Effects of Estradiol and Progesterone on IL-1β mRNA Expression in Fallopian Tube Tissue of LPS-induced Ovariectomized Mice

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Abstract **[Objective]** The paper was to study the effects of estradiol (E_2) and progesterone (P4) on the expression of interleukin-1β (IL-1β) mRNA in lipopolysaccharide (LPS)-induced acute salpingitis in ovariectomized mice, and preliminarily explore the anti-inflammatory mechanism associated with estrogen and progesterone. **[Method]** Healthy female KM mice were randomly assigned to several groups: the sham operation group (LPS+SHAM), the ovarian removal group (LPS+OVX), the ovarian removal + estradiol group (LPS+OVX+E_2), the ovarian removal + progesterone group (LPS +OVX+P4), the LPS group and the control group (control). HE staining was conducted to assess the pathological changes in the fallopian tubes of each group. Additionally, the expression levels of IL-1β mRNA in the fallopian tubes of the mice were quantified using RT-qPCR. **[Result]** The histopathological changes in the fallopian tubes were examined. Estrogen and progesterone demonstrated a significant capacity to mitigate salpingitis induced by LPS. In comparison to the control group, the expression of IL-1β mRNA in the LPS group, LPS+SHAM group, and LPS+OVX+E_2 group was significantly down-regulated (P<0.05). Furthermore, the expression of IL-1β mRNA in the LPS+OVX+P4 group exhibited an extremely significant down-regulation (P<0.01). When compared to the LPS+OVX group, the expression of IL-1β mRNA in the LPS+OVX+E_2 group was significantly down-regulated (P<0.05), while the expression in the LPS+OVX+P4 group was extremely significantly down-regulated (P<0.01). **[Conclusion]** Estrogen and progesterone have the capacity to inhibit the expression of IL-1β mRNA in the inflammatory tissue of the fallopian tubes in mice, consequently diminishing the inflammatory response induced by LPS.

Keywords Estradiol; Progesterone; LPS; Salpingitis; IL-1β

In humans and female animals, the fallopian tube serves as a crucial reproductive organ that orchestrates numerous fundamental functions of the host, thereby creating a conducive microenvironment for embryo conception. The tubal microenvironment is composed of secretions from the fallopian tubes, which encompass specific proteins, enzymes, cytokines, adhesion factors, and a diverse array of other substances that play a role in the physiopathological processes associated with the fallopian tubes. The incidence of fallopian tube diseases is significantly associated with these substances. Salpingitis is an acquired disease of the reproductive organs, primarily resulting from secondary infections of cervicitis and endometritis. Once inflammation occurs in the fallopian tubes, it adversely impacts the fertilization process, the transport of the fertilized egg, and the development of the embryo, ultimately contributing to female infertility[1–2]. The functional regulation of tubal biology is primarily governed by endocrine mechanisms that are exclusively modulated by the ovarian steroids, estrogen (E_2) and progesterone (P4)[3]. The innate and adaptive immune systems in the human and mammalian female reproductive tracts are hormonally regulated to prevent infections by foreign pathogens, while also facilitating successful reproduction. Immune protection within the female reproductive tract is influenced by varying levels of estrogen and progesterone[4]. Research has demonstrated that the reproductive steroid hormones estrogen and progesterone possess anti-inflammatory properties, facilitate the remodeling of immune cells, and enhance antibody production. The tubal microenvironment employs various mechanisms to defend against pathogens, including its innate immune antimicrobial functions, the secretion of antimicrobial factors, and the elimination of potential bacterial and viral pathogens[5-6]. Additionally, it has the capacity to regulate the recruitment and activity of immune cells from both the innate and adaptive immune systems[7]. These functions are partially mediated by the production of various innate effectors, including the pro-in-

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flammatory IL-1β, which is rapidly induced following the exposure of immune cells to lipopolysaccharide (LPS)[8]. The role of estrogen and progesterone in regulating the gene expression of IL-1β within the inflammatory tissues of the fallopian tubes in female animals remains unclear. Consequently, this study was undertaken to examine the effects of these hormones on the regulation of IL-1β cytokine expression in the inflammatory tissues of fallopian tubes. This investigation was based on the establishment of an inflammation model in ovariectomized animals, aimed at exploring the anti-inflammatory effects of estrogen and progesterone.

1 Materials and Methods

1.1 Animals A total of 35 SPF-grade female KM mice, aged between 6 to 8 weeks, were procured from SPF (Beijing) Bio-technology Co., Ltd., under Animal Production Licence [SCXK (Beijing) 2019-0010]. The mice were housed in the Experimental Animal Centre of Inner Mongolia Medical University, where they had *ad libitum* access to food and water throughout the duration of the experiment. The ambient temperature in the facility was consistently maintained at 20 ℃.

1.2 Drugs and agents LPS was acquired from Promega, estradiol benzoate was sourced from Ningbo Sansheng Biological Technology Co., Ltd., and progesterone was obtained from Coolaber. The TRIzon total RNA extraction reagent was utilized, along with the PrimeScript™ RT Reagent Kit for RNA reverse transcription and the SYBR Premix Ex Taq™ Kit for real-time fluorescence quantitative PCR, all of which were products of TaKaRa Bio-technology (Dalian) Co., Ltd.

1.3 Establishment of ovariectomized mouse model Mice in each group were intraperitoneally injected with 0.15 mL of 1% pentobarbital sodium. After a duration of 3 min, the mice were rendered completely anesthetized. Subsequently, the mice were positioned with their backs facing upwards, and their fur was clipped and disinfected. A transverse incision measuring 1–2 cm was made on the back, adjacent to the last rib. An opening was created at the site of the adipose tissue on both sides of the ribs, and the fat was extracted using ophthalmological forceps. The cysts were then incised, exposing the ovaries, which were excised. The uterine horns were returned to the abdominal cavity using forceps, and the incision was subsequently closed.

1.4 Grouping and interventions The mice were randomly assigned to one of four groups two weeks postoperatively: the sham operation group (SHAM), the ovary removal group (OVX), the ovary removal + estradiol group (OVX+E_2), and the ovary removal + progesterone group (OVX+P4). Each group consisted of five mice. The mice received intraperitoneal administration of the respective drugs, as required by the dosage regimen, once daily for one week (Tab.1).

Prior to the establishment of the LPS inflammation model, all mice were subjected to a fasting period of 10 h. Subsequently, each group received an intraperitoneal administration of 3 mg/kg of LPS. The experimental groups included the lipopolysaccharide +sham operation group (LPS +SHAM), the lipopolysaccharide + ovarian removal+vegetable oil group (LPS+OVX), the lipopolysaccharide+ovarian removal+estradiol group (LPS+OVX+E_2), and the lipopolysaccharide +ovarian removal + progesterone group (LPS+OVX+P4). Additionally, the remaining mice that did not undergo surgical or pharmacological interventions were categorized into a saline control group (control) and a lipopolysaccharide group (LPS). Each mouse in the LPS group received an intraperitoneal injection of 3 mg/kg of lipopolysaccharide, while each mouse in the control group was administered an equivalent volume of saline via the same route.

1.5 Specimen collection and pathological observations of tubal tissues After 24 h, the mice were euthanized via cervical dislocation, and both fallopian tube specimens were collected. One half of the specimens was immersed in a 10% formalin solution for temporary fixation, followed by paraffin embedding, sectioning, HE staining, and subsequent observation of histomorphological changes in the fallopian tubes of each group. The remaining half of the fallopian tube specimens was stored at −80 ℃ for future total RNA extraction.

1.6 RT-qPCR assay Total RNA was extracted from the fallopian tube tissues of each group of mice in accordance with the TRIzon Total RNA Extraction Instructions. cDNA was synthesized using the PrimeScript™ RT Reagent Kit. The cDNA served as a template for RT-qPCR amplification, which was conducted using the SYBR Premix Ex Taq™ II kit. The reaction conditions were as follows: an initial denaturation at 95.0°C for 1 min, followed by 40 cycles of denaturation at 95.0 ℃ for 10 sec, annealing at 63.0 ℃ for 20 sec, and extension at 72.0 ℃ for 10 sec, concluding with a final extension at 72.0 ℃ for 7 min.

The primer sequences for IL-1β were as follows: the sense primer was 5′-CA

Tab.1 Treatment of animals in each group (n=5)

Group	Surgical intervention	Drug	Dosage	Time//d	Approach
SHAM	Sham operation	Vegetable oil	0.1 mL/time/day	7	Intraperitoneal injection
OVX	Bilateral ovariectomy	Vegetable oil	0.1 mL/time/day	7	Intraperitoneal injection
OVX+E_2	Bilateral ovariectomy	Estradiol benzoate	1 μg/time/day	7	Intraperitoneal injection
OVX+P4	Bilateral ovariectomy	Progesterone	2 mg/time/day	7	Intraperitoneal injection

ACCAACAAGTGATATTCTCC-3′, and the antisense primer was 5′-GATCCACACTCT CCAGCTGCA-3′. Additionally, the primer sequences for the internal reference gene β-actin included the sense primer 5′-GA TTACTGCTCTCTGGCTCCTAGC-3′ and the antisense primer 5′-GACTCATCGTACTC-CTGCTTGC-3′.

1.7 Statistical methods The data were analyzed using the least significant difference (LSD) procedure within a one-way analysis of variance (ANOVA) in IBM SPSS Statistics 19.0. The results were presented as mean ± standard deviation ($\bar{x}\pm S$) and were deemed statistically significant when $P<0.05$, and highly significant when $P<0.01$.

2 Results and Analysis

2.1 Effect of oestradiol and progesterone on the histological structure of fallopian tube inflammation in ovariectomized mice HE staining revealed the absence of inflammatory cell infiltration in the mucosal, muscular, and plasma layers of the fallopian tube tissue of mice in the control group (Fig.1). Mucosal epithelial edema of the fallopian tube tissue in mice was observed in the LPS +OVX group, characterized by significant infiltration of inflammatory cells across all tissue layers. In the LPS+OVX+P4 and LPS+OVX+E_2 groups, partial infiltration of inflammatory cells was noted in the mucosal, muscular, and plasma membrane layers of the fallopian tube tissue. Additionally, a moderate degree of inflammatory cell infiltration was observed in both the LPS+SHAM and LPS groups.

2.2 qPCR melting curve The amplification of mouse IL-1β and β-actin genes via qPCR demonstrated melting curves characterized by a single peak, with no extraneous peaks observed at 82.5 ℃ and 84.5 ℃, respectively (Figs.2–3). Furthermore, the theoretical melting temperature (Tm) values for each amplification product closely aligned with those of the corresponding primary peaks. This alignment suggests that the qPCR products specifically corresponded to the IL-1β and β-actin genes of the mouse.

2.3 Effect of oestradiol and progesterone on IL-1β mRNA expression in inflammatory tissues of the fallopian tubes in ovariectomised mice IL-1β mRNA was observed in the fallopian tube tissues of mice across all experimental groups. When compared to the control group, the relative expression of IL-1β mRNA in the fallopian tube tissue was significantly elevated in the LPS group,

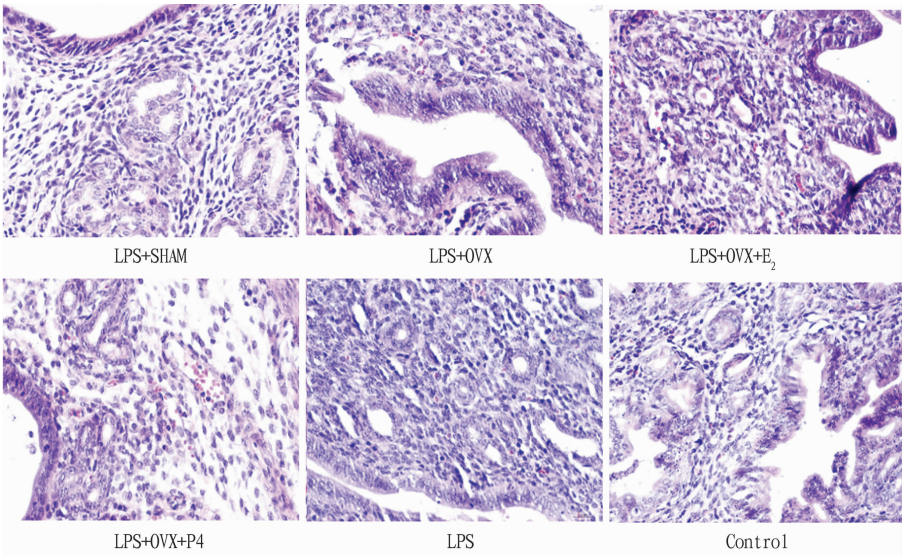


Fig.1 Histopathological sections of the fallopian tubes of mice in each group (HE staining, ×400)

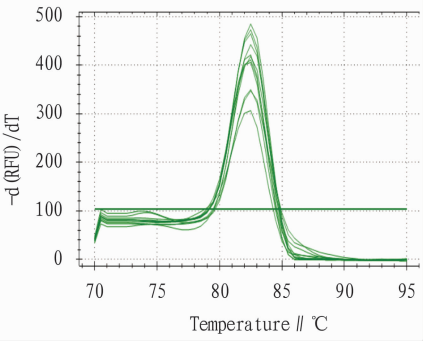


Fig.2 Melting curve of IL-1β

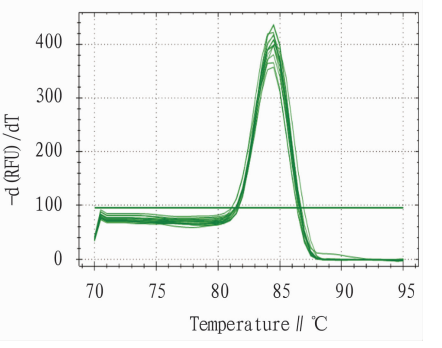
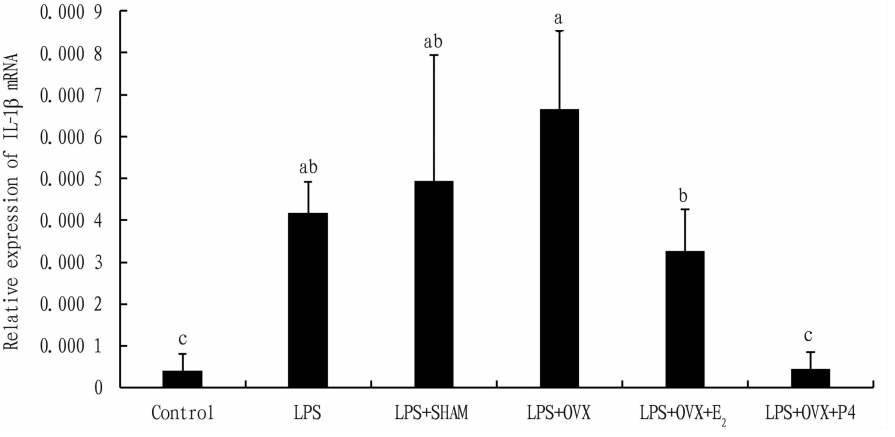


Fig.3 Melting curve of β-actin



Note: Different lowercase letters in the figure denote statistically significant differences ($P<0.05$).

Fig.4 Effect of oestradiol and progesterone on the relative expression of IL-1β mRNA in fallopian tube tissue of ovariectomized mice induced by LPS

the LPS +SHAM group, and the LPS + OVX+E_2 group (P<0.05). Furthermore, the relative expression of IL-1β mRNA in the fallopian tube tissue was markedly increased in the LPS+OVX group (P<0.01). However, no significant difference was found when comparing the LPS +OVX group to the LPS +OVX +P4 group (P >0.05). In comparison to the LPS+SHAM group, the relative expression of IL-1β mRNA in the fallopian tube tissue of the LPS +OVX group exhibited an upregulation, but this difference was not statistically significant (P>0.05). Conversely, when compared to the LPS+OVX group, the relative expression of IL-1β mRNA was significantly downregulated in the LPS+OVX+E_2 group (P<0.05). Furthermore, the LPS+OVX+P4 group demonstrated a highly significant downregulation of IL-1β mRNA expression in the fallopian tube tissue (P<0.01, Fig.4).

3 Discussion

Salpingitis is a prevalent gynecological condition characterized by pathology of the fallopian tube, which results in alterations in cytokine levels and functional abnormalities. These changes can lead to tubal infertility and ectopic pregnancy, posing significant risks to women's physical and mental health[1]. Reproductive tract infections are commonly attributed to gram-negative bacteria, including *Escherichia coli*, *Bartonella*, and *Salmonella*[9]. LPS, a significant component of the outer membrane of gram-negative bacterial cell walls, plays a crucial role in bacterial survival. It serves to inhibit the penetration of various antimicrobial agents by establishing an effective permeable barrier[10]. Currently, LPS has been extensively utilized in the development of animal models for studying inflammation and bacterial infections, as well as in *in vitro* cell culture systems. Consequently, LPS is employed to induce inflammatory models in fallopian tube tissue. The fallopian tube serves as the location for the capture of the ovum, fertilization, and the initial stages of embryonic development prior to implantation. The physiological functions and pathological processes of the fallopian tube are modulated by a diverse array of cytokines [2], which are a class of polypeptide proteins that facilitate inflammatory and immune responses, thereby playing a crucial role in the host's defense mechanisms against tissue damage[11]. Interleukin-1β (IL-1β) is a significant inflammatory cytokine, as it stimulates transcription and enhances the mRNA stability of other pro-inflammatory mediators[12]. The overproduction of IL-1β, in conjunction with other pro-inflammatory cytokines, results in an imbalance between pro-inflammatory and anti-inflammatory responses. This imbalance subsequently triggers both local and systemic responses to pathogen invasion. IL-1β is known to regulate a diverse array of biological functions within the female reproductive tract and is crucial in immunomodulation and inflammatory processes. Notably, studies measuring serum inflammatory cytokines have reported significantly elevated levels of serum IL-1β in patients diagnosed with tubal inflammatory infertility[13]. This finding suggests that IL-1β serves as a reliable indicator of the inflammatory status associated with tubal inflammatory disease. There exists both experimental and clinical evidence indicating that the ovarian steroid hormones, estrogen and progesterone, possess immunosuppressive properties and can exert anti-inflammatory effects through the modulation of the host immune response[14]. Consequently, the primary objective of the present study was to investigate the pathological alterations in the inflammatory tissues of the fallopian tubes in LPS-induced ovariectomized mice, as well as to assess the impact on the expression of IL-1β following the administration of exogenous estradiol and progesterone.

The results of HE staining indicated that histopathological alterations were present in the fallopian tubes. Furthermore, oestradiol and progesterone demonstrated the capacity to diminish the infiltration of inflammatory cells within the fallopian tube tissues of ovariectomized mice suffering from oviductitis. These hormones also contributed to the amelioration of inflammation severity and resulted in a reduction of overall inflammatory responses. The presence of IL-1β mRNA expression in the fallopian tubes of mice was confirmed through RT-qPCR analysis. A comparative assessment of the relative expression levels of IL-1β mRNA across various treatment groups revealed that the fallopian tube tissues from the LPS group, the LPS+ SHAM group, and the LPS +OVX +E_2 group exhibited a significant increase in IL-1β mRNA expression when compared to the control group (P<0.05). This finding indicates that LPS administration upregulated the secretion of IL-1β in the fallopian tube tissues of unspayed, sham-operated, ovariectomized, and ovariectomized mice treated with estradiol. Notably, the highest relative expression of IL-1β mRNA was observed in the LPS+OVX group (P<0.01), suggesting that the removal of the ovaries resulted in a reduction of ovarian steroid hormones, such as E_2 and P4. This hormonal deficiency appears to exacerbate the salpingitis infection process and further stimulate the secretion of the pro-inflammatory cytokine IL-1β. Ovary removal led to an increased relative expression of IL-1β mRNA in the tissues of the fallopian tubes. Conversely, the administration of oestradiol resulted in a down-regulation of IL-1β expression in these tissues. Notably, the injection of progesterone produced a highly significant reduction in IL-1β expression in the fallopian tube tissues of ovariectomized mice. These findings indicate that progesterone exerts a more pronounced down-regulatory effect on IL-1β mRNA under inflammatory conditions compared to estrogen, suggesting that progesterone plays a critical role in inhibiting IL-1β mRNA expression in mice. It is evident that oe-

strogen and progesterone significantly decrease the levels of the inflammatory cytokine IL-1β in inflamed tubal tissues, thereby demonstrating their anti-inflammatory properties. Notably, progesterone has been shown to effectively mitigate LPS-induced salpingitis, restoring the tissue's function to a state that is either normal or near-normal.

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