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Article

Immunosuppressant Cyclosporin A-Based Regulation of Lipopolysaccharide-Triggered Pro- and Anti-Inflammatory Cytokines in the Genital Tract of Female Rabbits

Li-Qin Yang[1], Qiu-Ying Wu[1], Xuan-Yu Chen[1], Chun Wang[1], Zhang Yan[1], Yu-Tian Zeng[1], Xu Ling[1], Chang-Jun Zeng[1,2], Guang-Bin Zhou[1,2], Izhar Hyder Qazi[2,3], Christiana Angel[4,5], Ming Zhang[1,2]*

[1] Farm Animal Genetic Resources Exploration and Innovation Key Laboratory of Sichuan Province, Sichuan Agricultural University, Chengdu-611130, P.R. China;
[2] College of Animal Science and Technology, Sichuan Agricultural University, Chengdu-611130, P.R. China;
[3] Department of Veterinary Anatomy & Histology, Shaheed Benazir Bhutto University of Veterinary and Animal Sciences, Sakrand-67210, Sindh, Pakistan; vetdr_izhar@yahoo.com (I.H.Q.);
[4] Department of Veterinary Parasitology, College of Veterinary Medicine, Sichuan Agricultural University, Chengdu-611130, P.R. China;
[5] Department of Veterinary Parasitology, Faculty of Veterinary Sciences, Shaheed Benazir Bhutto University of Veterinary and Animal Sciences, Sakrand-67210, Sindh, Pakistan.

*Correspondence: zhangming@sicau.edu.cn

Abstract: In this study, we evaluated the effects of Cyclosporine A (CsA) on Lipopolysaccharide (LPS)-induced cytokine production in the genital tract of female rabbits. Twelve sexually mature and healthy female rabbits were randomly divided into four groups (n = 3 each). The rabbits in the LPS group were given an intrauterine infusion of *Escherichia coli* LPS (4 mg/kg body weight (BW)). Rabbits in the CsA group were given CsA (20 mg/kg BW). Rabbits in the LPS + CsA group were given LPS (4 mg/kg BW) and CsA (20 mg/kg BW). The control group received only LPS and CsA carrier. The gene expression and protein levels of pro- and anti-inflammatory cytokines were observed using qRT-PCR and immuno-histochemical (IHC) assay, respectively. Our study showed that IL-1β, IL-6, IL-8, TNF-α, IFN-γ, IL-4, IL-10, IL-13, and TGF-β were expressed in female genital organs. The LPS challenge increased the mRNA expression of IL-6 and TNF-α in the uterine body and IL-1β in the uterotubal junction compared to the control group. CsA increased the basal mRNA expression of anti-inflammatory cytokines (i.e., IL-4 in the uterine body, uterotubal junction, and oviductal ampulla; IL-10 in the cervix, oviductal isthmus, and ampulla; and TGF-β in the uterotubal junction and oviductal ampulla) and pro-inflammatory cytokines (i.e., IL-6 and IL-8 in the cervix; IL-1β in the oviductal isthmus; TNF-α in the oviductal ampulla; and IFN-γ in the uterine body compared to the control group). In addition, CsA inhibited the mRNA expression of pro-inflammatory cytokines, such as IL-6 in the uterine body, uterotubal junction, and oviductal isthmus; TNF-α in the uterine body; and IFN-γ in the uterotubal junction and oviductal isthmus induced by the LPS challenge. The IHC assay showed the LPS-induced increase in protein production of IL-6 in the uterine body and oviductal isthmus. CsA increased the protein production

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of IL-10 in the cervix, uterine body, oviductal ampulla, and isthmus. Moreover, CsA decreased the protein production of IL-6 in the uterine body and oviductal isthmus induced by LPS.

Keywords: lipopolysaccharide; Cyclosporin A; pro-inflammatory cytokines; anti-inflammatory cytokines; rabbit

Key Contribution: Taken together, the results presented here will serve as a foundation for future research focused on the potential implication of CsA in regulating the delicate balance between pro- and anti-inflammatory cytokines, particularly in the context of Gram-negative bacteria-induced localized and sub-clinical infections invading the female genital tract and, as a consequence, affecting overall female reproduction and fertility.

1. Introduction

Female infertility, such as tubal factor infertility, is an increasing concern around the world. Most of the cases of tubal factor infertility are associated to undiagnosed and untreated sexually transmitted diseases. These diseases ascend through the female reproductive tract and are implicated in causing damage and inflammation in these organs. Past evidence has demonstrated that several pathogenic bacteria species are involved in causing reproductive tract disorders in females, such as tubal factor infertility and pelvic inflammatory disease [1]. Moreover, asymptomatic, undiagnosed, and untreated genital infections may have serious complications related to reproductive health in both humans and domestic animals [1–4]. Clinical and sub-clinical infections induced by Gram-negative bacteria such as *Pasteurella*, *Escherichia coli*, and *Salmonella* are common in rabbits and other female domestic animals [5] and reportedly cause severe economic losses to the farmers as a consequence of the resulting sub-fertility, infertility, increased culling rates due to repeated failure to conceive, reduced production, and increased rearing expenditures [2,6–8].

Lipopolysaccharide (LPS) is an essential building element of the outer membrane of the cell wall of Gram-negative bacteria which is released upon bacterial division and lysing and are recognized by mammalian cells via Toll-like receptors (TLR) 2 and 4 [9,10]. LPS is widely used to trigger the in vivo immune inflammatory response in several cells and organs [9,11–14] and could be used to elucidate the underlying mechanisms by which infections and/or inflammation impair occurs in the reproductive system in females [15]. Toll-like receptors (TLRs) are the members of pathogen-related molecular pattern receptors that are involved in recognizing microbial agents [9,16–18]. From these, TLR4, along with some adapter proteins (CD14 and MD2), recognizes the LPS on the surface of host immune cells. The interaction between TLR4 and LPS triggers a cascade of intracellular signaling pathways, resulting in production of immune cytokines such as interleukin (IL)-1β, IL-6, IL-8, and tumor necrosis factor-α (TNF-α) [15,19–22]. This, in turn, stimulates the production of chemokines and other mediators of inflammation, such as reactive oxygen species (ROS) and reactive nitrogen species (RNS) [23-25]. The expression of TLR4 on the surface of epithelial and stromal cells in female reproductive organs, i.e., vagina, cervix, uterus, and oviduct of women and other animal species, has been observed [15].

Cytokines from immune and non-immune cells are classically known to play a crucial role in the stimulation or inhibition of cell proliferation and cytotoxicity/apoptosis [26], antiviral activity [27], cell growth and differentiation [28], and inflammatory responses [29]. Although their specific biological functions may vary, cytokines may be categorized in two general classes, i.e., pro- and

anti-inflammatory [30]. In the host defense against Gram-negative bacterial infections and accompanying endotoxemia, a pivotal role is played by endogenous cytokines such as IL-1β, IL-6, IL-8, TNF-α, and interferon-γ (IFN-γ) [25,31–34]. Through complex feedback mechanisms, the excessive production of these mediators triggers immune cells to release anti-inflammatory cytokines (i.e., IL-4, IL-10, IL-13, and transforming growth factor-β (TGF-β)) to combat and down-regulate the pro-inflammatory response and to maintain homeostasis [35–42]. Since inflammatory and/or immune cytokine-mediated deregulations are related to a large number of reproductive pathologies, the appropriate functioning of cytokine cascade is indispensable for successful reproductive function [12,20,43–51].

Cyclosporine A (CsA) is a widely used, powerful immunosuppressant. It has been reported that CsA greatly ameliorates immunosuppressive therapy in organ transplantation and results in increased organ survival rate [52]. Moreover, it has been used to treat several autoimmune diseases such as rheumatoid arthritis [53], nephrotic syndrome [54,55], and systemic lupus erythematosus [56]. In addition, some previous studies have focused on the implications of immune therapy in reproduction related disorders, such as spontaneous miscarriage and induced pre-eclampsia [57–59].

Furthermore, previous studies in humans and mice have shown that CsA could inhibit the pro-inflammatory cytokines IL-1β, IL-6, IL-8, TNF-α, and IFN-γ and increase the production of anti-inflammatory cytokines IL-4 and IL-10 in response to LPS stimulation [19,59–64]. Recently Hu and colleagues, using a pregnant rat model, have demonstrated that CsA significantly ameliorates the clinical signs of LPS-induced pre-eclampsia and inhibits the inflammatory response [59]. However, little is known about the potential effects of CsA on LPS-induced immune cytokine production in the female genital tract in mammalian species, and further studies are awaited to continually explore the potential implication of this powerful immunosuppressant in regulating immune cytokine production in female reproductive organs.

In this study, using rabbit a model, we explored and elucidated the expression profile and localization of pro- and anti-inflammatory cytokines in female reproductive organs—i.e., cervix, uterine body, uterotubal junction, oviductal isthmus, and ampulla—and assessed whether LPS-induced gene expression and protein levels of pro- and anti-inflammatory cytokines could be affected by CsA in female rabbits.

2. Results

2.1. Expression Profile of Immune Cytokines

The expression profiles of immune cytokines in various tissues are depicted in Figure 1. Briefly, our real time PCR results confirmed the expression of immune cytokines IL-1β, IL-6, IL-8, TNF-α, IFN-γ, IL-4, IL-10, IL-13, and TGF-β in the female reproductive organs of rabbits (i.e., the cervix, uterine body, uterotubal junction, oviductal isthmus, and oviductal ampulla).

2.2. LPS Induced the Up-regulation of Pro-Inflammatory Cytokines but Did Not Affect Anti-Inflammatory Cytokines in the Female Genital Tract

The changes in the expression of pro-inflammatory cytokines IL-1β, IL-6, IL-8, TNF-α, and IFN-γ and anti-inflammatory cytokines IL-4, IL-10, IL-13, and TGF-β in the cervix, uterine body, uterotubal junction, oviductal isthmus, and oviductal ampulla following the LPS challenge are shown in Figure

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2. LPS significantly increased the expression of IL-6 ($p < 0.01$; Figure 2b) and TNF-α ($p < 0.05$; Figure 2d) in uterine body and IL-1β ($p < 0.05$; Figure 2a) in the uterotubal junction. However, the expressions of pro-inflammatory cytokines IL-8 ($p > 0.05$; Figure 3c) and IFN-γ ($p > 0.05$; Figure 3e) and the anti-inflammatory cytokines ($p > 0.05$; Figures 3f–3i) were not affected by the LPS challenge.

2.3. CsA Increased the mRNA Expression of Pro- and Anti-Inflammatory Cytokines in the Female Genital Tract

As shown in Figure 3, CsA significantly increased the expression of pro-inflammatory cytokines IL-6 ($p < 0.05$; Figure 3b) and IL-8 ($p < 0.05$; Figure 3c) in the cervix, IL-1β ($p < 0.05$; Figure 3a) in the oviductal isthmus, and TNF-α ($p < 0.05$; Figure 3d) in the oviductal ampulla. Interestingly, the expression of IFN-γ was significantly increased in the uterine body but decreased in the oviductal isthmus following CsA treatment ($p < 0.01$; Figure 3e). Moreover, the expressions of anti-inflammatory cytokines IL-4 in the uterine body, uterotubal junction, and oviductal ampulla ($p < 0.05$; Figure 3f); IL-10 in the cervix, oviductal isthmus, and oviductal ampulla ($p < 0.05$; Figure 3g); and TGF-β in uterotubal junction and oviductal ampulla ($p < 0.01$; Figure 3i) were significantly increased following CsA treatment, while the expression of cytokine IL-13 was not affected by CsA ($p > 0.05$; Figure 3h).

2.4. CsA Inhibited the mRNA Expression of LPS-Induced Pro-Inflammatory Cytokines in the Female Genital Tract

The results of CsA's effect on the mRNA expression profile of immune cytokines in various female reproductive tissues following the LPS challenge are shown in Figure 4. Briefly, CsA significantly decreased the expression of LPS-induced pro-inflammatory cytokines IL-6 in uterine body, uterotubal junction, and oviductal isthmus ($p < 0.05$; Figure 4b), TNF-α in the uterine body ($p < 0.05$; Figure 4d), and IFN-γ in the uterotubal junction and oviductal isthmus ($p < 0.05$; Figure 4e). However, the expressions of pro-inflammatory cytokines IL-1β ($p > 0.05$; Figure 4a) and IL-8 ($p > 0.05$; Figure 4c) and anti-inflammatory cytokines ($p > 0.05$; Figures 4f–4i) were not affected by CsA after the LPS challenge.

2.5. Effects of LPS and/or CsA on the Protein Expression of Pro-Inflammatory Cytokine IL-6 in the Female Genital Tract

Immuno-histochemical analysis revealed that pro-inflammatory cytokine IL-6 was expressed in the cervix (Figure 5), uterine body (Figure 6), uterotubal junction (Figure 7), oviductal isthmus (Figure 8), and oviductal ampulla (Figure 9) of female rabbits. Of note, it was localized in epithelial, glandular, and stromal cells of the cervix, uterine body, and oviduct. The intrauterine infusion of LPS increased the expression of IL-6 in the uterine body ($p < 0.05$; Figures 6f and 10) and the oviductal isthmus ($p < 0.05$; Figures 8f and 10) compared to the control group. Furthermore, CsA showed no effect on the basal expression of IL-6 ($p > 0.05$; Figures 5d–10d) but significantly decreased IL-6 expression induced by LPS in the uterine body ($p < 0.05$; Figures 6h and 10) and the oviductal isthmus ($p < 0.01$; Figures 8h and 10).

2.6. Effects of LPS and/or CsA on the Protein Expression of Anti-Inflammatory Cytokine IL-10 in the Female Genital Tract

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Similarly, IL-10 was expressed in the cervix (Figure 11), uterine body (Figure 12), uterotubal junction (Figure 13), oviductal isthmus (Figure 14), and oviductal ampulla (Figure 15) of female rabbits. It was localized in the epithelial cells of the cervix, uterine body, and uterotubal junction and epithelial and stromal cells of the oviductal isthmus and oviductal ampulla. However, the expression of IL-10 was unchanged in all of the tissues analyzed after the LPS challenge ($p > 0.05$; Figures 11f–15f and 16). Moreover, CsA treatment did not affect IL-10 expression following the LPS challenge ($p > 0.05$; Figures 11h–15h and 16) but significantly increased the basal expression in the cervix ($p < 0.01$; Figures 11d and 16), uterine body ($p < 0.01$; Figures 12d and 16), oviductal isthmus ($p < 0.05$; Figures 14d and 16), and oviductal ampulla ($p < 0.01$; Figures 15d and 16).

3. Discussion

The organs of the female genital tract are more likely to encounter invasion by microorganisms compared to other internal organs. The infection caused by Gram-negative bacteria is one of the major causes of infertility and sub-fertility in humans and domestic animals [1,2,4,65,66]. These infections and inflammatory conditions have relevance to several female reproduction-related complications, such as intrauterine growth retardation [67], premature birth, intrauterine fetal death and abortion [68], and fetal resorption [69,70]. The progression of these complications is reportedly mediated through endotoxins, including LPS, which triggers the production of immune cytokines and other mediators of inflammation by immune and non-immune cells [13,71]. However, at present, the exact underlying molecular mechanisms through which LPS-induced inflammatory responses are mediated in female reproductive organs are not yet sufficiently understood and require further elucidation [15].

The immune system of an organism is charged with defending the host against pathogenic microbes, however, this response must be appropriate and measured [26]. The success of the host immune response is by and large the outcome of both pro- and anti-inflammatory components that are cautiously tuned with the aim of scavenging pathogenic microbes and minimizing host damage [26]. Experimental studies are important to revealing the identities of pro- and anti-inflammatory players in immune responses and identifying the aftermaths of interrupting their balance [26]. The ultimate aim is to harness knowledge and isolate factors regulating this dynamic balance to furnish strategies for better patient results. For instance, analysis of models can identify immunomodulatory factors that, combined with antibiotics, enable patients to achieve pathogen clearance. While we now better understand how a balance of pro- and anti-inflammatory mediators might be needed to control infection, we have not yet fully applied that concept for therapeutic development [26]. In the present study, using rabbits as a model, we have demonstrated the CsA mediated regulation of immune and inflammatory responses following a local inoculation of LPS.

Our results showed that LPS significantly increased the mRNA expression of pro-inflammatory cytokines IL-6 and TNF-α in the uterine body and IL-1β in the uterotubal junction. However, in the cervix and oviduct, the expression of pro-inflammatory cytokines was unaffected. These results are in agreement with our previous studies demonstrating the LPS-induced up-regulated expressions of IL-1β, IL-6, and TNF-α in the uterine body and horn but not in the cervix following LPS challenge [19,72]. The tissue-specific differential expressions of pro-inflammatory cytokines may be attributed to the higher natural elicitation of an immune response and, perhaps, the relative anatomical

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location and histo-architecture of the cervix [72]. The findings are further supported by our present study which demonstrated that the expression of pro-inflammatory cytokine IL-6 in the cervix and oviduct of control group was higher compared to uterine body and uterotubal junction. Conversely, the expression of anti-inflammatory cytokine IL-10 contrasted that of IL-6 (Figure 17).

Even though there is certainly a commonality in the mucosal immune responses, there is also manifestations of a degree of location-specific immune mechanisms, dependent upon the physiological role and anatomical position of an organ [73]. There is also evidence that the upper "sterile" and the lower "non-sterile" female reproductive tract compartments may sustain a different immunological surveillance milieu and might also respond differentially to a pathogen challenge [73]. Albeit, the TLR patterns on endocervical epithelium are similar to the lower tract, the constitutive and induced cytokine profile in immortalized endocervical epithelium is considerably higher compared to matched immortalized ectocervical and vaginal epithelial cells [73]. Therefore, it reasonable to suggest that future research should also focus on clarifying the underlying mechanisms dictating the site-specific elicitation or up-regulation of immunological responses in specific reproductive tract sites. Moreover, a deeper apprehension of organ-specific, epithelial-derived early immune responses in the upper and lower reproductive tracts has significant implications for developing vaccines or immune-based remedies, since the elicitation and consequence of an acquired immune response are determined by the specific early immunological events which occur at a given site [73].

As for the LPS-induced expression of pro-inflammatory cytokines in uterine tissues, the findings of our study are also supported by results of previous in vitro studies reporting the increased expression of pro-inflammatory cytokine genes in cultured bovine endometrial epithelial and stromal cells, as well as in human endometrial epithelial cells following treatment with LPS [21,60,74,75]. Furthermore, few other reports have demonstrated that the intra-uterine infusion of LPS may induce an up-regulated expression of pro-inflammatory cytokine genes in mice and bovine animals [4,14].

Furthermore, it is well established that LPS is recognized by TLR4, which then triggers the expression of pro-inflammatory cytokines. Previous studies have demonstrated that LPS administration induced an increase in mRNA expression of TLR4 in the uterus, while no up-regulation in expression was found in the oviducts [15,72]. The differential expressions of TLR4 may be associated with different types of uterine and oviductal cells and/or the influx of inflammatory cells into the uterus and oviducts [15]. Additionally, it can be influenced by the concentrations of the reproductive hormones [76,77]. Recently, Menchetti and colleagues have reported a stronger signal for TLR4 protein in uterine stromal cells of LPS-challenged rabbits compared to the control group, indicating that these cells, in addition to epithelial cells, may have implications in triggering the inflammatory response to LPS challenge or invading microorganisms [15]. Additionally, it has also been reported that, following LPS challenge, mRNA expression of IL-1β is increased both in the uterus and oviduct, whereas the up-regulated expression of TNF-α was only observed in the rabbit uterus [15]. Therefore, it is reasonable to speculate that differential expression of immune cytokines in the uterus and oviduct following LPS challenge is tissue- and cell-specific and might be linked to the differential expression of TLR4 in these tissues. Nevertheless, studies are awaited to explicate the putative mechanisms by which LPS induces the differential expression of

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inflammatory cascades, including TLR4, in different female genital tract organs.

Inflammation elicited by the action of pro-inflammatory cytokines, such as IL-1, TNF, IFN-γ, and IL-6, is adjudicated by anti-inflammatory cytokines (i.e., IL-4, IL-10, IL-13, and TGF-β) [78]. Our results demonstrated that the expression of anti-inflammatory cytokines did not change in all tissues analyzed after LPS-challenge. These observations are consistent with the findings of Laura and colleagues who reported that LPS-treated endometrial cells demonstrated a significant increase in the mRNA expression of IL-1β, IL-6, and IL-8 but not IL-10 [79]. There is a dearth of information regarding the potential effects of LPS on the elicitation of anti-inflammatory cytokine expression. To our knowledge, our study is the first to report the effects of intrauterine administration of LPS on the protein and mRNA expression of anti-inflammatory cytokines in the uteruses and oviducts of female rabbits.

To understand the mode of action of CsA in regulating the immune and inflammatory responses, a thorough comprehension is needed of the underlying mechanisms by which it targets biochemical processes that have potential involvement in the stimulation of specified subsets of immune cells. Also needed is an understanding of how the interaction of these different subsets is mediated by a pathogen's antigens to result in a physiologically appropriate response [80]. Moreover, it has been demonstrated that CsA has implications in the inhibition of the essential antigen-dependent signal needed to stimulate T cells, as well as the production of interleukins by T cells and T cell clones. In addition to this, CsA is also implicated in inhibiting the antigen presentation by macrophages. The manifestation of such distinct activities warns one against an overly simplistic view of how CsA acts and of what immunoregulatory capacity it might possess [80]. CsA has been widely used as an anti-inflammatory agent in inflammatory conditions. The importance of CsA as an immunosuppressant is associated with its efficacy in suppressing the elicitation of immune responses at doses that do not impair the functioning of the hematopoietic system [80].

In the present study, we observed that CsA up-regulated the basal production of anti-inflammatory cytokines and down-regulated the LPS-triggered production of pro-inflammatory cytokines in reproductive organs of female rabbits. These enticing findings indicate that CsA might act as a regulator of the negative feedback between pro-inflammatory and anti-inflammatory cytokines to maintain tissue homeostasis [65,81]. To the best of our knowledge, no in vivo study investigated the effects of CsA on the production of inflammatory cytokines in the female genital tracts of mammals prior to this study. However, the results of our study conform with previous studies reporting the effect of CsA on the expression of inflammatory cytokines in other cell lines. For instance, consistent with our findings, it has been reported that CsA might inhibit the production of IL-6, IFN-γ, and TNF-α and up-regulate IL-10 and IL-4 expression in response to LPS stimulation in dendritic cells [60,61], macrophages [63], and PBMCs [59]. Interestingly, we further observed that CsA also up-regulated the basal production of pro-inflammatory cytokines. These findings may support the conjecture that CsA has potential implications in maintaining a delicate balance between pro- and anti-inflammatory cytokines, both in the resolution of inflammation and normal homeostasis. However, we are unaware of other precedents that report the implication of CsA in regulating pro-inflammatory and anti-inflammatory cytokines following LPS challenge in mammalian female genitals. Nevertheless, further research will be necessary to validate these

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findings.

In addition, previous research has indicated that CsA may act as immunomodulatory agent, however, this activity largely depends on the concentration of the antigen and dose of CsA used [80]. There have been reports that low and moderate concentrations of CsA increased, while therapeutic and high concentrations decreased, the basal production of pro-inflammatory cytokines in immune cells [82,83] and non-immune cells [84]. Furthermore, it has been demonstrated that high concentrations of CsA could decrease the viability of cells [85], thus decreasing pro-inflammatory cytokine production [84]. García and colleagues have shown that low and intermediate concentrations of CsA decreased, while high concentrations increased, the basal production of pro-inflammatory cytokines in human alveolar macrophages [62]. The discrepancy in these observations might be ascribed to diverse activities and differential effects of CsA on distinct cell lines [65,86,87]. Therefore, future studies focusing on this caveat are warranted to better comprehend the underlying complex feedback mechanisms, and they will add to the known effects of this potent immunosuppressant.

4. Conclusions

Interactions between the immune cascades and reproductive system have important consequences for successful reproduction and fertility. Our results show that CsA increased both mRNA expression of anti- and pro-inflammatory cytokines and inhibited the mRNA and protein expression of pro-inflammatory cytokines following LPS challenge. Our findings imply the potential role of CsA in downregulating the excessive production of pro-inflammatory cytokines as a part of the innate host immune response, enabling the inflammatory cascades to eliminate the invading agents through complex feedback mechanisms without compromising the immune system. Taken together, the results presented here will serve as a foundation for future research focused on the potential implications of CsA in regulating the delicate balance between pro-inflammatory and anti-inflammatory cytokines, particularly in the context of Gram-negative bacteria-induced localized and sub-clinical infections invading the female genital tract that consequently affect overall female reproduction and fertility. Moreover, properly powered experimental and clinical studies are needed to further explicate the effects of localized inflammatory conditions and sub-clinical infections on distinct aspects of female reproductive efficiency in different reproductive phases and to compound our understanding regarding the underlying mechanisms that dictate the crosstalk between the immune system and infertility.

5. Materials and Methods

5.1. Animals and Ethical Approval

The trial was carried out at the Laboratory of Animal Reproduction and Embryo Engineering, Institute of Animal Genetics and Breeding, Sichuan Agricultural University, Chengdu, Sichuan, China. All rabbits were provided with a similar feeding regime and environmental and sanitary conditions. The feeding was done as previously described [88]. All experimental procedures were performed strictly in accordance with the regulations for the Administration of Affairs Concerning Experimental Animals (Ministry of Science and Technology, China, revised in June 2004) and approved by the Institutional Animal Care and Use Committee in the College of Animal Science and Technology, Sichuan Agricultural University, Sichuan, China (approval No. SCAUS20163636).

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5.2. Experimental Design

Twelve healthy and sexually mature female New Zealand white rabbits of uniform body weight (2.5 ± 0.1 kg) and age (3.5 months) were randomly divided into four groups: control, LPS, CsA, and LPS + CsA group (n = 3 each). The rabbits in the LPS group were given an intrauterine infusion of *E. coli* LPS (055:B5, Sigma-Aldrich, USA) at 4 mg/kg BW, while rabbits in the CsA group were given CsA (A600352, BBI Life Sciences, Canada) at 20 mg/kg BW by intramuscular injection. The rabbits in the LPS + CsA group were treated with LPS (4 mg/kg BW) and CsA (20 mg/kg BW). The control group received only LPS and CsA carrier. The tissue specimen from the cervix, uterine body, uterotubal junction, oviductal isthmus, and ampulla were collected 3 h post-injection. Samples for RNA analysis were snap frozen in liquid nitrogen. For the immuno-histochemical assay, samples were fixed in 10% formaldehyde and kept at room temperature until further analysis.

5.3. RNA Isolation and cDNA Synthesis

The tissues were homogenized immediately in 1 ml TRzol Reagent (Ambion, USA) after collection, and total RNA were extracted according to the manufacturer's protocol. The quality of RNA was examined using 1.5% agarose gel electrophoresis, and the concentration of total RNA was determined with a NanoDrop ND-2000C (Thermo Scientific, USA). The purified RNA samples were reverse-transcribed using a PrimeScript® RT Reagent Kit with gDNA Eraser (Takara, Japan) following the manufacturer's protocol. Briefly, a 10 µl reaction mixture (3 µl of RNase free water, 2 µl 5 × gDNA Eraser Buffer, 1 µl of gDNA Eraser, and 4 µl RNA) was placed at room temperature for 5 min to remove genomic DNA. Then, 4 µl of RNase free water, 4 µl of 5 × PrimeScript Buffer 2 (for real time PCR), 1 µl of PrimeScript RT Enzyme Mix 1, and 1 µl of RT Primer Mix were added to the reaction mix (total volume = 20 µl). The samples were incubated at 42 °C for 2 min, followed by heat inactivation at 85°C for 5 s on a programmable thermal controller PTC-100 (Bio-Rad, USA) for cDNA synthesis.

5.4. Quantitative Real-Time PCR

Quantitative real-time PCR (RT-qPCR) was performed using a CFX 96 Real-Time PCR Detection System (Bio-Rad, USA) in a 10 µl reaction mixture containing 3 µl dH_2O, 5 µl 2 × SYBR Premix EX Taq II, 0.5 µl of each forward and reverse primer (primer sequences are provided in Table 1), and 1 µl cDNA. Following initial denaturation at 95 °C for 3 min, PCR was carried out with a thermal cycling protocol of 95 °C for 10 s and an annealing temperature of 60 °C (Table 1) for 30 s for 40 cycles. Specificity of the amplified products was verified by melting curve analysis. $2^{-\Delta\Delta Ct}$ method was used to calculate the expression of candidate genes. The GAPDH was used as an internal control. Each gene was analyzed in triplicate.

5.5. Immuno-Histochemical Assay of IL-6 and IL-10

After embedding, 4 µm-thick serial sections of tissues were cut and mounted on poly-lysine and 3-aminopropyl-triethoxysilane 3 (APES)-coated glass slides. Then, the sections were deparaffinized in xylene and rehydrated through decreasing concentrations of ethanol (i.e., 100, 95, 70, and 50% for 3 min each). The sections were washed in running tap water for 10 min and then heated at 121 °C for 3 min in Tris-EDTA (10 Mm Tris base, 1 mM EDTA solution, 0.05% Tween 20, pH of 9.0) for antigen retrieval in an autoclave (G154TW, Zealway, USA). To prevent non-specific binding of the primary antibody, the sections were pre-incubated for 2 h with normal goat serum (1:10, Boster, China) and endogenous peroxidase activity was blocked with 3% H_2O_2/phosphate-buffered saline

(PBS). Serial sections were then incubated with the corresponding primary antibodies diluted in Tris buffered saline (TBS) containing 1% BSA overnight at 4 °C. IL-6 and IL-10 were identified using following primary antibodies at specified dilutions: IL-6 mouse monoclonal antibody (1:500, 66146-1-lg, proteintech, USA) and IL-10 mouse monoclonal antibody (1:500, 60269-1-lg, proteintech, USA). Negative control slides omitting primary antibodies were prepared and used for all the assays. After rinsing with TBS with Tween 20 (TBST, 5 times in 5 min), the sections were incubated with peroxidase-labeled secondary antibody (goat anti-mouse IgG, 1:200, Boster, China) for 1 h at room temperature. After incubation, slides were rinsed with TBST and then exposed to an avidin biotin complex (ABC Kit, 1:100, Boster, China) for 1 h and rinsed again with TBST. To reduce variations in staining, tissue sections from all four groups were incubated together in a moist chamber during each immuno-histochemical procedure. The peroxidase activity sites were visualized using a DAB chromogenic substrate kit (TianGen, China). Sections were rinsed with distilled water and counterstained with Mayer's hematoxylin, washed in running tap water, and mounted in aqueous mounting agent (BBI Life Sciences, Canada). Specimen slides were randomly selected and observed under a light microscope (Nikon 90i, Japan). The representative images captured under high-power magnification (×200) using a digital camera (Nikon Dxm 1200, Japan) were obtained and processed in TIFF format. The positive areas of immune histochemical staining were analyzed using Image-Pro Plus 6 software (Media Cybernetics, Rockville, MD USA). Average optical density (AOD) was used to assess the expression intensity of IL-6 and IL-10 [89]. AOD = Integrated optical density (IOD)/Area. Three sections were examined from each tissue per animal, and three representative visual fields randomly selected on a section were analyzed for IL-6 and IL-10 immunoreactivity.

5.6. Statistical Analysis

Data was analyzed using SPSS statistical software (version 23.0; SPSS Inc, USA). Variations among groups were analyzed using one-way analysis of variance (ANOVA). Student's t-test was used to compare differences between two independent samples (control group vs. LPS group or CsA group; LPS group vs. LPS + CsA group). A p-value of < 0.05 was considered to be statistically significant. Data are presented as mean ± standard deviation (SD).

Supplementary Materials: All supporting data are included in this article.

Author contributions: MZ provided the conceptualization of the experiment and edited and reviewed the original draft. MZ and LQ designed the experiments, and LQ analyzed the data and wrote the original draft. LQ, QY, XY, CW, YT, and XL performed the experiments. IH and CA revised the manuscript. CJ, GB, and SY provided the help with the methodology for the experiments. YZ raised the animals.

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Conflicts of Interest: The authors declare no conflict of interest.

References

1. Tsevat, D.G.; Wiesenfeld, H.C.; Parks, C.; Peipert, J.F. Sexually transmitted diseases and infertility. *American journal of obstetrics and gynecology* **2017**, *216*, 1-9.
2. Boiti, C.; Canali, C.; Brecchia, G.; Zanon, F.; Facchin, E. Effects of induced endometritis on the life-span of corpora lutea in pseudopregnant rabbits and incidence of spontaneous uterine infections related to fertility of breeding does. *Theriogenology* **1999**, *52*, 1123-1132.
3. Frontoso, R.; De Carlo, E.; Pasolini, M.; van der Meulen, K.; Pagnini, U.; Iovane, G.; De

Martino, L. Retrospective study of bacterial isolates and their antimicrobial susceptibilities in equine uteri during fertility problems. *Research in veterinary science* **2008**, *84*, 1-6.

4. Sheldon, I.M.; Rycroft, A.N.; Dogan, B.; Craven, M.; Bromfield, J.J.; Chandler, A.; Roberts, M.H.; Price, S.B.; Gilbert, R.O.; Simpson, K.W. Specific strains of Escherichia coli are pathogenic for the endometrium of cattle and cause pelvic inflammatory disease in cattle and mice. *PLoS One* **2010**, *5*, e9192.
5. DiGiacomo, R.; Garlinghouse, J.L.; Van, J.H.G. Natural history of infection with Pasteurella multocida in rabbits. *Journal of the American Veterinary Medical Association* **1983**, *183*, 1172-1175.
6. Rosell, J.; De La Fuente, L. Culling and mortality in breeding rabbits. *Preventive veterinary medicine* **2009**, *88*, 120-127.
7. Cheong, S.; Nydam, D.; Galvão, K.; Crosier, B.; Gilbert, R. Cow-level and herd-level risk factors for subclinical endometritis in lactating Holstein cows. *Journal of Dairy Science* **2011**, *94*, 762-770.
8. Laven, R.; Peters, A. Bovine retained placenta: aetiology, pathogenesis and economic loss. *The veterinary record* **1996**, *139*, 465.
9. Boonen, B.; Alpizar, Y.; Meseguer, V.; Talavera, K. TRP Channels as Sensors of Bacterial Endotoxins. *Toxins* **2018**, *10*, 326.
10. Park, B.S.; Song, D.H.; Kim, H.M.; Choi, B.-S.; Lee, H.; Lee, J.-O. The structural basis of lipopolysaccharide recognition by the TLR4–MD-2 complex. *nature* **2009**, *458*, 1191.
11. Harris, T.G.; Battaglia, D.F.; E. Brown, M.; Brown, M.B.; Carlson, N.E.; Viguié, C.; Williams, C.Y.; Karsch, F.J. Prostaglandins mediate the endotoxin-induced suppression of pulsatile gonadotropin-releasing hormone and luteinizing hormone secretion in the ewe. *Endocrinology* **2000**, *141*, 1050-1058.
12. Hou, Y.; Wang, L.; Zhang, W.; Yang, Z.; Ding, B.; Zhu, H.; Liu, Y.; Qiu, Y.; Yin, Y.; Wu, G. Protective effects of N-acetylcysteine on intestinal functions of piglets challenged with lipopolysaccharide. *Amino Acids* **2012**, *43*, 1233-1242.
13. Williams, E.J.; Sibley, K.; Miller, A.N.; Lane, E.A.; Fishwick, J.; Nash, D.M.; Herath, S.; England, G.C.; Dobson, H.; Sheldon, I.M. The effect of Escherichia coli lipopolysaccharide and tumour necrosis factor alpha on ovarian function. *American journal of reproductive immunology* **2008**, *60*, 462-473.
14. Sheldon, I.M.; Roberts, M.H. Toll-like receptor 4 mediates the response of epithelial and stromal cells to lipopolysaccharide in the endometrium. *PLoS One* **2010**, *5*, e12906.
15. Menchetti, L.; Barbato, O.; Filipescu, I.E.; Traina, G.; Leonardi, L.; Polisca, A.; Troisi, A.; Guelfi, G.; Piro, F.; Brecchia, G. Effects of local lipopolysaccharide administration on the expression of Toll-like receptor 4 and pro-inflammatory cytokines in uterus and oviduct of rabbit does. *Theriogenology* **2018**, *107*, 162-174.
16. Molteni, M.; Bosi, A.; Rossetti, C. The Effect of Cyanobacterial LPS Antagonist (CyP) on Cytokines and Micro-RNA Expression Induced by Porphyromonas gingivalis LPS. *Toxins* **2018**, *10*, 290.
17. Molteni, M.; Gemma, S.; Rossetti, C. The role of toll-like receptor 4 in infectious and noninfectious inflammation. *Mediators of inflammation* **2016**, *2016*.
18. Zhang, M.; Nii, T.; Isobe, N.; Yoshimura, Y. Expression of Toll-like receptors and effects of

lipopolysaccharide on the expression of proinflammatory cytokines and chemokine in the testis and epididymis of roosters. *Poultry science* **2012**, *91*, 1997-2003.

19. Liu, S.; Shi, Y.; Liu, C.; Zhang, M.; Zuo, Z.; Zeng, C.; Zhou, G.; Xian, H.; Song, T. The upregulation of pro-inflammatory cytokines in the rabbit uterus under the lipopolysaccaride-induced reversible immunoresponse state. *Animal reproduction science* **2017**, *176*, 70-77.
20. Brecchia, G.; Menchetti, L.; Cardinali, R.; Castellini, C.; Polisca, A.; Zerani, M.; Maranesi, M.; Boiti, C. Effects of a bacterial lipopolysaccharide on the reproductive functions of rabbit does. *Animal reproduction science* **2014**, *147*, 128-134.
21. Cronin, J.G.; Turner, M.L.; Goetze, L.; Bryant, C.E.; Sheldon, I.M. Toll-like receptor 4 and MYD88-dependent signaling mechanisms of the innate immune system are essential for the response to lipopolysaccharide by epithelial and stromal cells of the bovine endometrium. *Biology of reproduction* **2012**, *86*, 51, 51-59.
22. Soboll, G.; Shen, L.; Wira, C.R. Expression of Toll-like receptors (TLR) and responsiveness to TLR agonists by polarized mouse uterine epithelial cells in culture. *Biology of reproduction* **2006**, *75*, 131-139.
23. Li, D.; Liu, Y.; Li, Y.; Lv, Y.; Pei, X.; Guo, D. Significance of nitric oxide concentration in plasma and uterine secretes with puerperal endometritis in dairy cows. *Veterinary research communications* **2010**, *34*, 315-321.
24. Jana, B.; Kozłowska, A.; Koszykowska, M.; Majewski, M. Expression of cyclooxygenase-2 in the inflammatory changed porcine uterus. *Polish journal of veterinary sciences* **2009**, *12*, 1-8.
25. Herath, S.; Lilly, S.T.; Santos, N.R.; Gilbert, R.O.; Goetze, L.; Bryant, C.E.; White, J.O.; Cronin, J.; Sheldon, I.M. Expression of genes associated with immunity in the endometrium of cattle with disparate postpartum uterine disease and fertility. *Reproductive Biology and Endocrinology* **2009**, *7*, 55.
26. Fukada, T.; Hibi, M.; Yamanaka, Y.; Takahashi-Tezuka, M.; Fujitani, Y.; Yamaguchi, T.; Nakajima, K.; Hirano, T. Two signals are necessary for cell proliferation induced by a cytokine receptor gp130: involvement of STAT3 in anti-apoptosis. *Immunity* **1996**, *5*, 449-460.
27. Heim, A.; Weiss, S. Interferons in enteroviral heart disease: modulation of cytokine expression and antiviral activity. *Medical microbiology and immunology* **2004**, *193*, 149-154.
28. Hirano, T.; Ishihara, K.; Hibi, M. Roles of STAT3 in mediating the cell growth, differentiation and survival signals relayed through the IL-6 family of cytokine receptors. *Oncogene* **2000**, *19*, 2548.
29. O'shea, J.J.; Murray, P.J. Cytokine signaling modules in inflammatory responses. *Immunity* **2008**, *28*, 477-487.
30. Cicchese, J.M.; Evans, S.; Hult, C.; Joslyn, L.R.; Wessler, T.; Millar, J.A.; Marino, S.; Cilfone, N.A.; Mattila, J.T.; Linderman, J.J. Dynamic balance of pro-and anti-inflammatory signals controls disease and limits pathology. *Immunological reviews* **2018**, *285*, 147-167.
31. Pivarcsi, A.; Nagy, I.; Koreck, A.; Kis, K.; Kenderessy-Szabo, A.; Szell, M.; Dobozy, A.; Kemeny, L. Microbial compounds induce the expression of pro-inflammatory cytokines, chemokines and human β-defensin-2 in vaginal epithelial cells. *Microbes and Infection* **2005**, *7*, 1117-1127.
32. Kawai, T.; Akira, S. Signaling to NF-κB by Toll-like receptors. *Trends in molecular medicine*

2007, *13*, 460-469.

33. Casey, L.C.; Balk, R.A.; Bone, R.C. Plasma cytokine and endotoxin levels correlate with survival in patients with the sepsis syndrome. *Annals of internal medicine* **1993**, *119*, 771-778.
34. Pinsky, M.R. Multiple systems organ failure: Malignant intravascular inflammation. *Critical care clinics* **1989**, *5*, 195-198.
35. Lin, E.; Calvano, S.E.; Lowry, S.F. Inflammatory cytokines and cell response in surgery. *Surgery* **2000**, *127*, 117-126.
36. Ni Choileain, N.; Redmond, H.P. Cell response to surgery. *Arch Surg* **2006**, *141*, 1132-1140.
37. Asimakopoulos, G. Mechanisms of the systemic inflammatory response. *Perfusion* **1999**, *14*, 269-277.
38. Loyer, P.; Ilyin, G.; Razzak, Z.A.; Banchereau, J.; Dezier, J.-F.; Campion, J.-P.; Guguen-Guillouzo, C.; Guillouzo, A. Interleukin 4 inhibits the production of some acute-phase proteins by human hepatocytes in primary culture. *FEBS letters* **1993**, *336*, 215-220.
39. Marchant, A.; Bruyns, C.; Vandenabeele, P.; Ducarme, M.; Gérard, C.; Delvaux, A.; De Groote, D.; Abramowicz, D.; Velu, T.; Goldman, M. Interleukin-10 controls interferon-γ and tumor necrosis factor production during experimental endotoxemia. *European journal of immunology* **1994**, *24*, 1167-1171.
40. Standiford, T.J.; Strieter, R.M.; Lukacs, N.W.; Kunkel, S.L. Neutralization of IL-10 increases lethality in endotoxemia. Cooperative effects of macrophage inflammatory protein-2 and tumor necrosis factor. *The Journal of Immunology* **1995**, *155*, 2222-2229.
41. Sano, C.; Shimizu, T.; Sato, K.; Kawauchi, H.; Tomioka, H. Effects of secretory leucocyte protease inhibitor on the production of the anti-inflammatory cytokines, IL-10 and transforming growth factor-beta (TGF-β), by lipopolysaccharide-stimulated macrophages. *Clinical & Experimental Immunology* **2000**, *121*, 77-85.
42. Kucharzik, T.; Lügering, N.; Pauels, H.; Domschke, W.; Stoll, R. IL-4, IL-10 and IL-13 down-regulate monocyte-chemoattracting protein-1 (MCP-1) production in activated intestinal epithelial cells. *Clinical and experimental immunology* **1998**, *111*, 152.
43. Rosa, L.; Scaini, G.; Furlanetto, C.B.; Galant, L.S.; Vuolo, F.; Dall'Igna, D.M.; Schuck, P.F.; Ferreira, G.C.; Dal-Pizzol, F.; Streck, E.L. Administration of branched-chain amino acids alters the balance between pro-inflammatory and anti-inflammatory cytokines. *International Journal of Developmental Neuroscience* **2016**, *48*, 24-30.
44. Schulze-Koops, H.; Kalden, J.R. The balance of Th1/Th2 cytokines in rheumatoid arthritis. *Best practice & research Clinical rheumatology* **2001**, *15*, 677-691.
45. Gilbert, R.O. The effects of endometritis on the establishment of pregnancy in cattle. *Reproduction, Fertility and Development* **2011**, *24*, 252-257.
46. Jaiswal, Y.K.; Jaiswal, M.K.; Agrawal, V.; Chaturvedi, M.M. Bacterial endotoxin (LPS)–induced DNA damage in preimplanting embryonic and uterine cells inhibits implantation. *Fertility and sterility* **2009**, *91*, 2095-2103.
47. Daniel, J.; Abrams, M.; Wagner, C.; Whitlock, B.; Sartin, J. Endotoxin inhibition of luteinizing hormone in sheep. *Domestic animal endocrinology* **2003**, *25*, 13-19.
48. Magata, F.; Horiuchi, M.; Echizenya, R.; Miura, R.; Chiba, S.; Matsui, M.; Miyamoto, A.; Kobayashi, Y.; Shimizu, T. Lipopolysaccharide in ovarian follicular fluid influences the steroid production in large follicles of dairy cows. *Animal reproduction science* **2014**, *144*, 6-13.

49. Bridges, G.; Day, M.; Geary, T.; Cruppe, L. Triennial Reproduction Symposium: deficiencies in the uterine environment and failure to support embryonic development. *Journal of animal science* **2013**, *91*, 3002-3013.
50. Herath, S.; Lilly, S.T.; Fischer, D.P.; Williams, E.J.; Dobson, H.; Bryant, C.E.; Sheldon, I.M. Bacterial lipopolysaccharide induces an endocrine switch from prostaglandin F2α to prostaglandin E2 in bovine endometrium. *Endocrinology* **2008**, *150*, 1912-1920.
51. Laird, S.; Tuckerman, E.; Li, T. Cytokine expression in the endometrium of women with implantation failure and recurrent miscarriage. *Reproductive biomedicine online* **2006**, *13*, 13-23.
52. Kamar, N.; Allard, J.; Ader, J.; Rostaing, L. Cyclosporine-A-based immunosuppression and renal functional reserve in organ-transplant patients. In Proceedings of Transplantation proceedings; pp. S248-S250.
53. Hetland, M.L. Modern treatment strategies in rheumatoid arthritis. *Dan Med Bull* **2011**, *58*, B4320.
54. Sketris, I.; Yatscoff, R.; Keown, P.; Canafax, D.M.; First, M.R.; Holt, D.W.; Schroeder, T.J.; Wright, M. Optimizing the use of cyclosporine in renal transplantation. *Clinical biochemistry* **1995**, *28*, 195-211.
55. Fujinaga, S.; Hirano, D.; Murakami, H.; Ohtomo, Y.; Shimizu, T.; Kaneko, K. Nephrotoxicity of once-daily cyclosporine A in minimal change nephrotic syndrome. *Pediatric Nephrology* **2012**, *27*, 671-674.
56. Germano, V.; Picchianti, A.D.; Ferlito, C.; Podestà, E.; Salemi, S.; Migliore, A.; Laganà, B. Cyclosporine A in the long-term management of systemic lupus erythematosus. *Journal of biological regulators and homeostatic agents* **2011**, *25*, 397-403.
57. Tang, C.; Chen, L.; Gu, W.; Du, M.; Li, M.; Chen, Q.; Li, D. Cyclosporin A enhances the ability of trophoblasts to displace the activated human umbilical vein endothelial cell monolayers. *International journal of clinical and experimental pathology* **2013**, *6*, 2441.
58. Wang, S.-C.; Yu, M.; Li, Y.-H.; Piao, H.-L.; Tang, C.-L.; Sun, C.; Zhu, R.; Li, M.Q.; Jin, L.-P.; Li, D.-J. Cyclosporin A promotes proliferating cell nuclear antigen expression and migration of human cytotrophoblast cells via the mitgen-activated protein kinase-3/1-mediated nuclear factor-κB signaling pathways. *International journal of clinical and experimental pathology* **2013**, *6*, 1999.
59. Hu, B.; Yang, J.; Huang, Q.; Bao, J.; Brennecke, S.P.; Liu, H. Cyclosporin A significantly improves preeclampsia signs and suppresses inflammation in a rat model. *Cytokine* **2016**, *81*, 77-81.
60. Duperrier, K.; Farre, A.; Bienvenu, J.; Bleyzac, N.; Bernaud, J.; Gebuhrer, L.; Rigal, D.; Eljaafari, A. Cyclosporin A inhibits dendritic cell maturation promoted by TNF-α or LPS but not by double-stranded RNA or CD40L. *Journal of leukocyte biology* **2002**, *72*, 953-961.
61. Szabo, G.; Gavala, C.; Mandrekar, P. Tacrolimus and cyclosporine A inhibit allostimulatory capacity and cytokine production of human myeloid dendritic cells. *Journal of Investigative Medicine* **2001**, *49*, 442-449.
62. Garcia, J.L.; Rodriguez, F.M.; Lopez, A.J.; Salgado, M.G.; De Cabo, M.M.; Losada, J.P.; Arellano, J.P. Effect of cyclosporin A on inflammatory cytokine production by human alveolar macrophages. *Respiratory medicine* **1998**, *92*, 722-728.

63. Le Contel, C.; Beigneux, A.P.; Huang, J.; Parant, M.A. Regulation of lipopolysaccharide-induced tumor necrosis factor production by cyclosporin A in mice primed with muramyl dipeptide. *FEMS Immunology & Medical Microbiology* **1995**, *11*, 297-305.
64. Ge, Y.; Xu, Y.; Sun, W.; Man, Z.; Zhu, L.; Xia, X.; Zhao, L.; Zhao, Y.; Wang, X. The molecular mechanisms of the effect of Dexamethasone and Cyclosporin A on TLR4/NF-κB signaling pathway activation in oral lichen planus. *Gene* **2012**, *508*, 157-164.
65. Fiorentino, D.F.; Zlotnik, A.; Mosmann, T.; Howard, M.; O'garra, A. IL-10 inhibits cytokine production by activated macrophages. *The Journal of Immunology* **1991**, *147*, 3815-3822.
66. Laufer, N.; Simon, A.; Schenker, J.; Sekeles, E.; Cohen, R. Fallopian tubal mucosal damage induced experimentally by Escherichia coli in the rabbit: a scanning electron microscopic study. *Pathology-Research and Practice* **1984**, *178*, 605-610.
67. Zalewska-Piatek, B.; Piatek, R.; Kur, J. Adhesive and invasive factors of uropathogenic Escherichia coli strains-urinary tract infections and mechanisms of pathogenesis. *POSTEPY MIKROBIOLOGII* **2008**, *47*, 199-206.
68. Xu, D.-X.; Wang, H.; Zhao, L.; Ning, H.; Chen, Y.-H.; Zhang, C. Effects of low-dose lipopolysaccharide (LPS) pretreatment on LPS-induced intra-uterine fetal death and preterm labor. *Toxicology* **2007**, *234*, 167-175.
69. Aisemberg, J.; Vercelli, C.; Billi, S.; Ribeiro, M.; Ogando, D.; Meiss, R.; McCann, S.; Rettori, V.; Franchi, A. Nitric oxide mediates prostaglandins' deleterious effect on lipopolysaccharide-triggered murine fetal resorption. *Proceedings of the National Academy of Sciences* **2007**, *104*, 7534-7539.
70. Ogando, D.; Paz, D.; Cella, M.; Franchi, A. The fundamental role of increased production of nitric oxide in lipopolysaccharide-induced embryonic resorption in mice. *Reproduction* **2003**, *125*, 95-110.
71. Swangchan-Uthai, T.; Lavender, C.R.; Cheng, Z.; Fouladi-Nashta, A.A.; Wathes, D.C. Time course of defense mechanisms in bovine endometrium in response to lipopolysaccharide. *Biology of reproduction* **2012**, *87*, 135, 131-113.
72. Chen, C.; Zibiao, H.; Ming, Z.; Shiyi, C.; Ruixia, L.; Jie, W.; SongJia, L. Expression pattern of Toll-like receptors (TLRs) in different organs and effects of lipopolysaccharide on the expression of TLR 2 and 4 in reproductive organs of female rabbit. *Developmental & Comparative Immunology* **2014**, *46*, 341-348.
73. Quayle, A.J. The innate and early immune response to pathogen challenge in the female genital tract and the pivotal role of epithelial cells. *Journal of Reproductive Immunology* **2002**, *57*, 61-79, doi:https://doi.org/10.1016/S0165-0378(02)00019-0.
74. Cronin, J.; Kanamarlapudi, V.; Thornton, C.; Sheldon, I. Signal transducer and activator of transcription-3 licenses Toll-like receptor 4-dependent interleukin (IL)-6 and IL-8 production via IL-6 receptor-positive feedback in endometrial cells. *Mucosal immunology* **2016**, *9*, 1125.
75. Krikun, G.; Trezza, J.; Shaw, J.; Rahman, M.; Guller, S.; Abrahams, V.M.; Lockwood, C.J. Lipopolysaccharide appears to activate human endometrial endothelial cells through TLR-4-dependent and TLR-4-independent mechanisms. *American journal of reproductive immunology* **2012**, *68*, 233-237.
76. Jursza, E.; Kowalewski, M.; Boos, A.; Skarzynski, D.; Socha, P.; Siemieniuch, M. The role of toll-like receptors 2 and 4 in the pathogenesis of feline pyometra. *Theriogenology* **2015**, *83*,

596-603.

77. Wira, C.R.; Grant-Tschudy, K.S.; Crane-Godreau, M.A. Epithelial cells in the female reproductive tract: a central role as sentinels of immune protection. *American journal of reproductive immunology* **2005**, *53*, 65-76.
78. Yoshimura, A.; Mori, H.; Ohishi, M.; Aki, D.; Hanada, T. Negative regulation of cytokine signaling influences inflammation. *Current opinion in immunology* **2003**, *15*, 704-708.
79. Healy, L.L.; Cronin, J.G.; Sheldon, I.M. Endometrial cells sense and react to tissue damage during infection of the bovine endometrium via interleukin 1. *Scientific reports* **2014**, *4*, 7060.
80. Bretscher, P.A.; Havele, C. Cyclosporin A can switch the immune response induced by antigen from a humoral to a cell-mediated mode. *European journal of immunology* **1992**, *22*, 349-355.
81. Armstrong, L.; Jordan, N.; Millar, A. Interleukin 10 (IL-10) regulation of tumour necrosis factor alpha (TNF-alpha) from human alveolar macrophages and peripheral blood monocytes. *Thorax* **1996**, *51*, 143-149.
82. Garcia, J.E.L.; de Cabo, M.R.M.; Rodríguez, F.M.; Losada, J.P.; López, A.J.; Arellano, J.L.P. Effect of cyclosporin A on inflammatory cytokine production by U937 monocyte-like cells. *Mediators of inflammation* **2000**, *9*, 169-174.
83. López-Flores, R.; Bojalil, R.; Benítez, J.C.; Ledesma-Soto, Y.; Terrazas, C.A.; Rodríguez-Sosa, M.; Terrazas, L.I. Consecutive low doses of cyclosporine A induce pro-inflammatory cytokines and accelerate allograft skin rejection. *Molecules* **2011**, *16*, 3969-3984.
84. Borger, P.; Kauffman, H.; Timmerman, J.; Scholma, J.; Van den Berg, J.; Koeter, G. Cyclosporine, FK506, mycophenolate mofetil, and prednisolone differentially modulate cytokine gene expression in human airway-derived epithelial cells. *Transplantation* **2000**, *69*, 1408-1413.
85. Hossain, M.; Okubo, Y.; Sekiguchi, M. Effects of various drugs (staurosporine, herbimycin A, ketotifen, theophylline, FK506 and cyclosporin A) on eosinophil viability. *Japanese Journal of Allergology* **1994**, *43*, 711-717.
86. Alberti, S.; Boraschi, D.; Luini, W.; Tagliabue, A. Effects of in vivo treatments with cyclosporin-A on mouse cell-mediated immune responses. *International journal of immunopharmacology* **1981**, *3*, 357-364.
87. Pores-Fernando, A.T.; Gaur, S.; Doyon, M.Y.; Zweifach, A. Calcineurin-dependent lytic granule exocytosis in NK-92 natural killer cells. *Cellular immunology* **2009**, *254*, 105-109.
88. Zhang, G.-W.; Wang, H.-Z.; Chen, S.-Y.; Li, Z.-C.; Zhang, W.-X.; Lai, S.-J. A reduced incidence of digestive disorders in rabbits is associated with allelic diversity at the TLR4 locus. *Veterinary immunology and immunopathology* **2011**, *144*, 482-486.
89. Liu, D.-P.; He, Q.-J.; Liu, C.-G. Correlations among Helicobacter pylori infection and the expression of cyclooxygenase-2 and vascular endothelial growth factor in gastric mucosa with intestinal metaplasia or dysplasia. *Journal of Gastroenterology and Hepatology* **2010**, *4*, 795-799.

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Table 1. Primer information and PCR conditions.

Gene	Accession No.	Primer sequences	PCR conditions	Product size (bp)
IL-1β	M26295	F: 5′- AAGAAGAACCCGTCCTCTGCAACA -3′ R: 5′- TCAGCTCATACGTGCCAGACAACA -3′	95 °C/3 min → (95 °C/10 s →60 °C/30 s) × 40 → 68 °C/3 min	128
IL-6	AF169176	F: 5′- CTGAAGACGACCACGATCCA -3′ R: 5′- AAGGACACCCGCACTCCAT -3′	95 °C/3 min → (95 °C/10 s → 60 °C/30 s) × 40 → 68 °C/3 min	98
IL-8	KT279656	F: 5′- CTCTCTTGGCAACCTTCCTG-3′ R: 5′- TTGCACAGTGAGGTCCACTC-3′	95 °C/3 min → (95 °C/10 s →60 °C/30 s) × 40 → 68 °C/3 min	165
TNF-α	M12845	F: 5′-CTCCTACCCGAACAAGGTCA-3′ R: 5′- CGGTCACCCTTCTCCAACT-3′	95 °C/3 min → (95 °C/10 s → 60 °C/30 s) × 40 → 68 °C/3 min	138
IFN-γ	AB010386	F: 5′-TTCCCAAGGATAGCAGTGGT-3′ R: 5′- TGAAGCCAGAAGTCCTCAAAA-3′	95 °C/3 min → (95 °C/10 s →60 °C/30 s) × 40 → 68 °C/3 min	160
IL-4	DQ852343	F: 5′-AGAGCTCGGTGACCTCAGAC-3′ R: 5′- CTTGCATGGCGGTCTTTAG-3′	95 °C/3 min → (95 °C/10 s → 60 °C/30 s) × 40 → 68 °C/3 min	140
IL-10	KT279661	F: 5′-GAGAACCACAGTCCAGCCAT -3′ R: 5′-CATGGCTTTGTAGACGCCTT -3′	95 °C/3 min → (95 °C/10 s → 60 °C/30 s) × 40 → 68 °C/3 min	177
IL-13	XM-002710092.3	F: 5′-ATTGCTGTGACCTGCCTT-3′ R: 5′- AGCCTTCTGGTTGTGTGTG-3′	95 °C/3 min → (95 °C/10 s → 60 °C/30 s) × 40 → 68 °C/3 min	111
TGF-β	AF068058	F: 5′-TGG ACA CCA ACT ACT GCT-3′ R: 5′- TGT GCT GGT TGT ACA GG-3′	95 °C/3 min → (95 °C/10 s → 60 °C/30 s) × 40 → 68 °C/3 min	201
GAPDH	NC-013676	F: 5′-TGTTTGTGATGGGCGTGAA -3′ R: 5′-CCTCCACAATGCCGAAGT -3′	95 °C/3 min → (95 °C/10 s → 60 °C/30 s) × 40 → 68 °C/3 min	150

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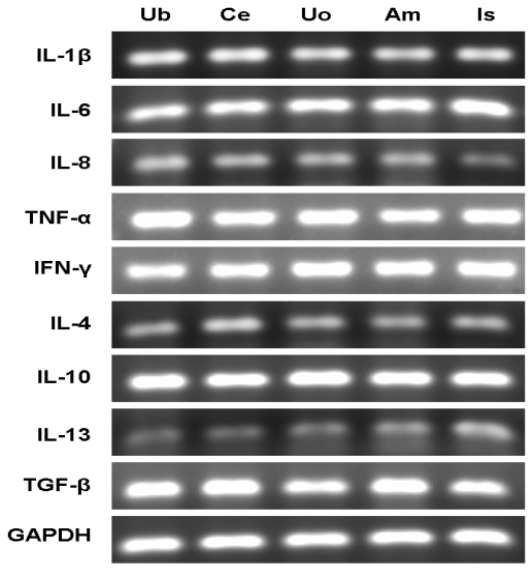

Figure 1. Expression profile of immune cytokines in the genital tract of a female rabbit. RT-PCR was performed to examine the expression of immune cytokines IL-1β, IL-6, IL-8, IL-4, IL-10, IL-13, TNF-α, IFN-γ, and TGF-β of control group animals. Ub: uterine body; Ce: cervix; Uo: uterotubal junction; Am: oviductal ampulla; Is: oviductal isthmus.

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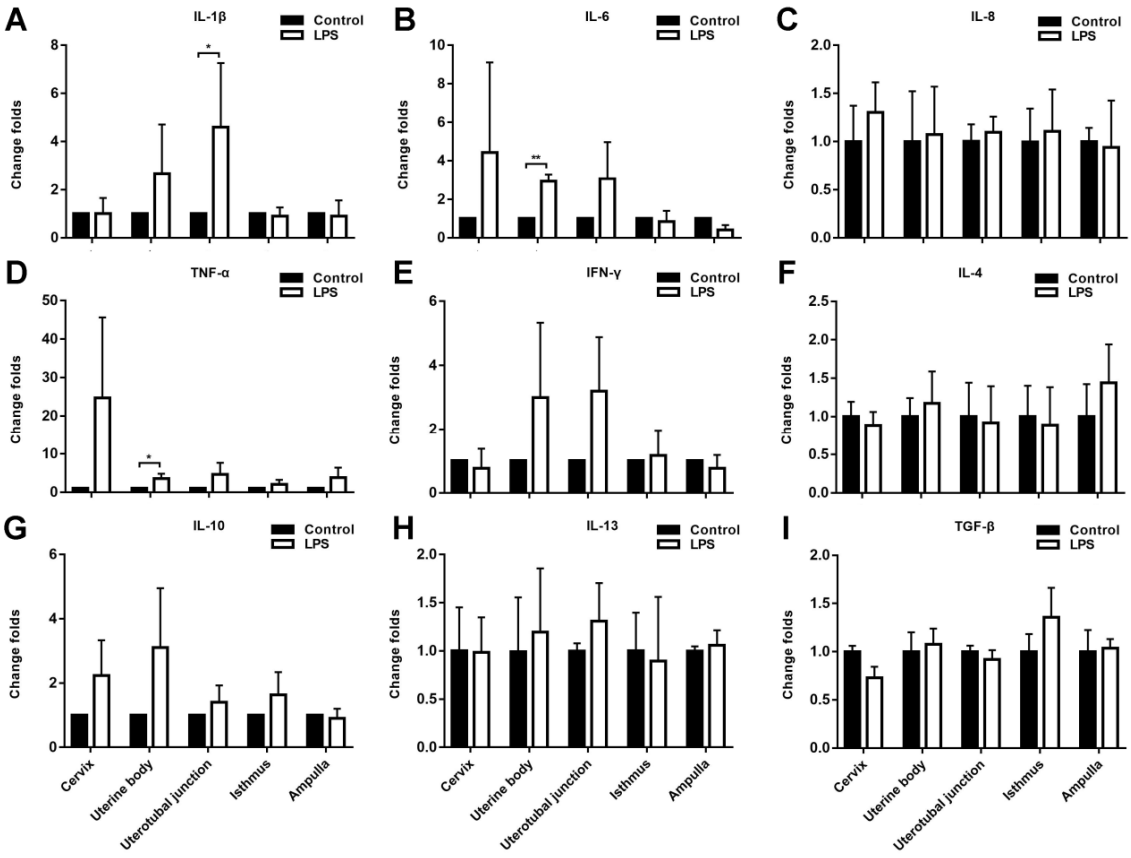

Figure 2. Lipopolysaccharide (LPS) induced the up-regulation of pro-inflammatory cytokines but did not affect anti-inflammatory cytokines in the genital tract of female rabbits. The rabbits were given an intrauterine infusion of *E. coli* LPS (4 mg/kg body weight (BW)) or LPS carrier. The tissue specimens from cervix, uterine body, uterotubal junction, oviductal isthmus, and ampulla were collected 3 h after LPS administration, and the mRNA expression of immune cytokines was analyzed using RT-qPCR. The GAPDH was used as an internal control. Each gene was analyzed in triplicate. The data were analysed using SPSS statistical software, and the results were presented as the mean ± SD ($n = 3$). *$p < 0.05$ and **$p < 0.01$.

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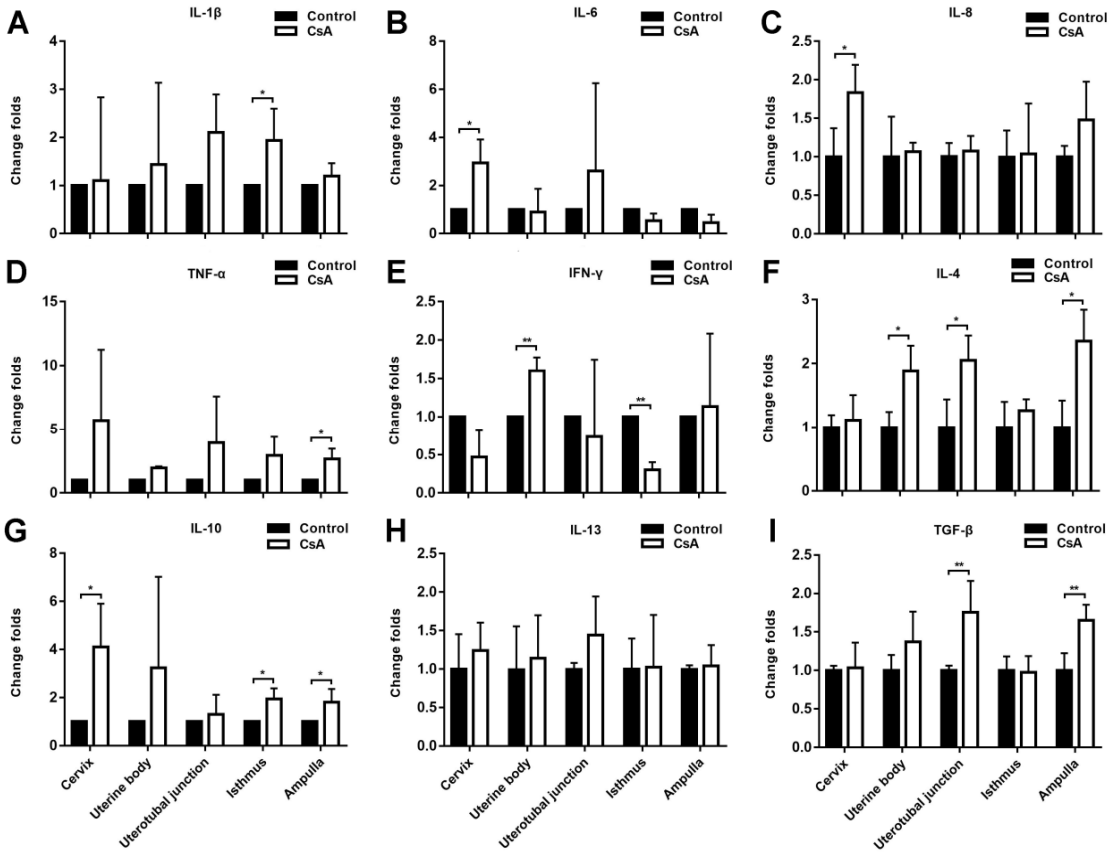

Figure 3. Cyclosporine A (CsA) increased the mRNA expression of pro- and anti-inflammatory cytokines in the genital tract of female rabbits. The rabbits were given CsA (20 mg/kg BW) or CsA carrier via intramuscular injection. The tissue specimens from the cervix, uterine body, uterotubal junction, oviductal isthmus, and ampulla were collected 3 h post-injection, and the mRNA expression of immune cytokines was analyzed using RT-qPCR. The GAPDH was used as an internal control. Each gene was analyzed in triplicate. The data were analyzed using SPSS statistical software, and the results are presented as the mean ± SD (n=3). *$p < 0.05$ and **$p < 0.01$.

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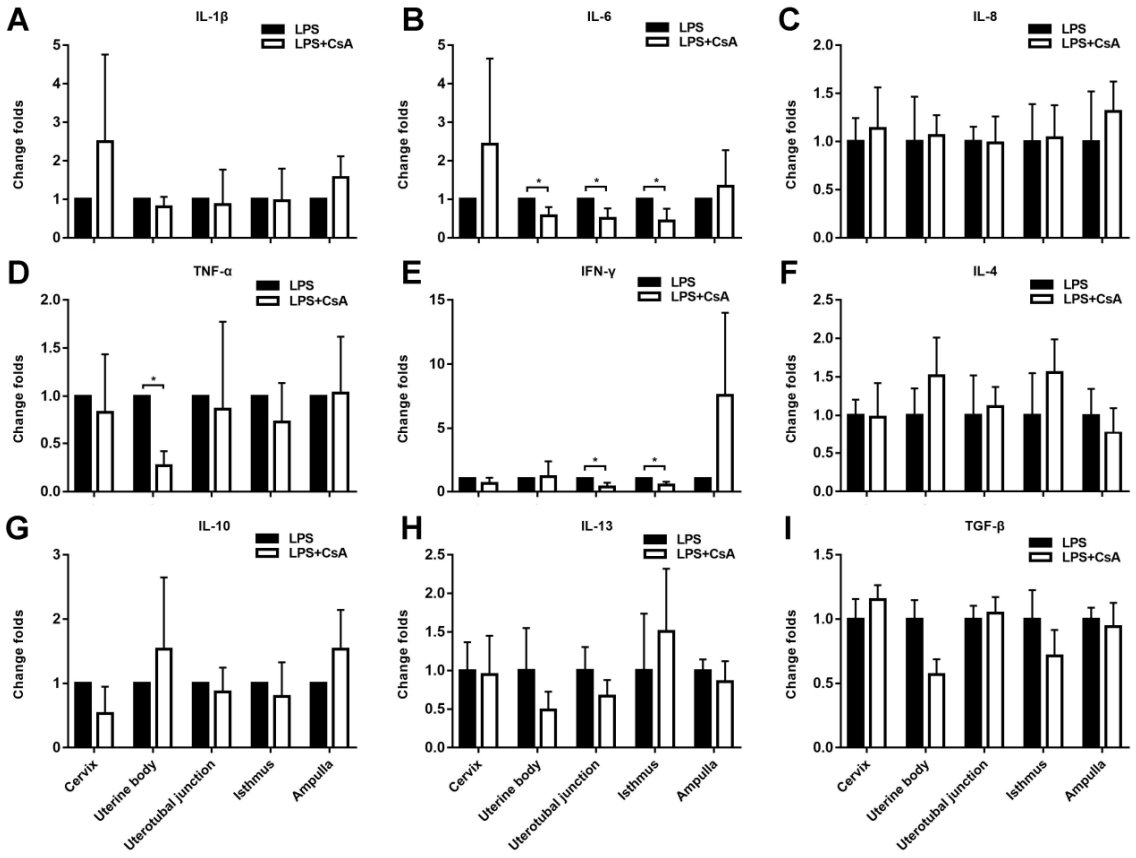

Figure 4. CsA inhibited the mRNA expression of LPS-induced pro-inflammatory cytokines. LPS rabbits were given intrauterine infusion of *E. coli* LPS (4 mg/kg BW). LPS + CsA rabbits were given an intrauterine infusion of *E. coli* LPS (4 mg/kg BW) and an intramuscular injection of CsA (20 mg/kg BW). The tissue specimens from cervix, uterine body, uterotubal junction, oviductal isthmus, and ampulla were collected 3 h post-injection, and the mRNA expression of immune cytokines was analyzed using RT-qPCR. The GAPDH was used as an internal control. Each gene was analyzed in triplicate. The data were analyzed using SPSS statistical software, and the results are presented as the mean ± SD (n=3). *$p < 0.05$ and **$p < 0.01$.

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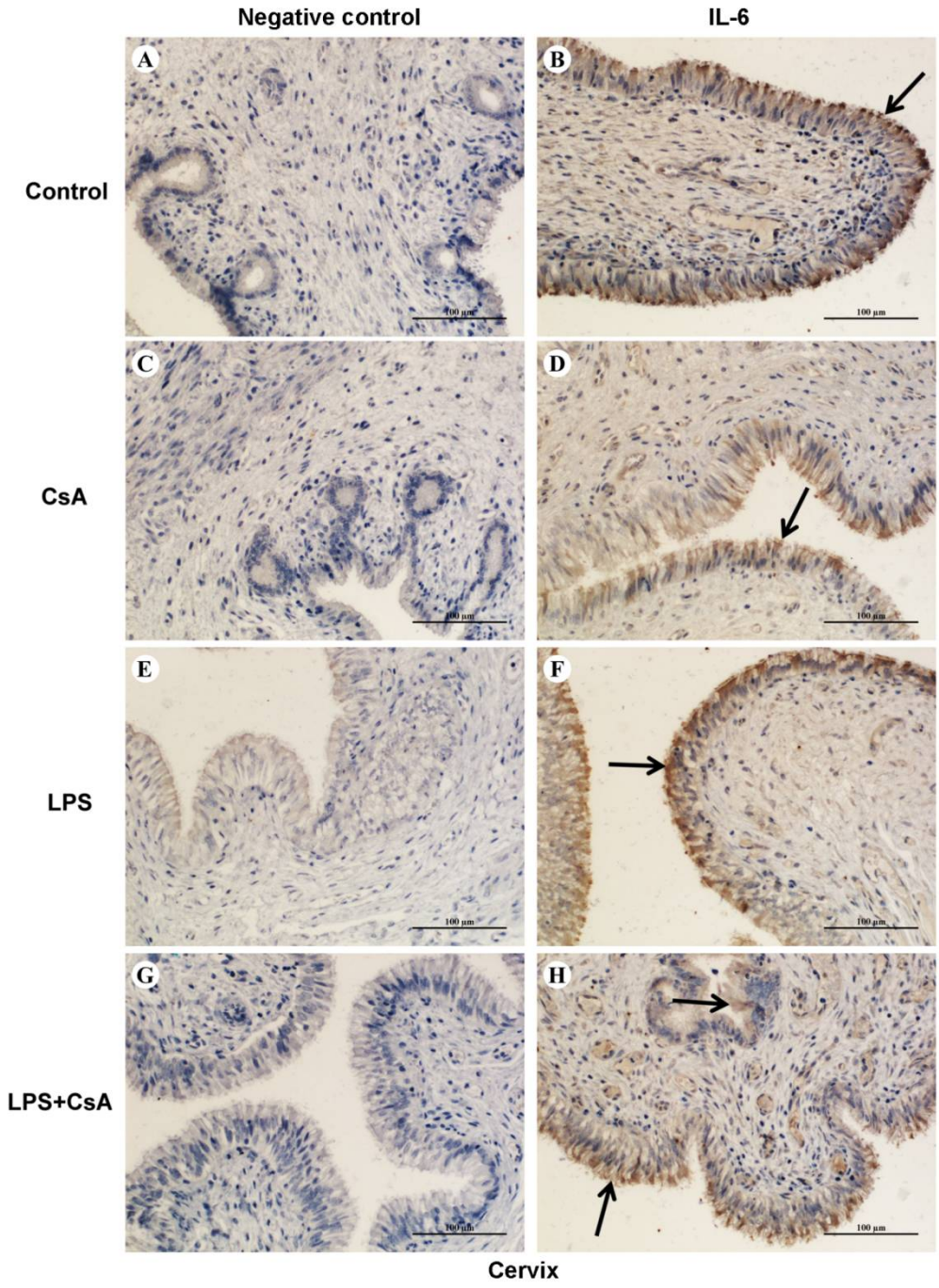

Figure 5. Immuno-histochemical localization of IL-6 in the cervix of a female rabbit. Twelve rabbits (n = 3 each) were given an intrauterine infusion of *E. coli* LPS (4 mg/kg BW) and/or an intramuscular injection of CsA (20 mg/kg BW). The tissue specimen from the cervix was collected 3 h post-injection. The distribution of IL-6 in the cervix was identified through an immuno-histochemical assay using IL-6 mouse monoclonal antibody (1:500) and goat anti-mouse secondary antibody (1:200). Mouse IgG (1:200) was used as a negative control. Nuclear counterstaining was done with hematoxylin. Black arrows indicate the positive areas of IL-6 in the cervix. (**a,c,e,g**) Negative control. (**b,d,f,h**) IL-6. (a) and (b) represent control groups; (c) and (d) represent the CsA groups; (e) and (f) represent the LPS groups; and (g) and (h) represent the LPS + CsA groups.

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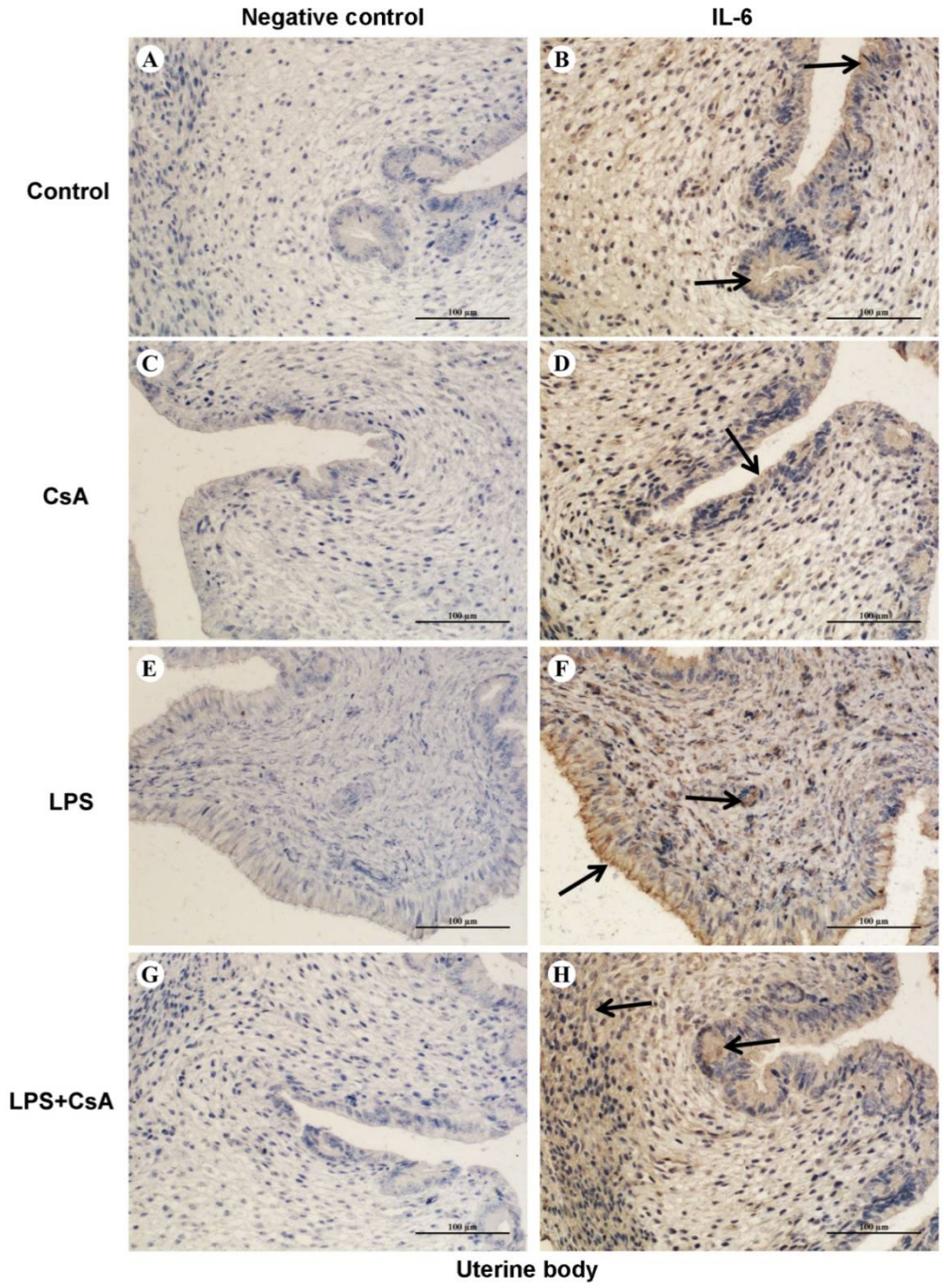

Figure 6. Immuno-histochemical localization of IL-6 in the uterine body of a female rabbit. Twelve rabbits (n = 3 each) were given an intrauterine infusion of *E. coli* LPS (4 mg/kg BW) and/or an intramuscular injection of CsA (20 mg/kg BW). The tissue specimen from the uterine body was collected 3 h post-injection. The distribution of IL-6 in uterine body was identified through an immuno-histochemical assay using IL-6 mouse monoclonal antibody (1:500) and goat anti-mouse secondary antibody (1:200). Mouse IgG (1:200) was used as a negative control. Nuclear counterstaining was done with hematoxylin. Black arrows indicate the positive areas of IL-6 in the uterine body. (**a,c,e,g**) Negative control. (**b,d,f,h**) IL-6. (a) and (b) represent control groups; (c) and (d) represent the CsA groups; (e) and (f) represent the LPS groups; and (g) and (h) represent the LPS + CsA groups.

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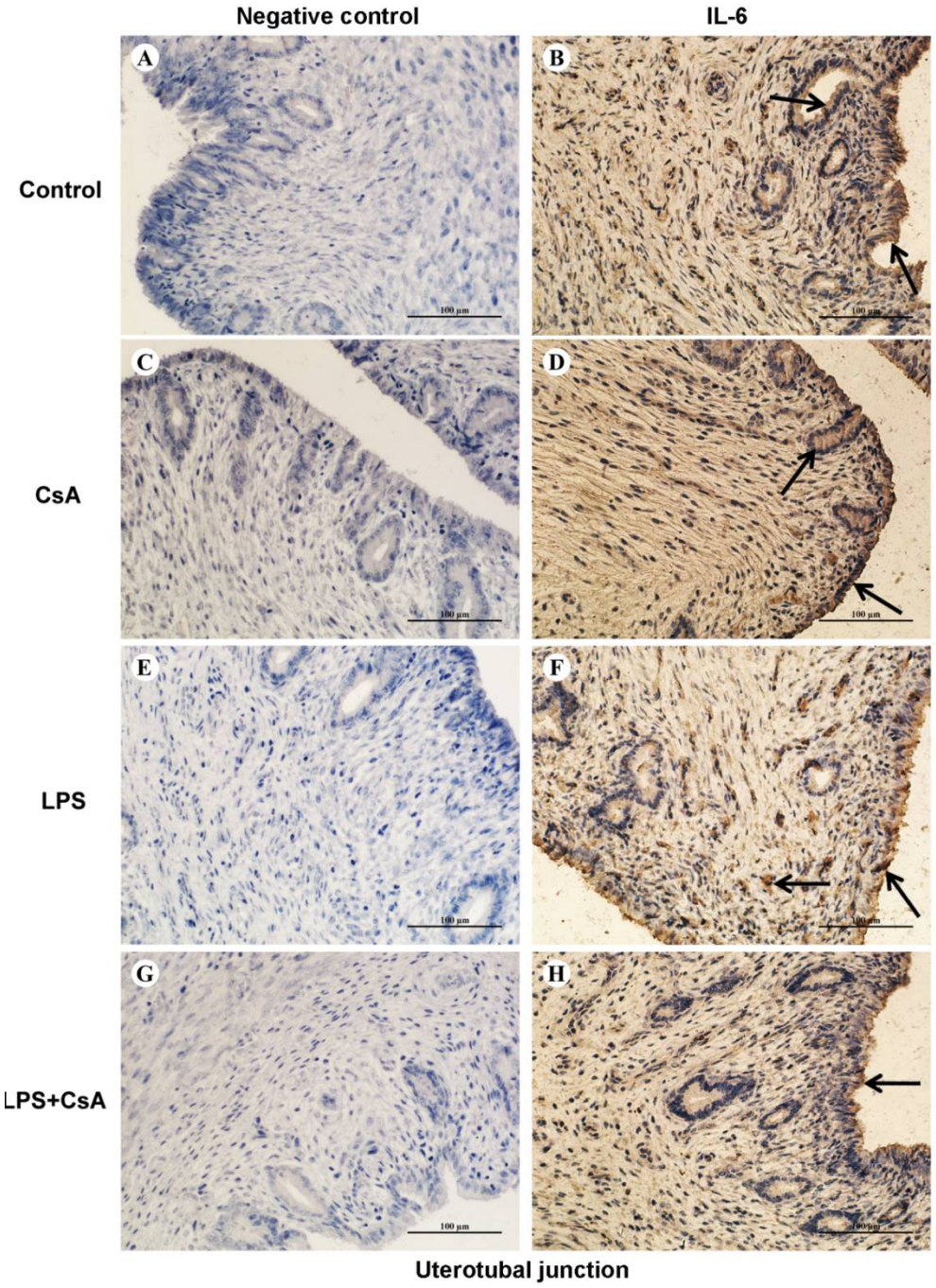

Figure 7. Immuno-histochemical localization of IL-6 in the uterotubal junction of a female rabbit. Twelve rabbits (n = 3 each) were given an intrauterine infusion of *E. coli* LPS (4 mg/kg BW) and/or an intramuscular injection of CsA (20 mg/kg BW). The tissue specimen from the uterotubal junction was collected 3 h post-injection. The distribution of IL-6 in the uterotubal junction was identified through an immuno-histochemical assay using IL-6 mouse monoclonal antibody (1:500) and goat anti-mouse secondary antibody (1:200). Mouse IgG (1:200) was used as a negative control. Nuclear counterstaining was done with hematoxylin. Black arrows indicate the positive areas of IL-6 in the uterotubal junction. (**a,c,e,g**) Negative control. (**b,d,f,h**) IL-6. (a) and (b) represent control groups; (c) and (d) represent the CsA groups; (e) and (f) represent the LPS groups; and (g) and (h) represent the LPS + CsA groups.

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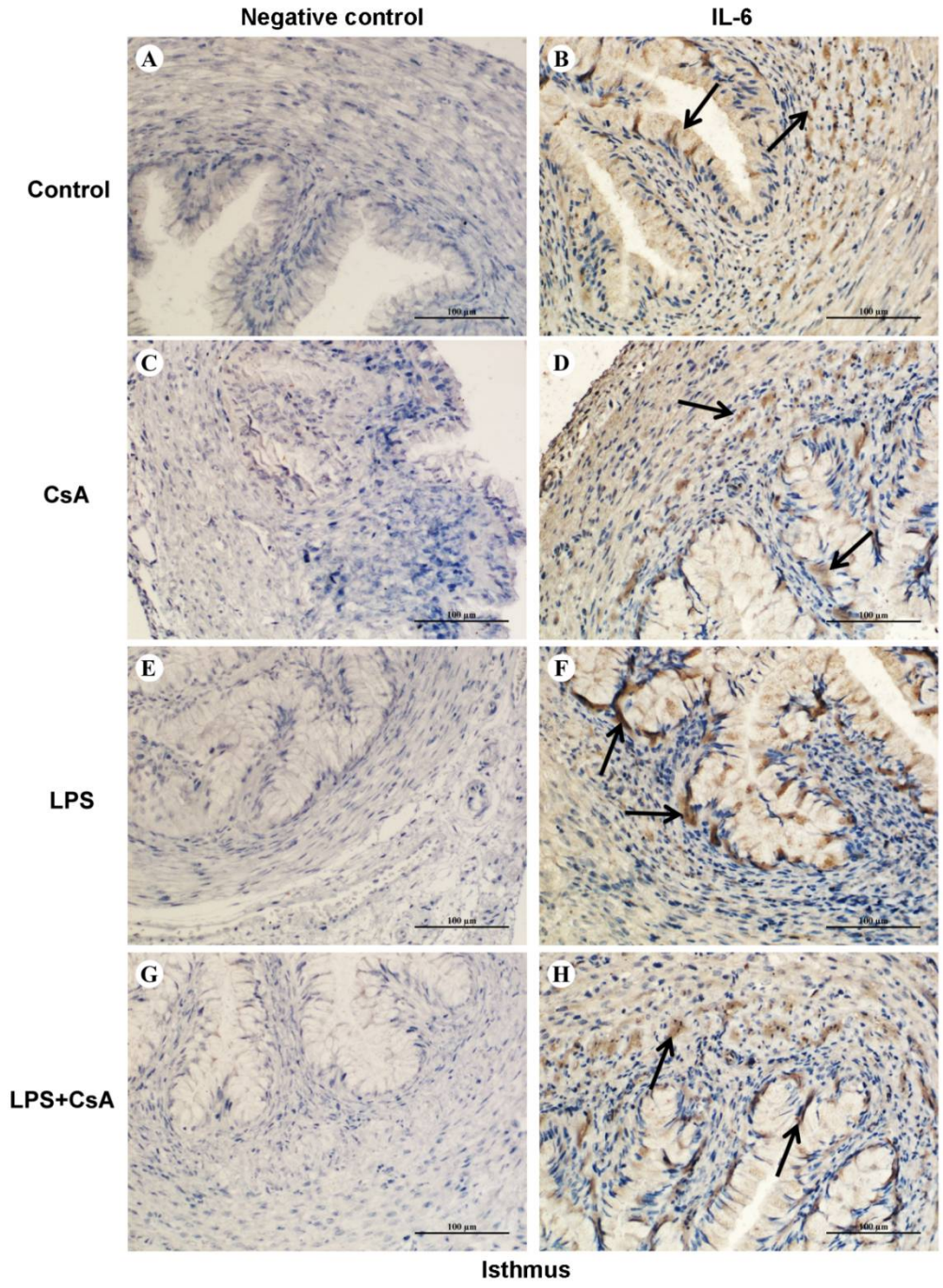

Figure 8. Immuno-histochemical localization of IL-6 in the oviductal isthmus of a female rabbit. Twelve rabbits (n = 3 each) were given an intrauterine infusion of *E. coli* LPS (4 mg/kg BW) and/or an intramuscular injection of CsA (20 mg/kg BW). The tissue specimen from the oviductal isthmus was collected 3 h post-injection. The distribution of IL-6 in the oviductal isthmus was identified through an immuno-histochemical assay using IL-6 mouse monoclonal antibody (1:500) and goat anti-mouse secondary antibody (1:200). Mouse IgG (1:200) was used as a negative control. Nuclear counterstaining was done with hematoxylin. Black arrows indicate the positive areas of IL-6 in the oviductal isthmus. (**a,c,e,g**) Negative control. (**b,d,f,h**) IL-6. (a) and (b) represent control groups; (c) and (d) represent the CsA groups; (e) and (f) represent the LPS groups; and (g) and (h) represent the LPS + CsA groups.

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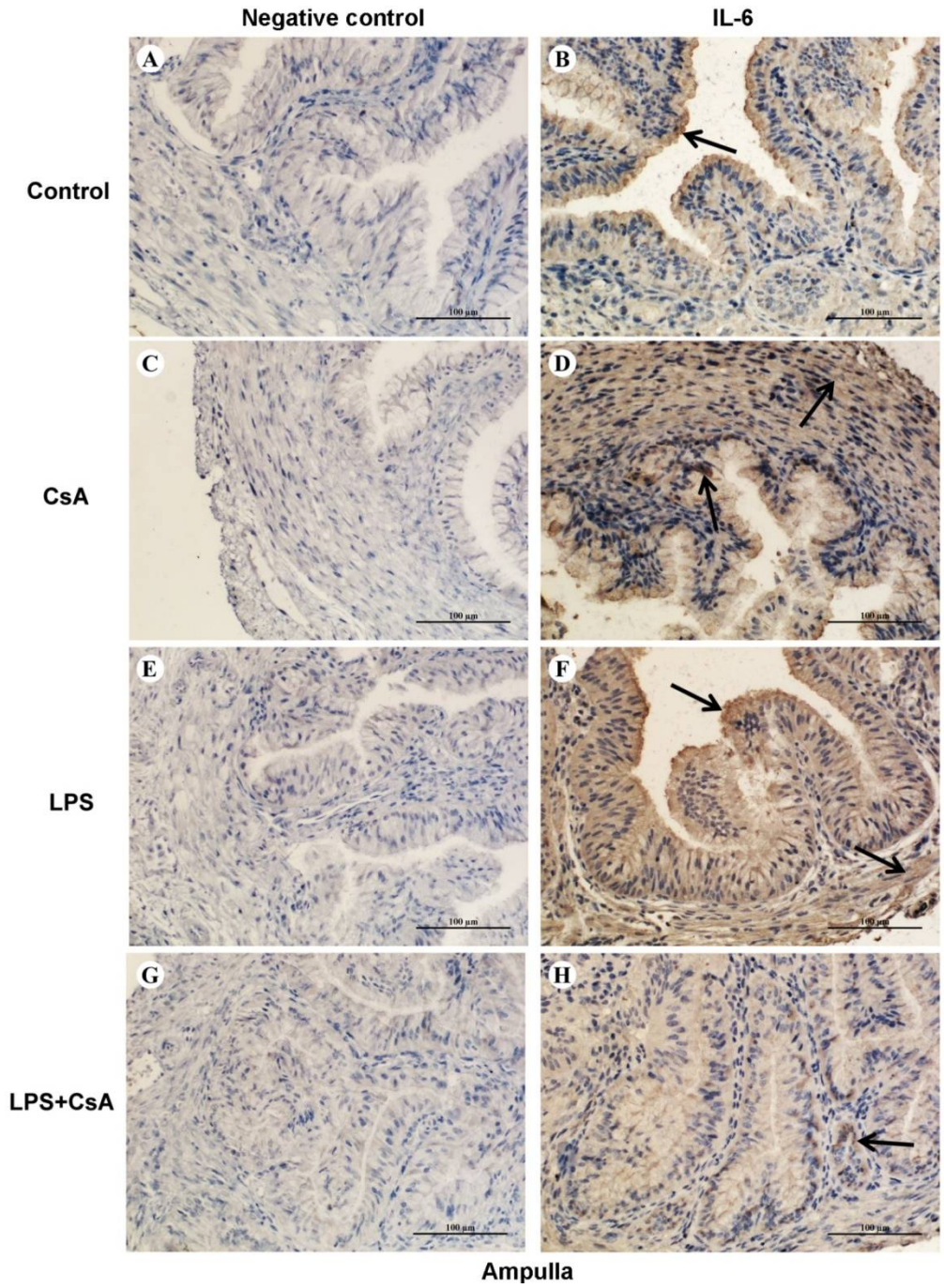

Figure 9. Immuno-histochemical localization of IL-6 in the oviductal ampulla of a female rabbit. Twelve rabbits (n = 3 each) were given an intrauterine infusion of *E. coli* LPS (4 mg/kg BW) and/or an intramuscular injection of CsA (20 mg/kg BW). The tissue specimen from the oviductal ampulla was collected 3 h post-injection. The distribution of IL-6 in the oviductal ampulla was identified through an immuno-histochemical assay using IL-6 mouse monoclonal antibody (1:500) and goat anti-mouse secondary antibody (1:200). Mouse IgG (1:200) was used as a negative control. Nuclear counterstaining was done with hematoxylin. Black arrows indicate the positive areas of IL-6 in the oviductal ampulla. (**a,c,e,g**) Negative control. (**b,d,f,h**) IL-6. (a) and (b) represent control groups; (c) and (d) represent the CsA groups; (e) and (f) represent the LPS groups; and (g) and (h) represent the LPS + CsA groups.

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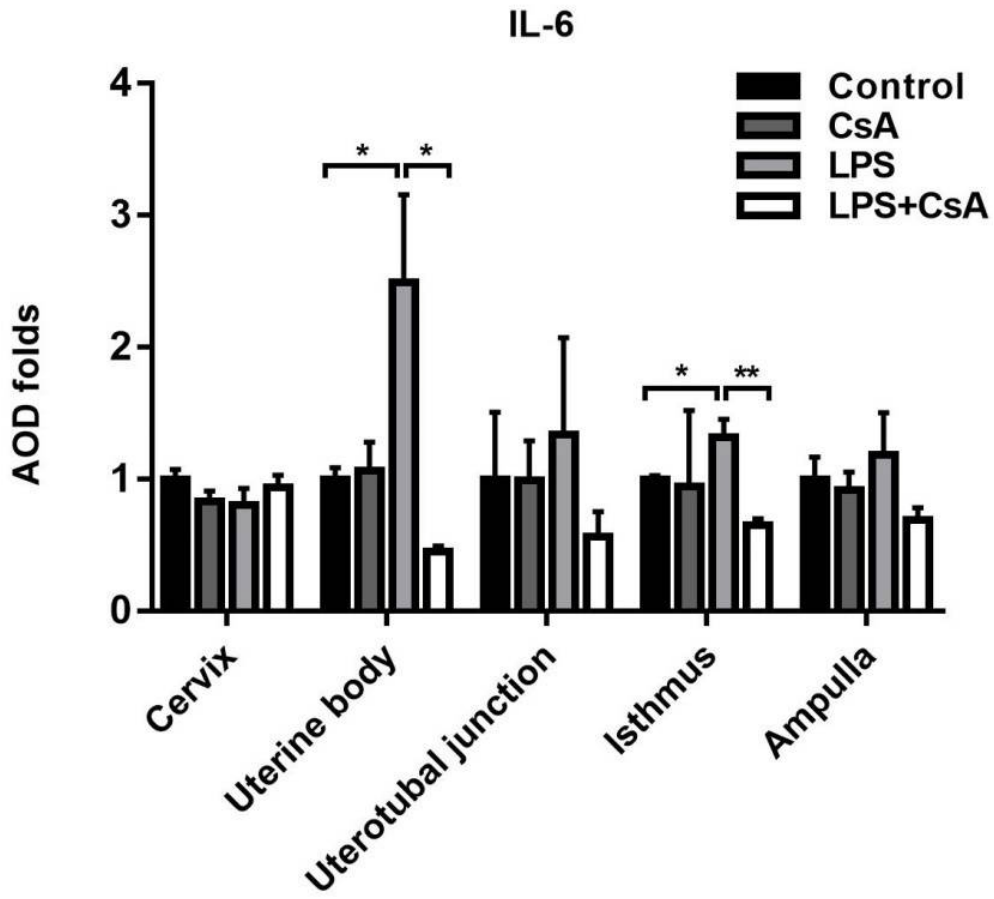

Figure 10. The protein expression of IL-6 in the genital tract of female rabbits. Twelve rabbits (n = 3 each) were given an intrauterine infusion of *E. coli* LPS (4 mg/kg BW) and/or an intramuscular injection of CsA (20 mg/kg BW). The tissue specimens from the cervix, uterine body, uterotubal junction, oviductal isthmus, and ampulla were collected 3 h post-injection. The protein expression of IL-6 was assessed using an immuno-histochemical assay. The positive areas of immune-histochemical staining were analyzed using Image-Pro Plus 6 software, and the data were analysed using SPSS statistical software. AOD: average optical density; AOD = Integrated optical density (IOD)/Area. The results are presented as the mean ± SD (n=3). *$p < 0.05$ and **$p < 0.01$.

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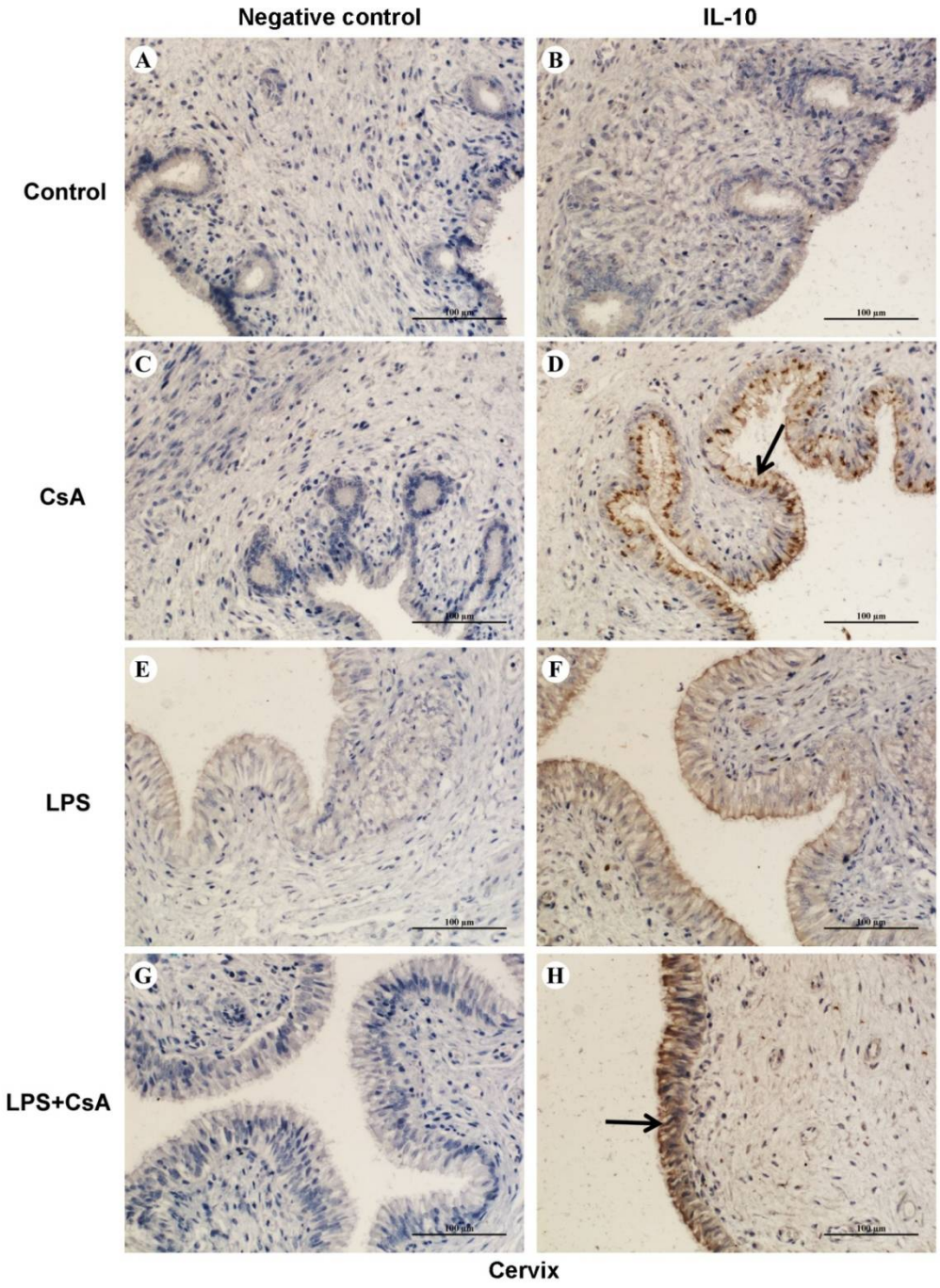

Figure 11. Immuno-histochemical localization of IL-10 in the cervix of a female rabbit. Twelve rabbits ($n = 3$ each) were given an intrauterine infusion of *E. coli* LPS (4 mg/kg BW) and/or an intramuscular injection of CsA (20 mg/kg BW). The tissue specimen from the cervix was collected 3 h post-injection. The distribution of IL-10 in the cervix was identified through an immuno-histochemical assay using IL-10 mouse monoclonal antibody (1:500) and goat anti-mouse secondary antibody (1:200). Mouse IgG (1:200) was used as a negative control. Nuclear counterstaining was done with hematoxylin. Black arrows indicate the positive areas of IL-10 in the cervix. (**a,c,e,g**) Negative control. (**b,d,f,h**) IL-10. (a) and (b) represent control groups; (c) and (d) represent the CsA groups; (e) and (f) represent the LPS groups; and (g) and (h) represent the LPS + CsA groups.

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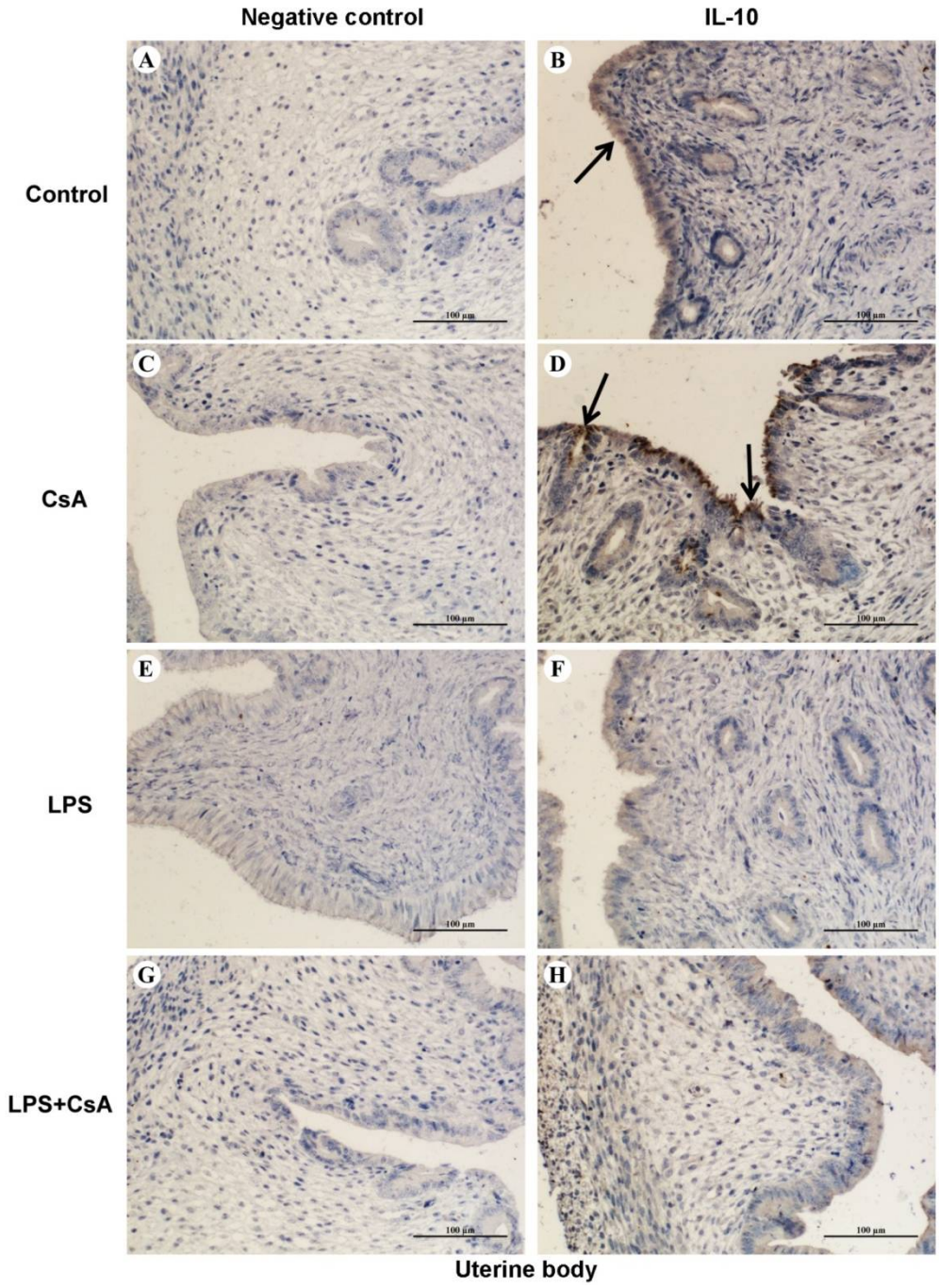

Figure 12. Immuno-histochemical localization of IL-10 in the uterine body of a female rabbit. Twelve rabbits (n = 3 each) were given an intrauterine infusion of *E. coli* LPS (4 mg/kg BW) and/or an intramuscular injection of CsA (20 mg/kg BW). The tissue specimen from the uterine body was collected 3 h post-injection. The distribution of IL-10 in the uterine body was identified through an immuno-histochemical assay using IL-10 mouse monoclonal antibody (1:500) and goat anti-mouse secondary antibody (1:200). Mouse IgG (1:200) was used as a negative control. Nuclear counterstaining was done with hematoxylin. Black arrows indicate the positive areas of IL-10 in the uterine body. (**a,c,e,g**) Negative control. (**b,d,f,h**) IL-10. (a) and (b) represent control groups; (c) and (d) represent the CsA groups; (e) and (f) represent the LPS groups; and (g) and (h) represent the LPS + CsA groups.

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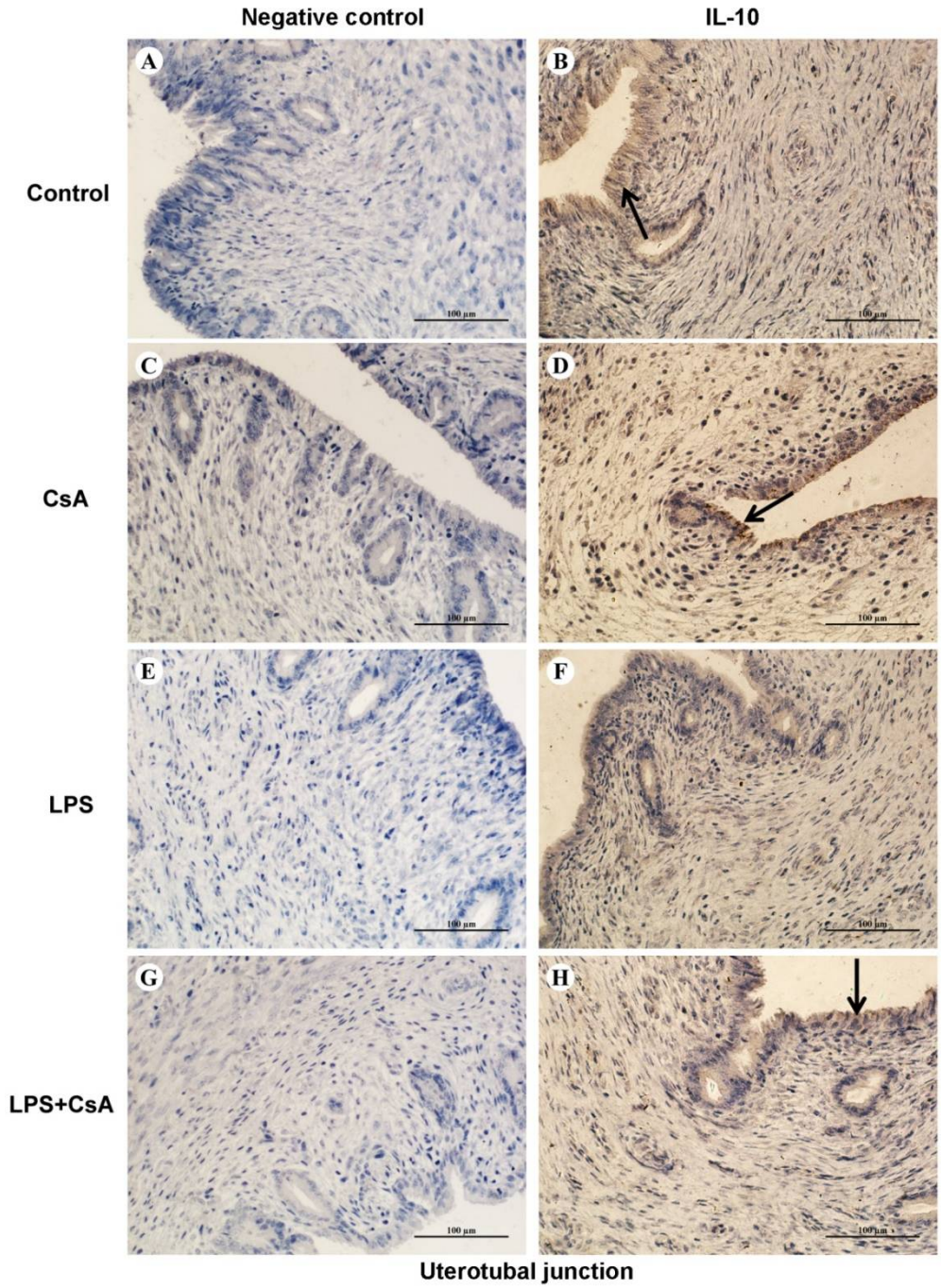

Figure 13. Immuno-histochemical localization of IL-10 in the uterotubal junction of a female rabbit. Twelve rabbits (n = 3 each) were given an intrauterine infusion of *E. coli* LPS (4 mg/kg BW) and/or an intramuscular injection of CsA (20 mg/kg BW). The tissue specimen from the uterotubal junction was collected 3 h post-injection. The distribution of IL-10 in the uterotubal junction was identified through an immuno-histochemical assay using IL-10 mouse monoclonal antibody (1:500) and goat anti-mouse secondary antibody (1:200). Mouse IgG (1:200) was used as a negative control. Nuclear counterstaining was done with hematoxylin. Black arrows indicate the positive areas of IL-10 in the uterotubal junction. (**a,c,e,g**) Negative control. (**b,d,f,h**) IL-10. (a) and (b) represent control groups; (c) and (d) represent the CsA groups; (e) and (f) represent the LPS groups; and (g) and (h) represent the LPS + CsA groups.

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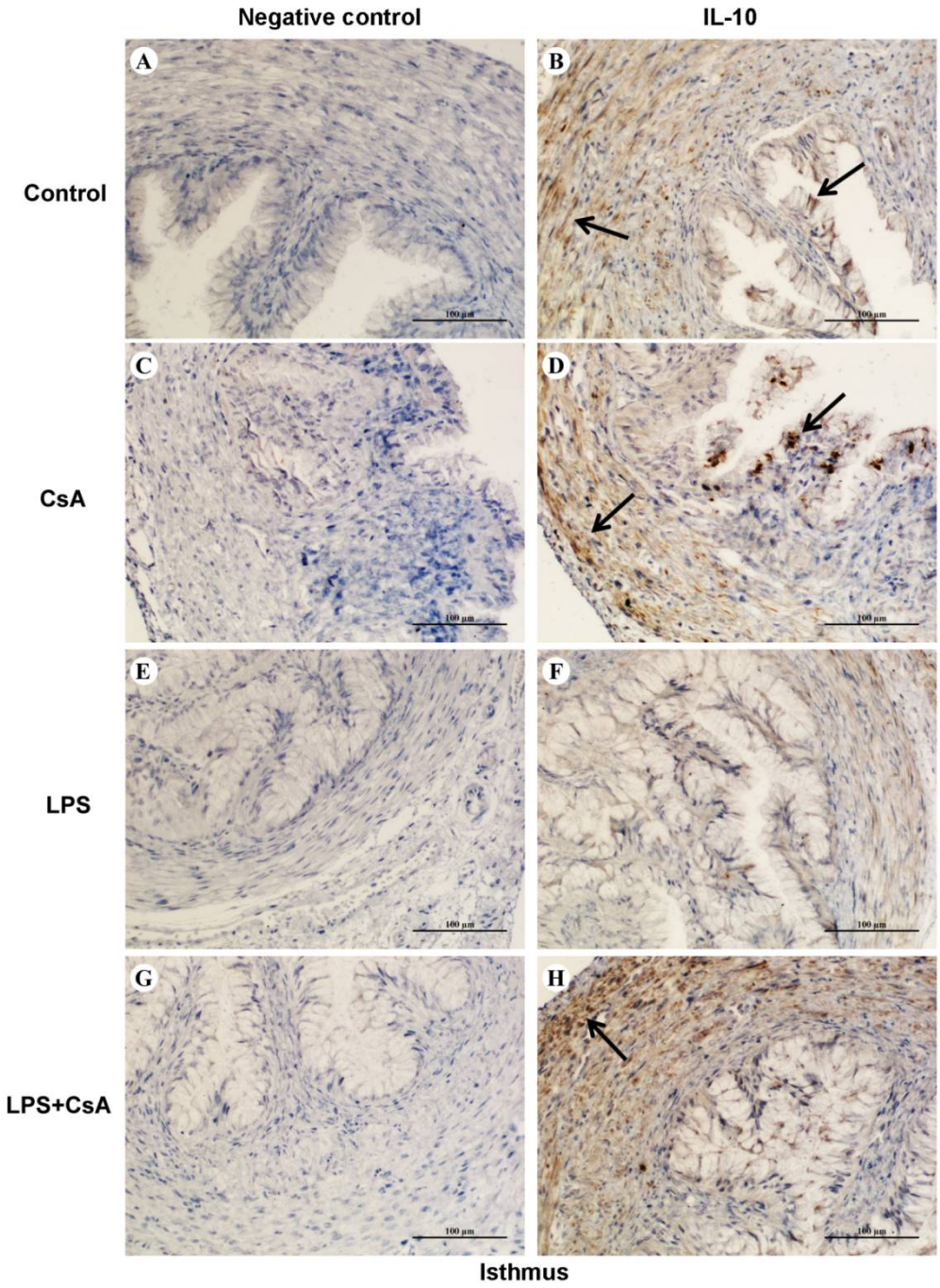

Figure 14. Immuno-histochemical localization of IL-10 in the oviductal isthmus of a female rabbit. Twelve rabbits (n = 3 each) were given an intrauterine infusion of *E. coli* LPS (4 mg/kg BW) and/or an intramuscular injection of CsA (20 mg/kg BW). The tissue specimen from the oviductal isthmus was collected 3 h post-injection. The distribution of IL-10 in the oviductal isthmus was identified through an immuno-histochemical assay using IL-10 mouse monoclonal antibody (1:500) and goat anti-mouse secondary antibody (1:200). Mouse IgG (1:200) was used as a negative control. Nuclear counterstaining was done with hematoxylin. Black arrows indicate the positive areas of IL-10 in the oviductal isthmus. (**a,c,e,g**) Negative control. (**b,d,f,h**) IL-10. (a) and (b) represent control groups; (c) and (d) represent the CsA groups; (e) and (f) represent the LPS groups; and (g) and (h) represent the LPS + CsA groups.

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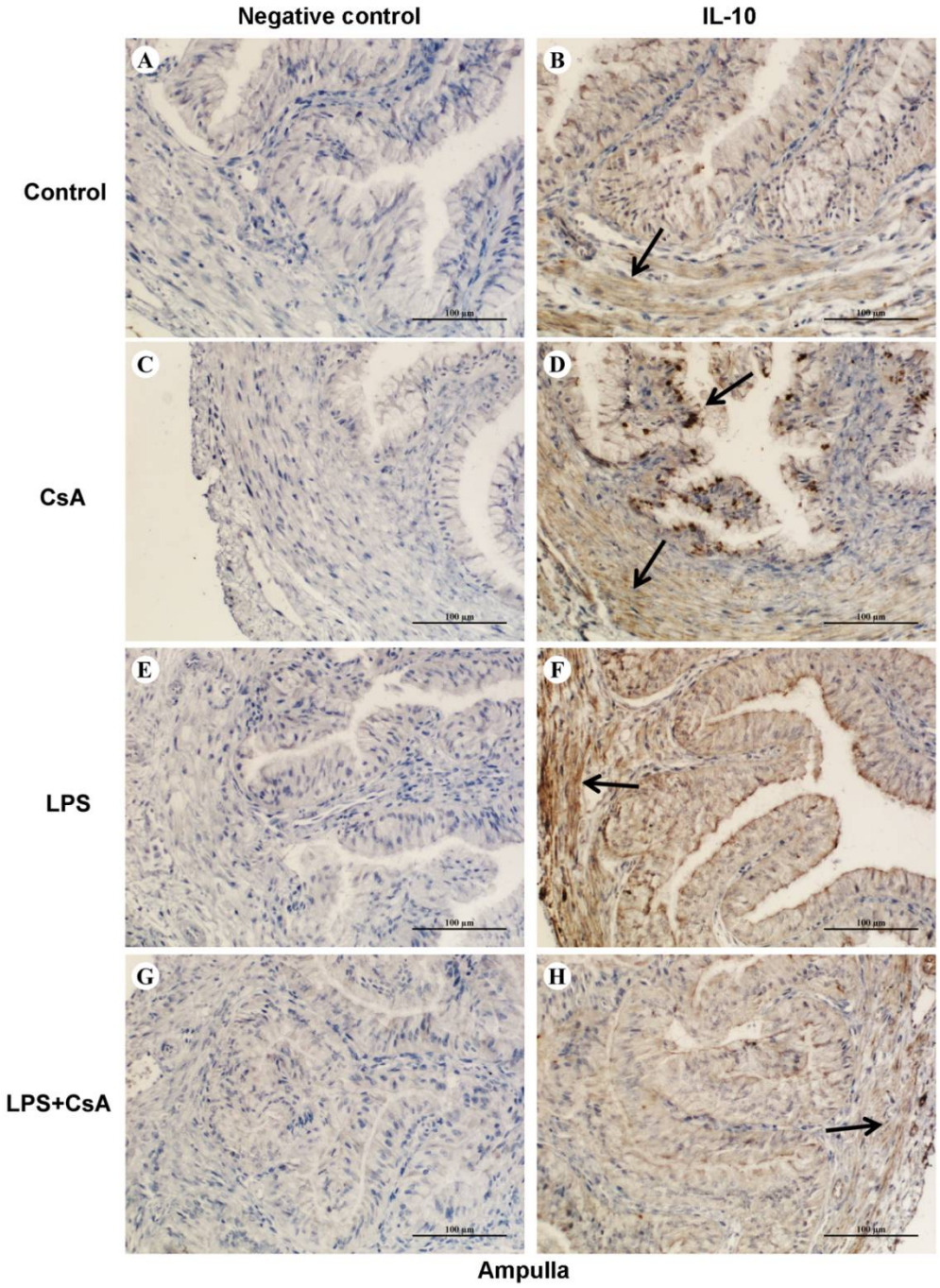

Figure 15. Immuno-histochemical localization of IL-10 in the oviductal ampulla of a female rabbit. Twelve rabbits (n = 3 each) were given an intrauterine infusion of *E. coli* LPS (4 mg/kg BW) and/or an intramuscular injection of CsA (20 mg/kg BW). The tissue specimen from the oviductal ampulla was collected 3 h post-injection. The distribution of IL-10 in the oviductal ampulla was identified through an immuno-histochemical assay using IL-10 mouse monoclonal antibody (1:500) and goat anti-mouse secondary antibody (1:200). Mouse IgG (1:200) was used as a negative control. Nuclear counterstaining was done with hematoxylin. Black arrows indicate the positive areas of IL-10 in the oviductal ampulla. (**a,c,e,g**) Negative control. (**b,d,f,h**) IL-10. (a) and (b) represent control groups; (c) and (d) represent the CsA groups; (e) and (f) represent the LPS groups; and (g) and (h) represent the LPS + CsA groups.

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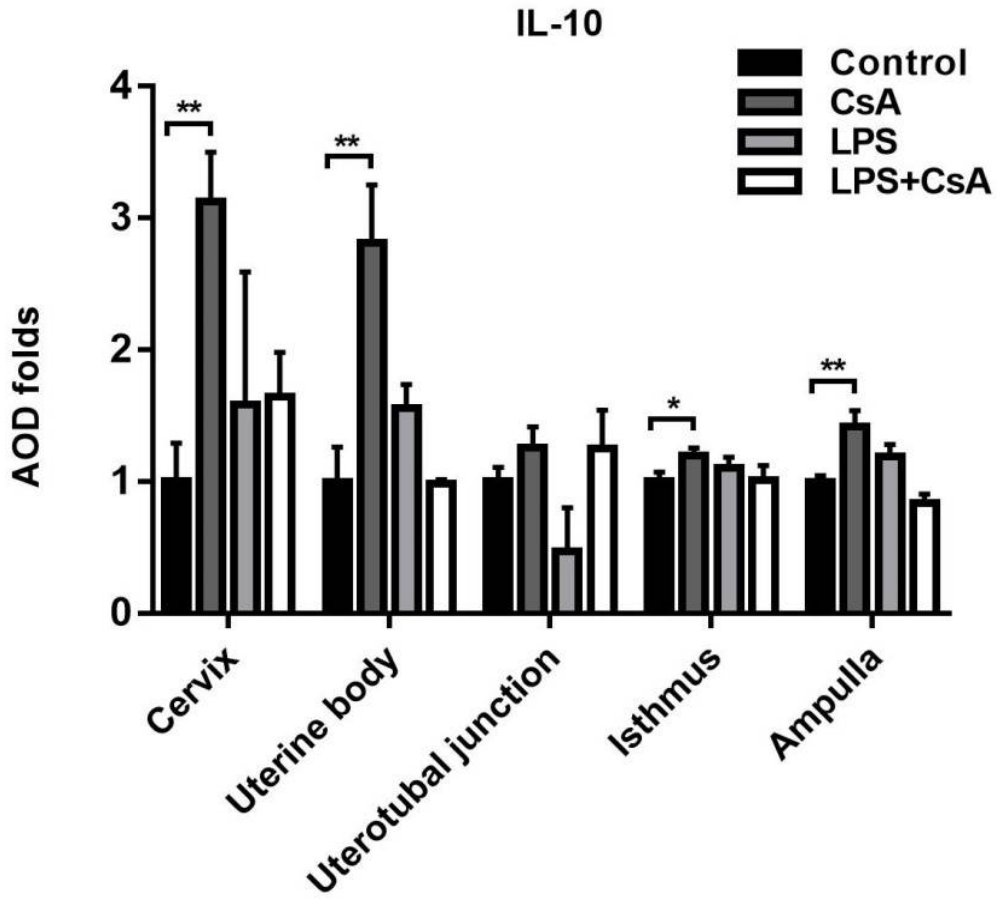

Figure 16. The protein expression of IL-10 in the genital tract of female rabbits. Twelve rabbits (n = 3 each) were given an intrauterine infusion of *E. coli* LPS (4 mg/kg BW) and/or an intramuscular injection of CsA (20 mg/kg BW). The tissue specimens from the cervix, uterine body, uterotubal junction, oviductal isthmus, and ampulla were collected 3 h post-injection. The protein expression of IL-10 was assessed using an immuno-histochemical assay. The positive areas of immune-histochemical staining were analyzed using Image-Pro Plus 6 software, and the data were analysed using SPSS statistical software. AOD: average optical density; AOD = Integrated optical density (IOD)/Area. The results are presented as the mean ± SD (n=3). *$p < 0.05$ and **$p < 0.01$.

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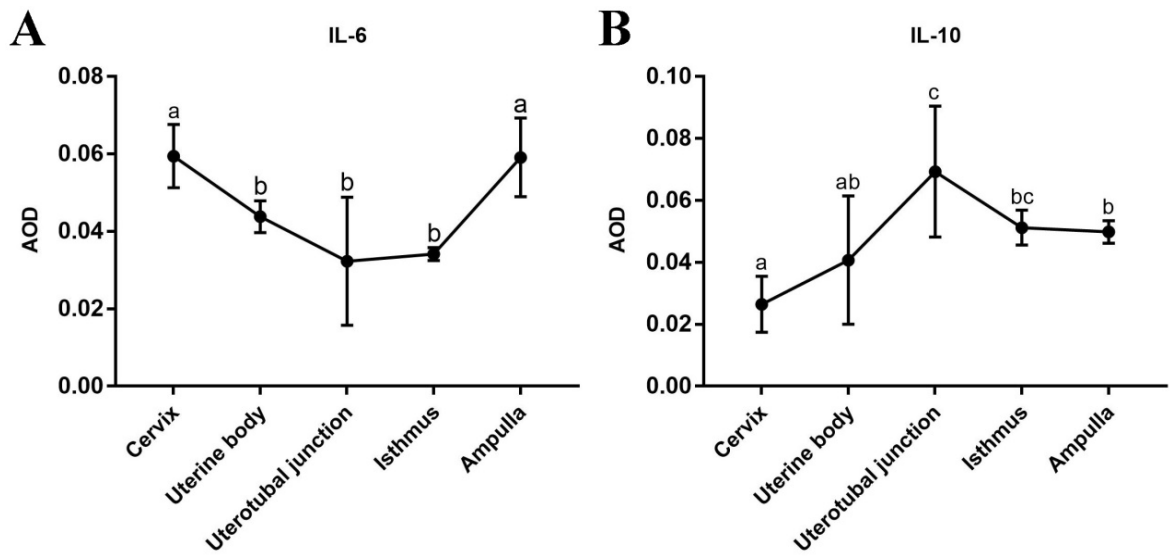

Figure 17. The protein expression of IL-6 and IL-10 in the genital tracts of control group animals. The rabbits received only LPS and CsA carrier. The tissue specimens from the cervix, uterine body, uterotubal junction, oviductal isthmus, and ampulla were collected 3 h post-injection. The protein expressions of IL-6 and IL-10 were assessed using an immuno-histochemical assay. The positive areas of immune-histochemical staining were analyzed using Image-Pro Plus 6 software, and the data were analysed using SPSS statistical software. The results are presented as the mean ± SD (n = 3). (**a**) IL-6. (**b**) IL-10. $^{a, b, c}$ in this figure means the difference is significant among these tissues ($p < 0.05$).