe containing DCFH-DA (10 μM). The cells were incubated in 37°C cell incubator for 30 min. Then the cells were washed three times with serum-free cell culture medium. Flow cytometry was performed using 488 nm excitation wavelength and 525 nm emission wavelength. Data analysis was performed using the FlowJo software V10.0 (Ashland, KY, USA).

5.4 Determination of MDA, SOD, CAT and T-AOC

After treatment for 12 h, the collected cells were lysed by ultrasound on ice and then centrifuged at 6000×*g* for 10 min at 4°C. The supernatant was collected for oxidative damage labeling and antioxidant enzyme activity analysis. The MDA (A003-4-1), SOD (A001-3-2), CAT (A007-1-1), and T-AOC (A015-2-1) assay kits were purchased from Nanjing Jiancheng Bioengineering Institute.

5.5 RNA Extraction and Quantitative PCR

The cells were inoculated in a six-well plate. Total RNA was extracted using Trizol reagent (ET111, Tran, China) after treatment. The quantity and purity of RNA was detected by a Nanodrop 2000 spectrophotometer (Thermo, USA). The RNA absorption ratios (A260/A280) between 1.8 and 2.1 can be used for subsequent experiments. The total RNA was converted into cDNA using the primescript RT Regent kit gDNA eraser (DRR047A, Takara, Japan). Real-time PCR was used to detect the expression of mRNA according to SYBR Premix Ex Taq™ II (RR820A, TaKaRa, Japan) on the CFX 96 Real-Time PCR Detection System (BIO-RAD, USA). The SYBR Premix, primers of target gene and template DNA were added into the amplification mixtures. The following cycling conditions were performed: 95°C for 2 min; 95°C for 5 s and 60°C for 34 s, 40 cycles; 95°C for 15 s; 60°C for 5 s; 60°C~95°C, 0.5°C gradient heating. The $2^{-\triangle\triangle Ct}$ method was carried out to measure the relative abundance of mRNA transcripts. A single product was amplified by each primer pair. The products were purified and sequenced (TsingKe Biotech, Beijing, China), and then the sequence results were analysed through BLAST (http://blast.ncbi.nlm.nih.gov/blast.cg) and compared to GenBank database. The sequences of primers were shown in Table 1.

Table 1
Primer sequences used for quantitative real−time PCR

Gene	Primer sequence (5' → 3')	Length (bp)	NCBI accession
NFE2L2	F: CCCAGTCTTCACTGCTCCTC	165	NM_001011678.2
	R: TCAGCCAGCTTGTCATTTTG		
KEAP1	F: TCACCAGGGAAGGATCTACG	199	NM_001101142.1
	R: AGCGGCTCAACAGGTACAGT		
HMOX1	F: GGCAGCAAGGTGCAAGA	221	NM_001014912.1
	R: GAAGGAAGCCAGCCAAGAG		
NQO1	F: AACCAACAGACCAGCCAATC	154	NM_001034535.1
	R: CACAGTGACCTCCCATCCTT		
GPX2	F: CTTCAACCTGTCCTCCCT	98	NM_174076.3
	R: GGTCATTCATCTGGGTGT		
GCLC	F: ATTGGGTGGAGAGTGGAA	133	NM_001083674
	R: ACAGCGGGATGAGAAAGT		
GSTP1	F: TTTGCGGACTACAACCTG	186	NM_177516.1
	R: CCCTCACTGTTTCCCATT		
ACTB	F: CAGCAAGCAGGAGTACGATGAG	85	NM_173979.3
	R: AAGGGTGTAACGCAGCTAACAGT		

5.6 Western Blot Assay

Total protein and nuclear protein were extracted separately. Ripa lysate (P0013b, Beyotime, China) was used for total protein, and its main component was 50 mM Tris (pH7.4), 150 mM NaCl, 1% Triton X-100, 1% sodium deoxyholate, 0.1% SDS, sodium orthovanadate, sodium fluoride, EDTA, leupeptin and other inhibitors. Nuclear protein was extracted by nuclear protein and cytoplasmic protein extraction kit (P0013b, Beyotime, China). Both were quantified by BCA protein assay kit (Beyotime, China). The quantified protein (20~30 μg) was separated on 10% SDS polyacrylamide gel after electrophoresis, and was transferred to a PVDF membrane. The PVDF membrane was incubated in 5% skimmed milk for 1.5 h at room temperature. After completion of the sealing, the PVDF membrane was washed with TBST (0.05% Tween-20 Tris-HCl buffer) for six times with each 5 min. Then the PVDF membranes were incubated with corresponding primary antibodies specific for Nrf2 (#12721, Cell Signaling Technology, USA), Keap1 (ab227828, Abcam, UK), HO-1 (ab227828, Abcam, UK), NQO1 (ab80588, Abcam, UK), LaminB1(#13435, Cell Signaling Technology, USA) and β-actin (#4970, Cell Signaling Technology, USA) at 4°C overnight. These antibodies were diluted to 1:1000 with 5% bovine serum albumin. Then the PVDF

membrane was incubated with the secondary antibody (diluted to 1:2000 with 5% skimmed milk) for 1.5 h at room temperature. The protein bands were detected using a chemiluminescence assay. The antigen-antibody complexes were visualized on horseradish peroxidase substrate (Millipore, Billerica, MA, USA) by ChemiScope 5300Pro CCD camera (Clinx Science Instruments, Shanghai, China). The band intensity was quantified by Quantity One software (Bio-Rad, California, USA).

5.7 Immunofluorescence Staining

The cells were inoculated into a 24-well plate and was treated according to the experiment design. Then the cells were covered with 4% formaldehyde to a depth of 2~3 mm. The cells were fixed at room temperature for 15 min and was washed with PBS three times with each 5 min. Then the cell membrane was penetrated with 0.4% Triton X-100 (ST797, Beyotime, China) for 15 min. After washing with PBS, the cells were blocked in blocking buffer (5% BSA) for 60 min at room temperature. The primary antibody for Nrf2 (dilution ratio 1:200) was prepared in antibody dilution buffer (5% BSA). The cells were incubated with primary antibody at 4°C overnight and were washed with PBS three times with each 5 min. The fluorescein coupled secondary antibody (A0423, beyotime, China) was diluted with antibody dilution buffer, and the cells were incubated in dark for 1.5 h at room temperature. The nuclei were stained with DAPI (C1005, Beyotime, China). The fluorescence microscope (Leica TCS Sp8, Leica company, Germany) was used for observation.

5.8 Statistical Analysis

The experiment was repeated at least three times. All data were analyzed using the SPSS-Statistics 21.0 software (IBM, NY, USA). Statistically significant differences were calculated by one-way ANOVA, followed by Dunnett's test. The data were presented as means ± standard error of the means (SEM). A two-sided $P < 0.05$ was considered statistically significant.

Abbreviations

BEEC: Bovine endometrial epithelial cells; LPS: lipopolysaccharide; ROS: Reactive oxygen species; MDA: malondialdehyde; GSH-PX: glutathione peroxidase；P4: Progesterone; ARE: anti-oxidative response element; PR: progesterone receptor; DMEM/F-12: Dulbeccos's Modified Eagle Media/Nutrient Mixture F-12; cDNA: Complementary DNA; *E.coli*: Escherichia coli; qPCR: Quantitative polymerase chain reaction; DCFH-DA: 2,7-Dichlorodihy drofluorescein diacetate; SOD: Superoxide Dismutase; CAT: Catalase; T-AOC: Total antioxidant capacity; BSA: Bovine serum albumin; PVDF: Poly(vinylidene fluoride).

Declarations

Ethics approval and consent to participate

This study was conducted according to the guidelines of College of Veterinary Medicine, Yangzhou University.

Consent for publication

Not applicable

Competing interests

The authors declared that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Authors' contributions

LC conceived and designed the study. QZ performed the experiments and wrote the manuscript. QZ and JZ analyzed the data. HW, JD, JL, JJL, and CQ supervised and edited the article. All authors contributed to the article and approved the final version of the article.

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Availability of data and materials

All datasets presented in the study are available from LC, QZ and JJL on request.

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Not applicable

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Figures

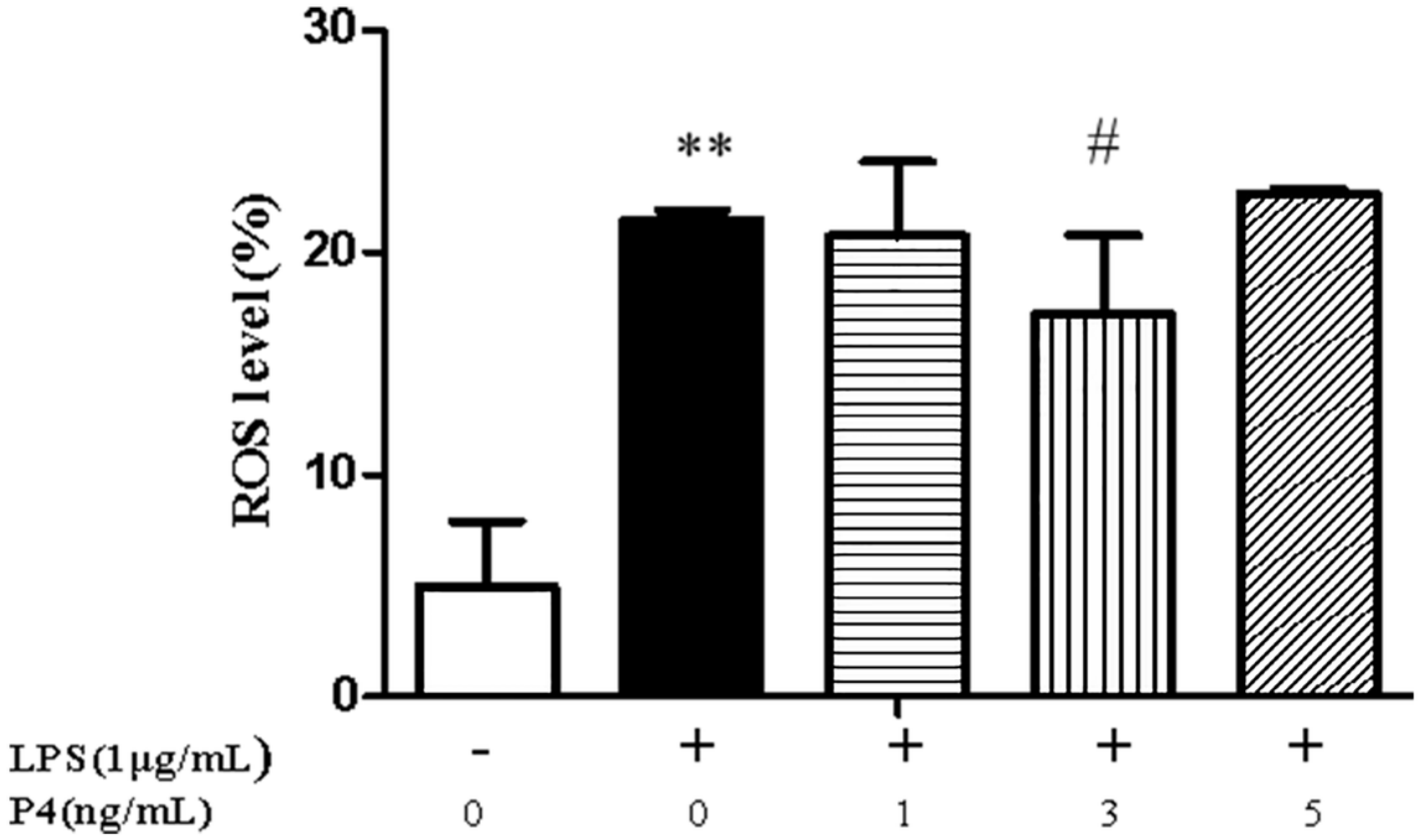

Figure 1

The effect of progesterone (P4) on the level of reactive oxygen species (ROS) in primary bovine endometrial epithelial cells (BEEC) stimulated by lipopolysaccharide (LPS). The cells were cotreated with LPS (1 μg/mL) and P4 (1, 3, and 5 ng/mL), and were collected 12 h after treatment. All data were presented as means ± SEM (n = 3). ** $P < 0.01$, difference compared with the control; # $P < 0.05$, difference compared with the LPS group.

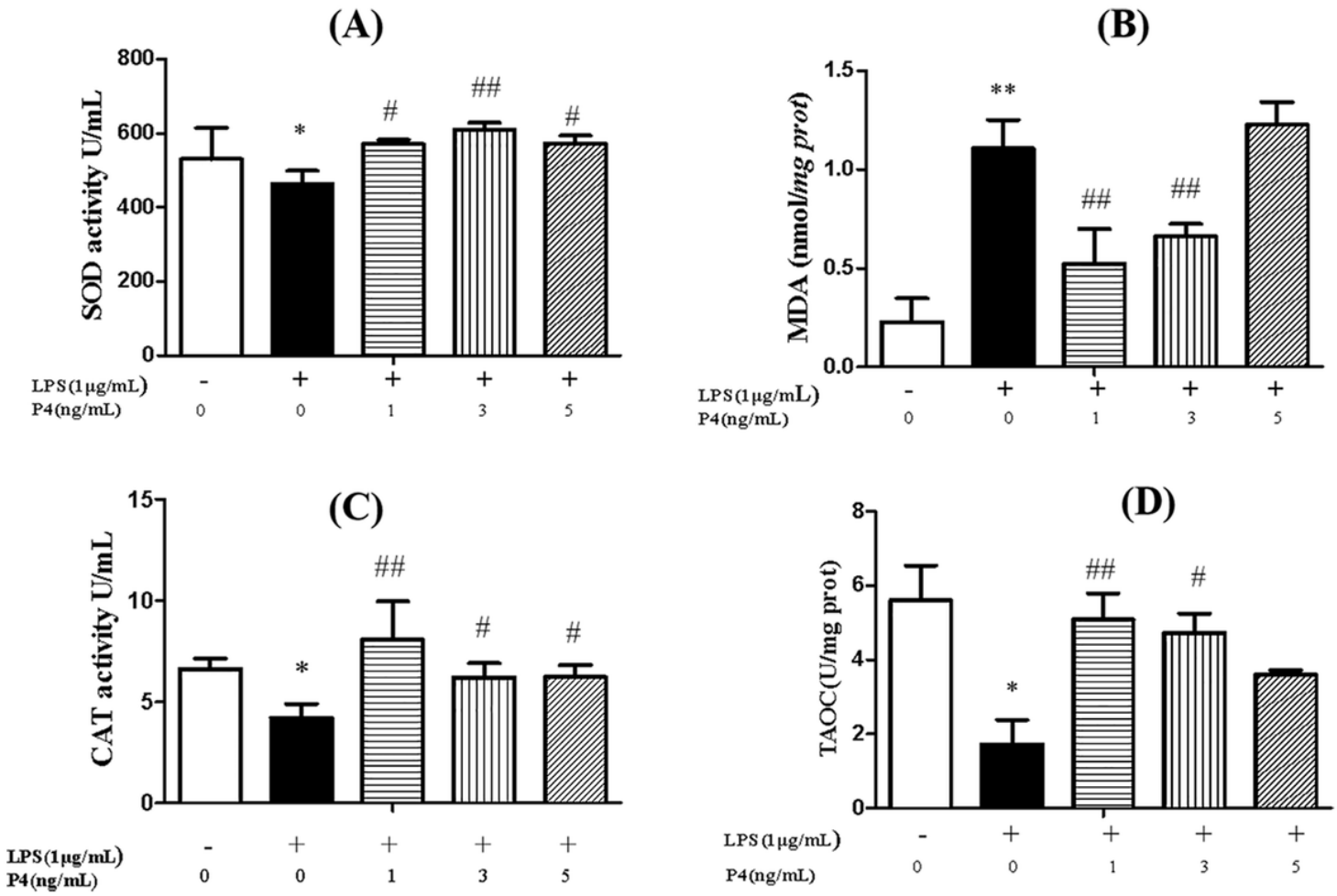

Figure 2

The effect of progesterone (P4) on the level of malondialdehyde (A), the activities of superoxide dismutase (B) and catalase (C), and the total antioxidant capacity (D) in primary bovine endometrial epithelial cells (BEEC) stimulated by lipopolysaccharide (LPS). The cells were cotreated with LPS (1 μg/mL) and P4 (1, 3, and 5 ng/mL), and were collected 12 h after treatment. MDA, malondialdehyde; SOD, superoxide dismutase; CAT, catalase; T-AOC, total antioxidant capacity. All data were presented as means ± SEM (n = 3). * $P < 0.05$, ** $P < 0.01$, difference compared with the control; # $P < 0.05$, ##$P < 0.01$, difference compared with the LPS group.

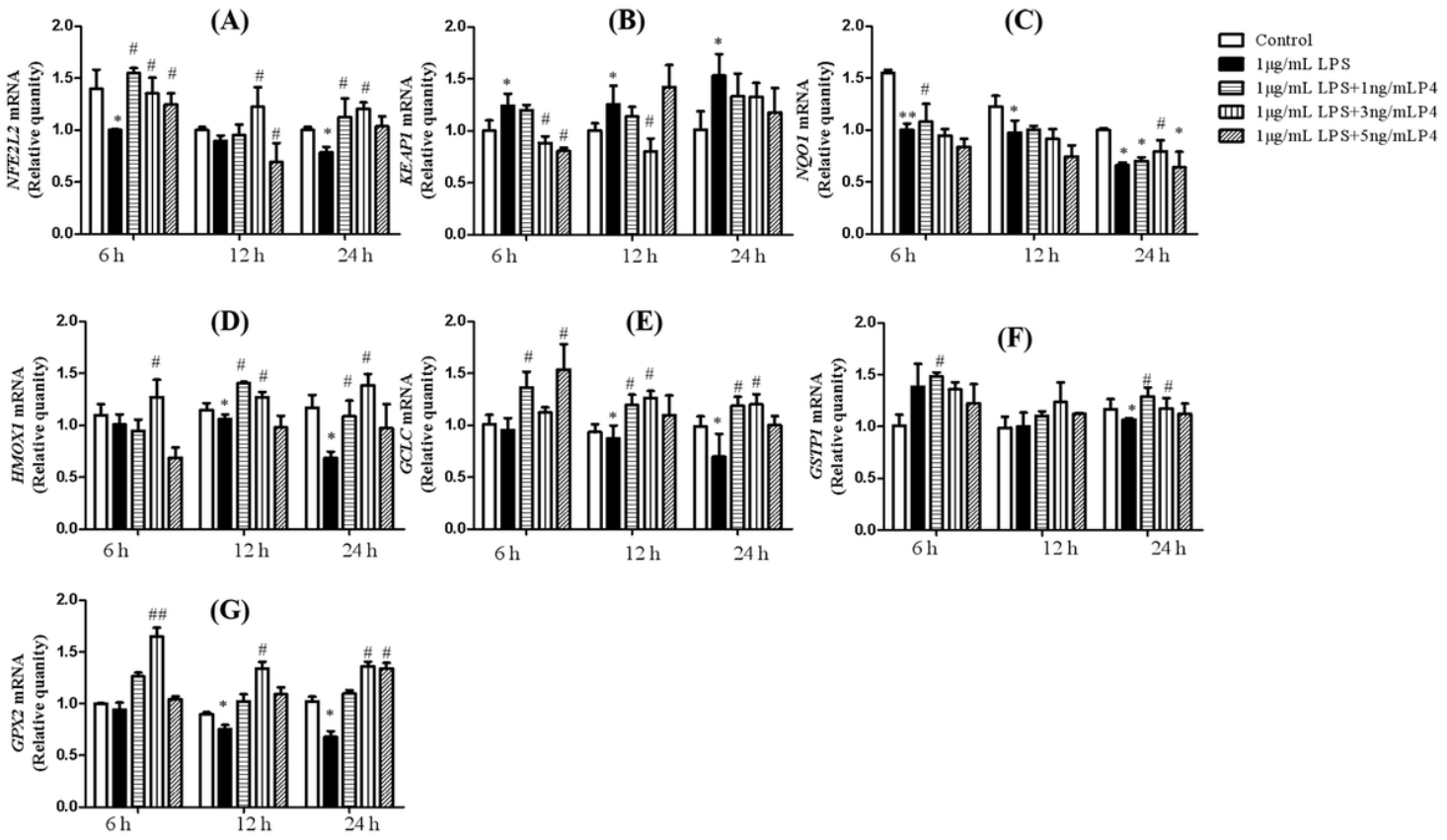

Figure 3

The effect of progesterone (P4) on the relative mRNA abundance of NFE2L2 (A), KEAP1 (B), NQO1 (C), HMOX1 (D), GCLC (E), GSTP1 (F), and GSTP2 (G) in primary bovine endometrial epithelial cells (BEEC) stimulated by lipopolysaccharide (LPS). The cells were cotreated with LPS (1 μg/mL) and P4 (1, 3, and 5 ng/mL), and were collected at 6, 12, and 24 h. All data were presented as means ± SEM (n = 3). * $P < 0.05$, ** $P < 0.01$, difference compared with the control; # $P < 0.05$, ##$P < 0.01$, difference compared with the LPS group.

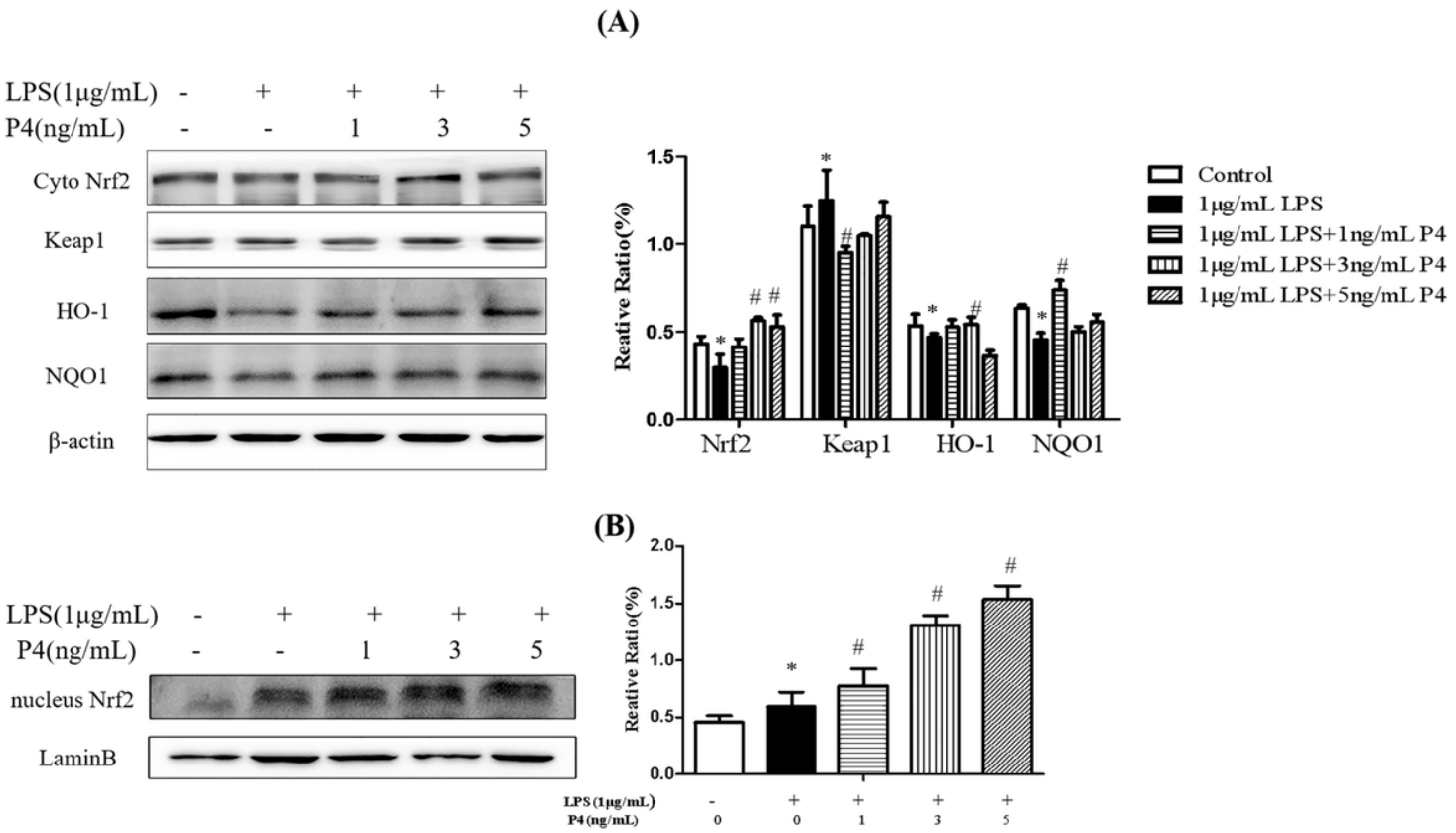

Figure 4

The effect of progesterone (P4) on the protein expression of Nrf2/Keap1 pathway in primary bovine endometrial epithelial cells (BEEC) stimulated by lipopolysaccharide (LPS). The cells were cotreated with LPS (1 μg/mL) and P4 (1, 3, and 5 ng/mL), and were collected at 90 min. (A), protein expression of cyto Nrf2, Keap1, HO-1 and NQO1; (B), protein expression of nucleus Nrf2. * $P < 0.05$, ** $P < 0.01$, difference compared with the control; # $P < 0.05$, ##$P < 0.01$, difference compared with the LPS group.

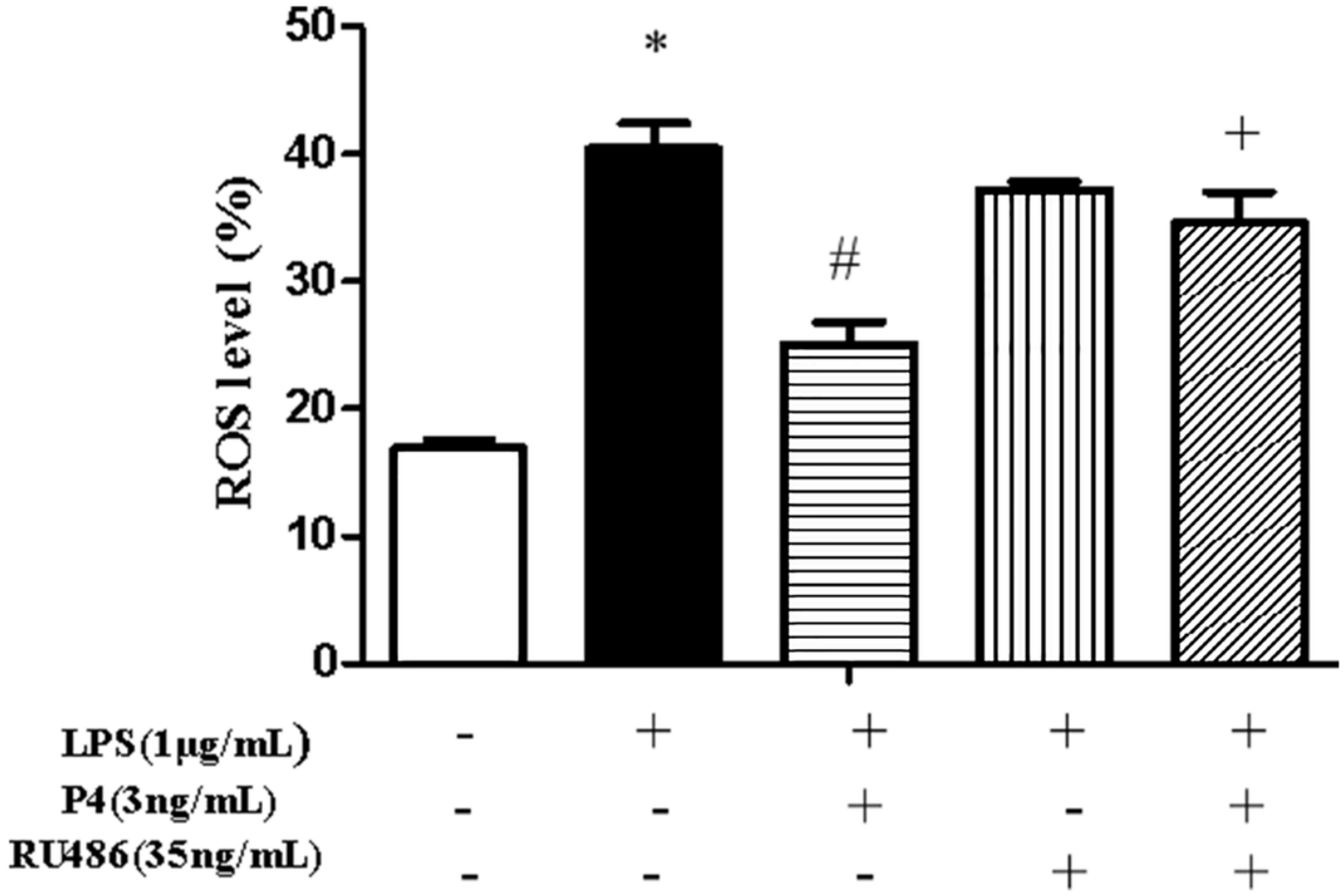

Figure 5

The effect of progesterone (P4) on the level of reactive oxygen species (ROS) in primary bovine endometrial epithelial cells (BEEC) stimulated by lipopolysaccharide (LPS). The cells were cotreated with LPS (1 μg/mL), P4 (3 ng/mL), and RU486 (35 ng/mL), and were collected 12 h after treatment. All data were presented as means ± SEM (n = 3). * $P < 0.05$, difference compared with the control; # $P < 0.05$, difference compared with the LPS group; + $P < 0.05$, difference compared with the cotreatment group of LPS and P4.

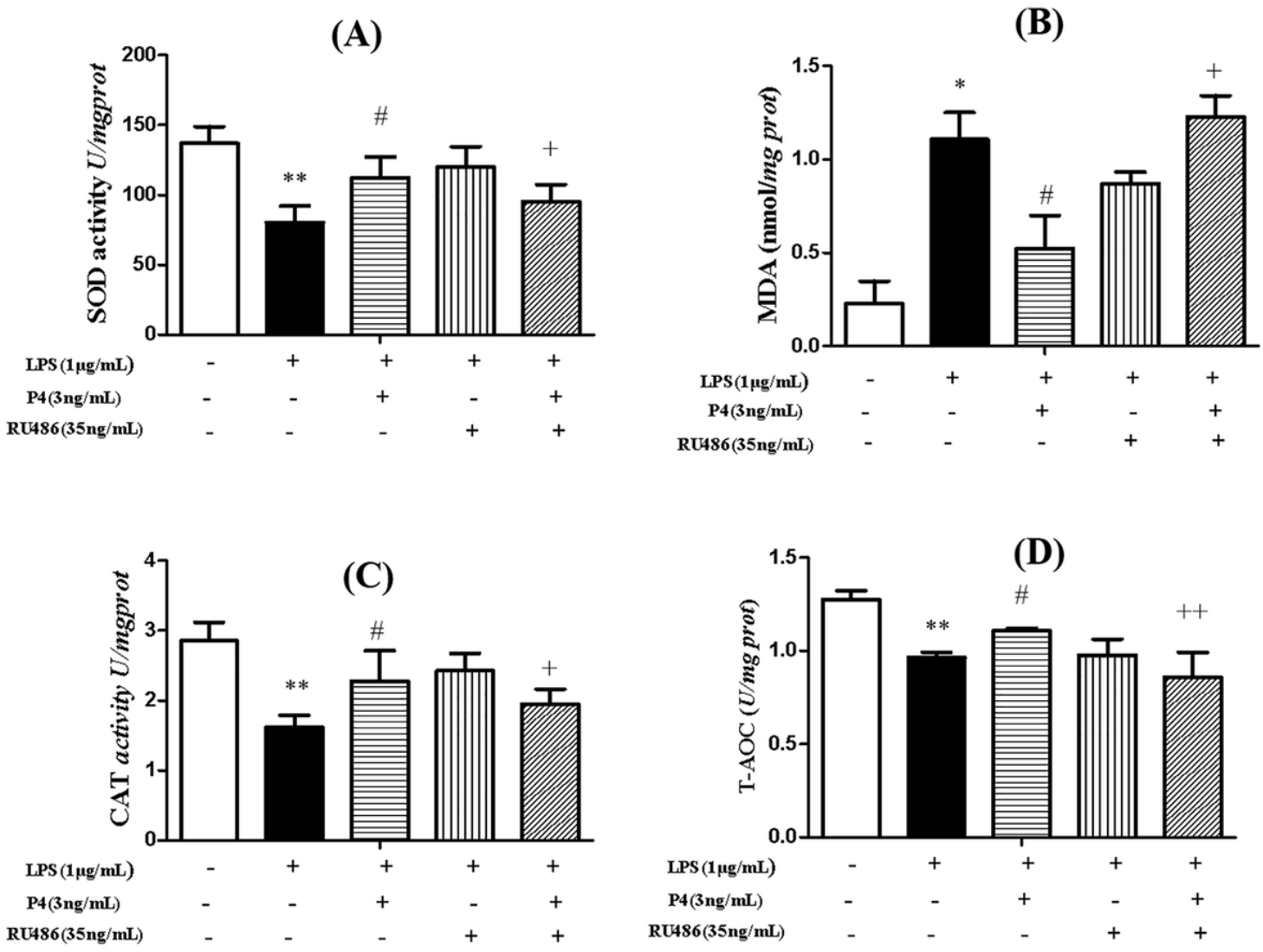

Figure 6

The effect of progesterone (P4) on the level of malondialdehyde (A), the activities of superoxide dismutase (B) and catalase (C), and the total antioxidant capacity (D) in primary bovine endometrial epithelial cells (BEEC) stimulated by lipopolysaccharide (LPS). The cells were cotreated with LPS (1 μg/mL), P4 (3 ng/mL), and RU486 (35 ng/mL), and were collected 12 h after treatment. All data were presented as means ± SEM (n = 3). * $P < 0.05$, difference compared with the control; # $P < 0.05$, difference compared with the LPS group; + $P < 0.05$, difference compared with the cotreatment group of LPS and P4.

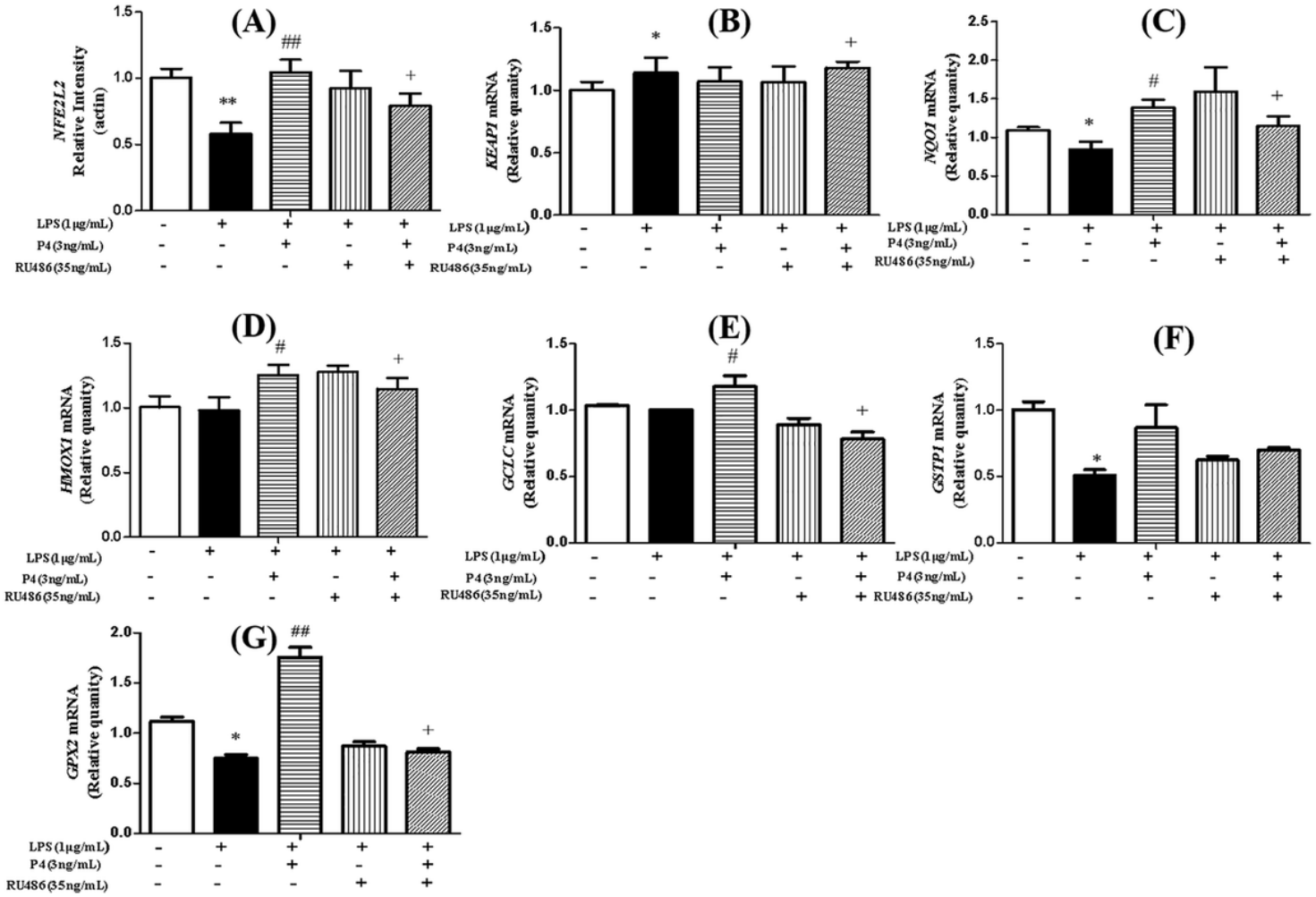

Figure 7

The effect of progesterone (P4) on the relative mRNA abundance of NFE2L2 (A), KEAP1 (B), NQO1 (C), HMOX1 (D), GCLC (E), GSTP1 (F), and GSTP2 (G) in primary bovine endometrial epithelial cells (BEEC) stimulated by lipopolysaccharide (LPS). The cells were cotreated with LPS (1 μg/mL), P4 (3 ng/mL), and RU486 (35 ng/mL), and were collected 6, 12, 24 h after treatment. All data were presented as means ± SEM (n = 3). * $P < 0.05$, difference compared with the control; # $P < 0.05$, difference compared with the LPS group; + $P < 0.05$, difference compared with the cotreatment group of LPS and P4.

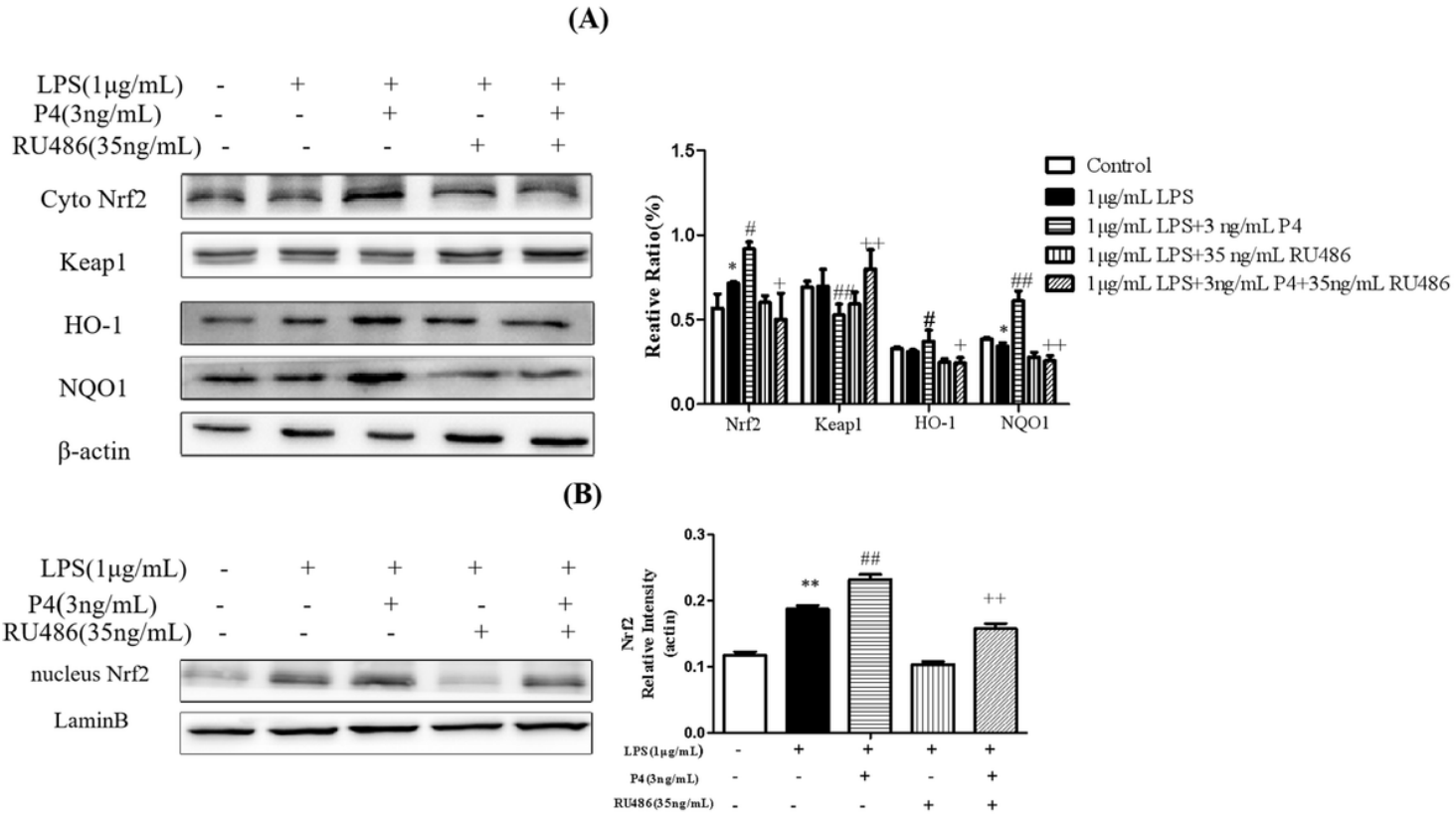

Figure 8

The effect of progesterone (P4) on the key protein expressions in Nrf2/Keap1 pathway in primary bovine endometrial epithelial cells (BEEC) stimulated by lipopolysaccharide (LPS). The cells were cotreated with LPS (1 μg/mL), P4 (3 ng/mL), and RU486 (35 ng/mL), and were collected 90 min after treatment. All data were presented as means ± SEM (n = 3). * $P < 0.05$, difference compared with the control; # $P < 0.05$, difference compared with the LPS group; + $P < 0.05$, difference compared with the cotreatment group of LPS and P4.

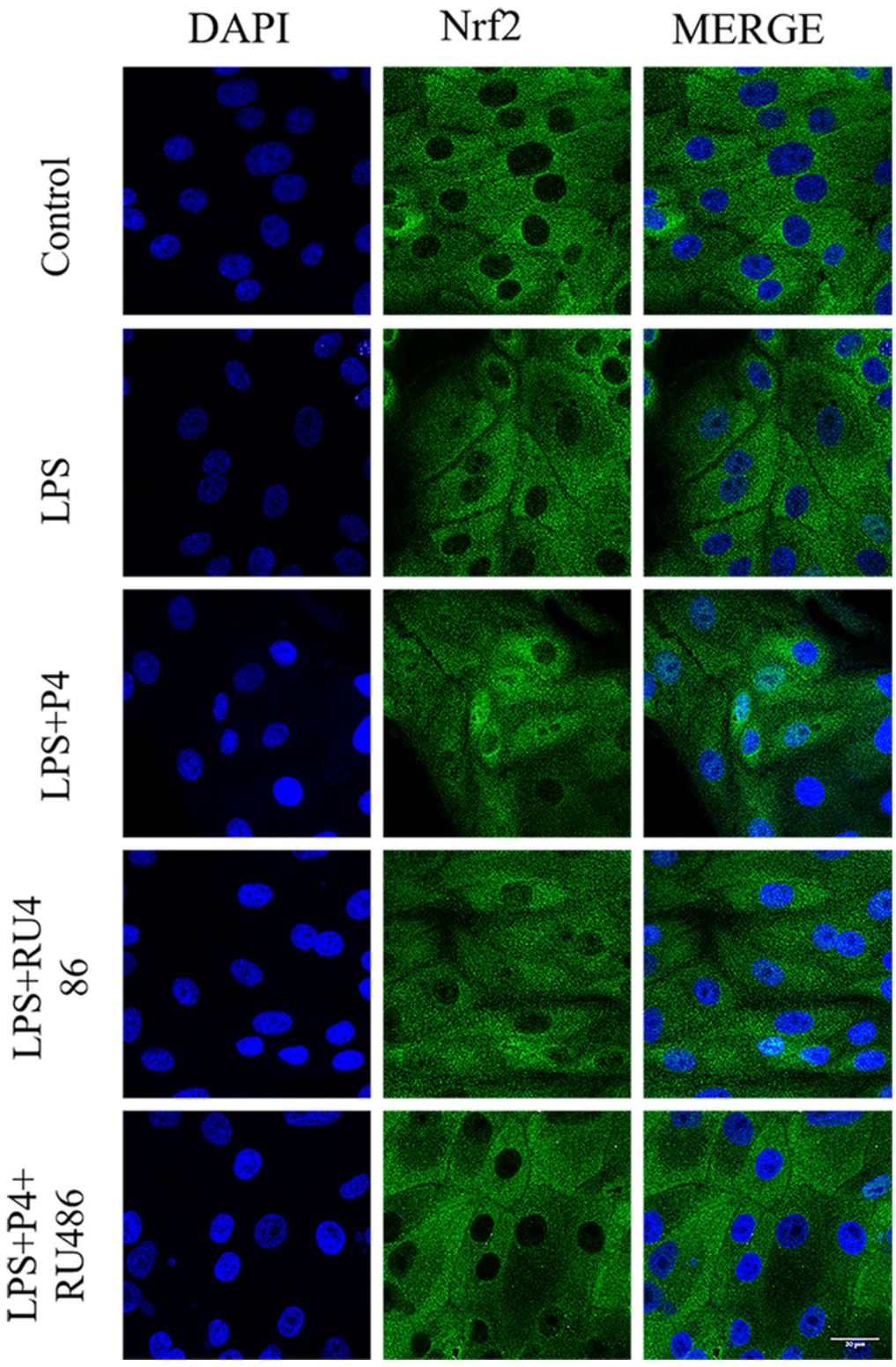

Figure 9

The effect of progesterone and RU486 on Nrf2 translocation in primary bovine endometrial epithelial cells. The cells were cotreated with LPS (1 μg/mL), P4 (3 ng/mL), and RU486 (35 ng/mL), and were collected at 90 min. The Nrf2 were observed by confocal microscopy.

Supplementary Files

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