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SHORT COMMUNICATION

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Essential role of ATG7 in LPS-induced endometrial inflammatory injury in goats

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Abstract

The economic losses caused by uterine infections in ruminants have received increasing attention. Autophagy-related 7 (*ATG7*) has been demonstrated to be capable of regulating apoptosis, but surprisingly, *ATG7* can both promote and inhibit apoptosis in different cellular contexts. However, the role of *ATG7* in endometrial cell apoptosis during the postpartum period remains unclear. Herein, the location and expression of *ATG7* was determined after mice were treated with lipopolysaccharide (LPS) in the uterus. The results showed that lipopolysaccharide (LPS) increased *ATG7* expression in endometrial epithelial cells (EECs) but not endometrial stromal cells. The apoptosis of goat EECs was increased under LPS treatment, and LPS further led to an increase in *bax* expression and a decline in *bcl-2* expression in goat EECs. Silencing of *ATG7* inhibited goat EEC apoptosis with LPS treatment. The role of *ATG7* in the regulation of goat EEC apoptosis was further confirmed by overexpression. *ATG7* may serve as an essential regulatory factor in the process of endometrial epithelial cell apoptosis in ruminants under inflammatory injury. The findings help elucidate the pathogenesis of postpartum endometritis in ruminants.

Keywords Ruminants, Endometritis, *ATG7*, Endometrial epithelial cell (EEC)

Main text

In ruminants, uterine infections occur frequently during the postpartum period. Endometritis is one of the principal causes of infertility in goats and dairy cows. The incidence of uterine infection in cattle and goats is 50% and 5.6%, respectively, and it causes substantial economic losses (Cui et al. 2020). Although the biology of the endometrium in different animals has unique mechanisms, pathogenic bacteria (such as *Escherichia coli* and *Streptococcus*) are the main cause of postpartum endometritis (Yin et al. 2021). Lymphocyte infiltration and increased macrophages and plasma cells in the lamina propria are common features of endometritis. Surface epithelial shedding and endometrial cell death are widely observed histopathological features (Beena et al. 2017). However, the precise mechanism linking endometritis to endometrial epithelial cell (EEC) apoptosis remains unclear.

Autophagy-related 7 (*ATG7*) is a core protein that regulates the classical autophagic degradation pathway through *ATG8* lipidation. According to previous reports, autophagy-related functions of *ATG7* participate in many physiological and pathological processes, such as the redistribution of tight junction proteins in EECs to preserve barrier function (Yang et al. 2022). Recent data have demonstrated that *ATG7* regulates cell function independent of autophagy-associated signaling (Collier et al. 2021). Several studies have suggested that *ATG7* is capable of regulating apoptosis, but surprisingly, *ATG7* can both promote and inhibit apoptosis in different cellular contexts (He et al. 2021; Wang et al. 2021). To date, whether *ATG7* mediates endometrial cell apoptosis in ruminants suffering from endometritis remains unclear.

In this study, we used an lipopolysaccharide (LPS) induced mouse endometritis injury model and a goat endometrial epithelial cell inflammation model to

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study ruminant endometritis. The location and expression level of ATG7 in mouse endometrial tissue under LPS treatment were analysed. To further explore the potential roles of ATG7 in EEC apoptosis, lentiviruses were selected to knock down or overexpress *ATG7*.

Increased expression of *ATG7* in endometrial tissue of endometritis

To verify that the uteri suffered from inflammatory damage, histological analysis was performed using H&E staining. The abscission of endometrial luminal epithelial cells, necrosis and hemorrhages were observed in the diseased uterine tissues compared with healthy tissues (Fig. 1A). Fig. 1B showed that the fluorescence intensity of ATG7 in the endometrial luminal and glandular epithelial cells of the diseased uteri was greater than that in the healthy uteri. Western blot analysis also demonstrated that there was an increase in ATG7 expression in uterine tissues after LPS treatment (Fig. 1C). The above results provide evidence that ATG7 might participate in endometrial inflammatory injury.

LPS induces high levels of *ATG7* in goat EECs

Due to the increased expression of *ATG7* in the diseased uterine tissues, we next wondered whether it could be upregulated in the endometrial inflammatory injury model *in vitro*. As shown in Fig. 2A, LPS increased *ATG7* expression in goat EECs at 24 h. Similar results were obtained by Western blot: ATG7 expression was upregulated under LPS treatment in EECs at 12 and 24 h (Fig. 2B). Then, qPCR was used to examine the expression of apoptosis-related genes, including *bcl-2* and *bax*, in EECs after treatment with LPS. Results showed that treatment reduced the mRNA levels of *bcl-2* but increased *bax* expression in EECs at 24 h (Fig. 2C). Western blot analysis also confirmed these results (Fig. 2D). Similarly, there were more TUNEL-positive cells in the EECs treated with LPS than in those without LPS treatment, with a maximum at approximately 24 h after treatment (Fig. 2E). Therefore, the experimental conditions for LPS treatment were selected as 24 h to explore the roles of *ATG7* in endometrial inflammatory injury.

Silencing *ATG7* inhibits apoptosis in goat EECs

Considering that LPS treatment increased *ATG7* expression in EECs, whether *ATG7* defects caused apoptosis were further explored. The interference efficiency of three constructed *ATG7* lentivirus interference vectors were analyszed. As shown in Fig. 3A, the third *ATG7* interfering vector (pCD513B-U6-ATG7-shRNA-3) significantly reduced the transcription level of *ATG7*. Western blotting further confirmed these results (Fig. 3B). Therefore, pCD513B-U6-ATG7-shRNA-3 were selected for subsequent testing. qPCR was performed to measure the levels of bax and *bcl-2*, the key genes responsible for regulating apoptosis. Silencing of *ATG7* led to an increase in *bcl-2* expression but declined *bax* expression in EECs (Fig. 3C and D). Correspondingly, silencing of *ATG7* induced a reduction in apoptosis in EECs compared with shN under LPS treatment, as evidenced by decreased TUNEL staining (Fig. 3E).

Overexpression *ATG7* induces excessive apoptosis in goat EECs

To further validate the role of *ATG7* in regulating the apoptosis of EECs with LPS treatment, *ATG7* were overexpressed. ATG7-lentivirus infection,

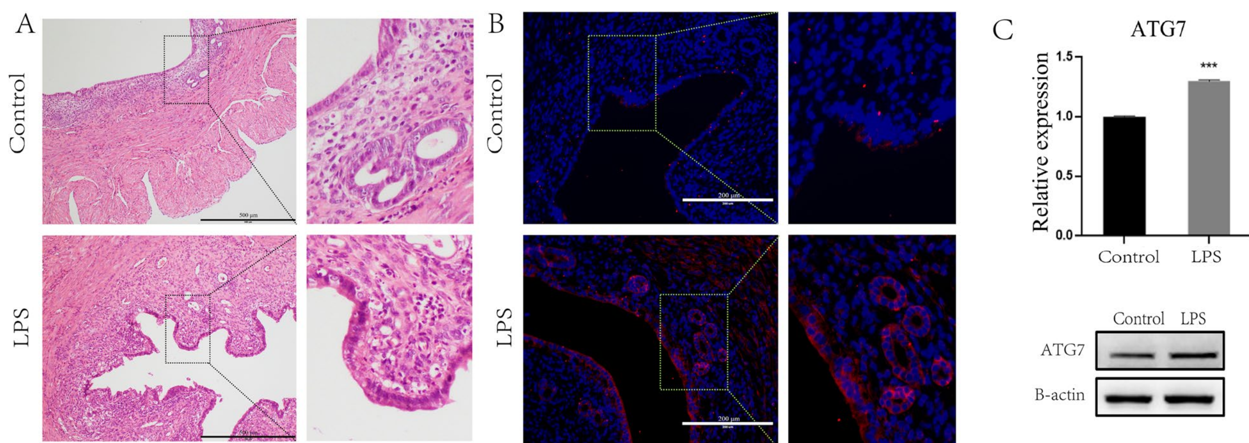

Fig. 1 The expression of ATG7 in mouse uterine tissues. **A** H&E staining of uterine tissue. **B** The expression and location of ATG7 in uterine tissue. Red: ATG7. Blue: DAPI. **C** Western blot analysis of ATG7 protein levels with LPS treatment in mouse uterus tissues. LPS, lipopolysaccharide. The data are presented as the means ± SD of three independent experiments. * Significant difference ($p < 0.05$) compared with other groups. ** Significant difference ($p < 0.01$) compared with other groups. *** Significant difference ($p < 0.001$) compared with other groups

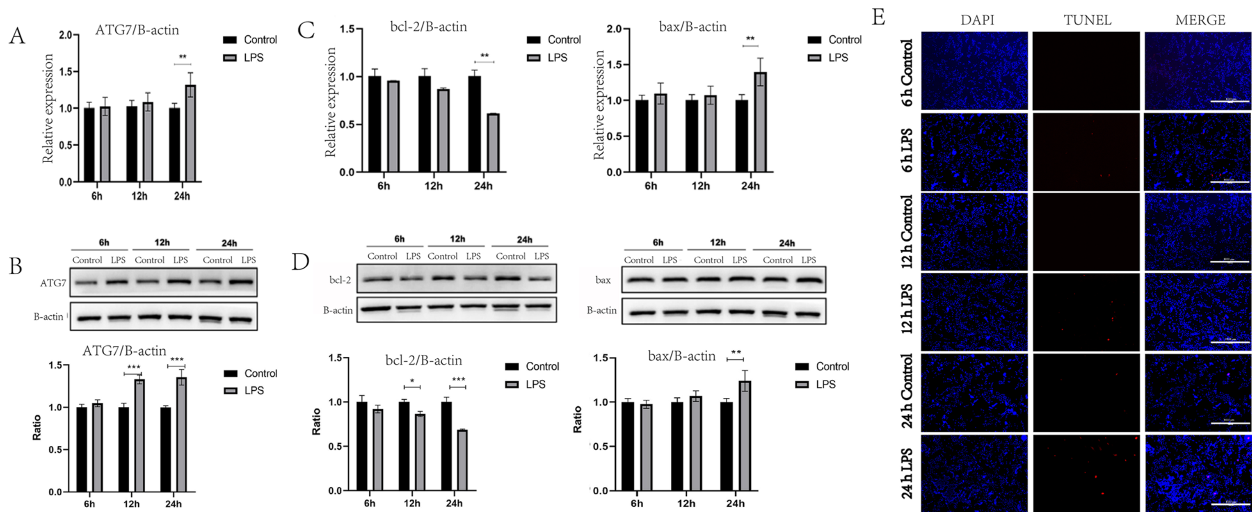

Fig. 2 LPS-induced apoptosis in goat EECs. **A** and **B** The expression levels of ATG7 in LPS-treated goat EECs at different time points. **C** and **D** The expression levels of bcl-2 and bax in LPS-treated goat EECs at different time points. **E** Representative merged fluorescence images of TUNEL in goat EECs with or without LPS treatment. EECs, endometrial epithelial cell. LPS, lipopolysaccharide. The data are presented as the means ± SD of three independent experiments. * Significant difference ($p < 0.05$) compared with other groups. ** Significant difference ($p < 0.01$) compared with other groups. *** Significant difference ($p < 0.001$) compared with other groups

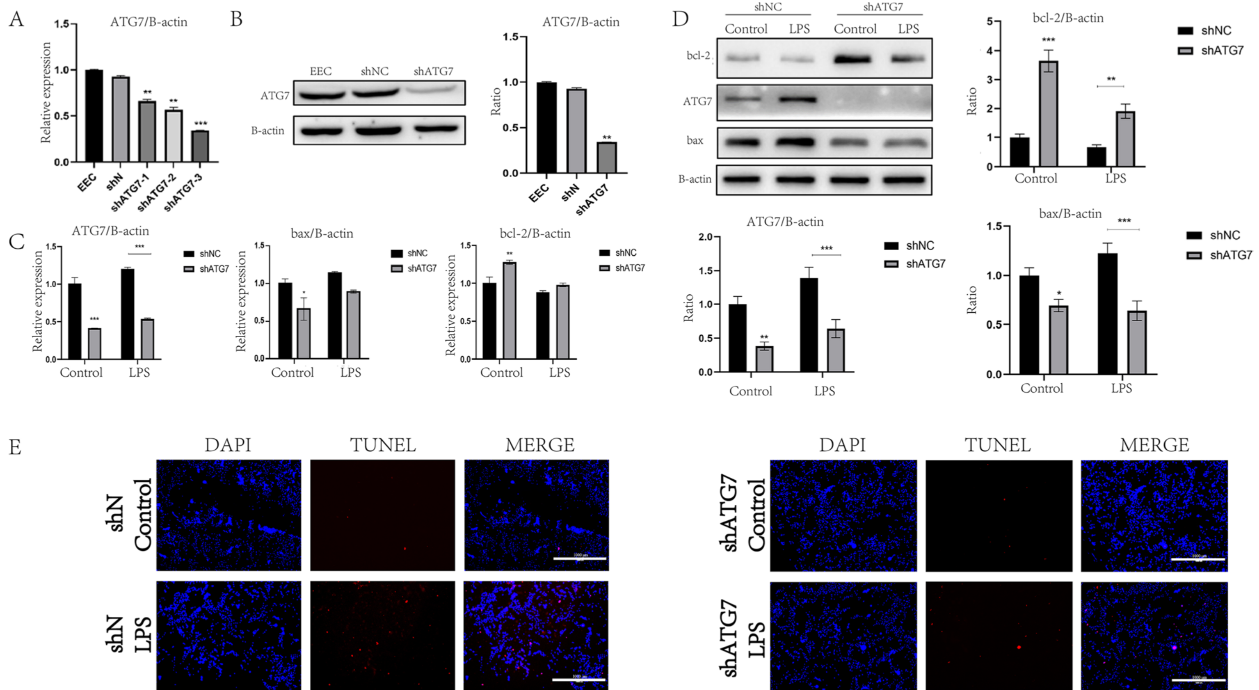

Fig. 3 Silencing *ATG7* inhibits apoptosis in goat EECs. **A** Real-time PCR analysis of *atg7* mRNA levels in goat EECs. **B** Western blot analysis of ATG7 protein levels in goat. EECs, pCD513B-U6-ATG7-shRNA-1 and mock control were transduced for 48 h. **C** and **D** Real-time PCR and Western blot analysis of the bax, bcl-2 and ATG7 levels following LPS treatment. **E** Representative merged fluorescence images of TUNEL in goat EECs with or without LPS treatment. EECs, endometrial epithelial cell. LPS, lipopolysaccharide. The data are presented as the means ± SD of three independent experiments. * Significant difference ($p < 0.05$) compared with other groups. ** Significant difference ($p < 0.01$) compared with other groups. *** Significant difference ($p < 0.001$) compared with other groups

but not vector infection, produced a significant increase in *ATG7* expression (Fig. 4A and B). *ATG7* overexpression significantly increased the expression of *bax* and decreased *bcl-2* expression (Fig. 4A and B). As shown in Fig. 4C, there were more TUNEL-positive cells in the ATG7-overexpressing EECs than in the vector EECs under LPS treatment. These results provide unequivocal evidence that *ATG7* activation enhanced EEC apoptosis under LPS treatment.

Inflammatory injury of the endometrium is an obstetric disease with a high incidence in ruminants after delivery. The inflammatory uterus causes pain to female animals and is harmful to the next round of embryo implantation, which causes major economic losses to animal husbandry. Apoptosis of endometrial epithelial cells caused by excessive inflammation is a typical pathological feature of endometritis. Previous studies have found that *ATG7* was involved in the process of apoptosis of various tissues and cells (She et al. 2022; Wang et al. 2022). Hence, in the present study, the relationship between *ATG7* and apoptosis in EEC was investigated.

ATG7 is a key protein that regulates the function of goat endometrial epithelial cells (Yang et al. 2021). The loss of ATG7 led to inhibition of endometrial epithelial cell proliferation, decreased cell adhesion activity and decreased prostaglandin secretion (Yang et al. 2021). Yang reported that ATG7 plays a critical role in baicalin protecting the endometrial barrier by promoting the redistribution of tight junction proteins under LPS stimulation (Yang et al. 2022). The above studies suggest that ATG7 plays an important role in the physiological and pathological processes of the ruminant endometrium. However, whether ATG7 directly participates in the apoptosis of endometrial cells caused by endometritis injury has not been reported. In this study, we found for the first time that lipopolysaccharide-mediated endometrial inflammatory injury can lead to increased expression of ATG7 in endometrial luminal and glandular epithelial cells rather than endometrial stromal cells, suggesting that lipopolysaccharide might damage the integrity of the barrier of endometrial luminal epithelia and affect the secretion of uterine milk proteins.

Originally, autophagy was considered to be an important mechanism for protecting cells from apoptosis during nutritional deficiency. However, an increasing number of reports have found that sustained and excessive autophagy might also induce apoptosis (Wu et al. 2022). Liao demonstrated that there is crosstalk between apoptosis and autophagy (Liao et al. 2023). ATG7 is a well-known key autophagic protein that participates in classic autophagic degradation through ATG8 lipidation. Knocking out *ATG7* caused an obstacle in the degradation of the inner autophagosomal membrane, which in turn blocked autophagic flux (Collier et al. 2021). As an

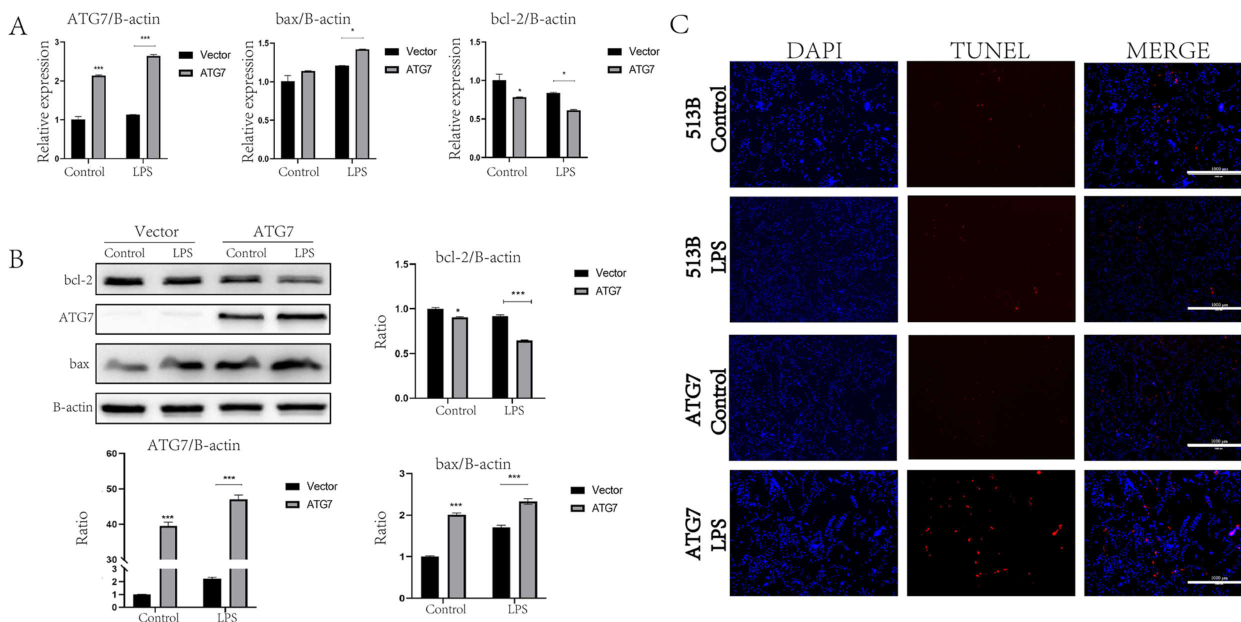

Fig. 4 Overexpression *ATG7* induces excessive apoptosis in goat EECs. **A** and **B** Real-time PCR and Western blot analysis of bax, bcl-2 and ATG7 following LPS treatment. **C** Representative merged fluorescence images of TUNEL in goat EECs with or without LPS treatment.EECs, endometrial epithelial cell. LPS, lipopolysaccharide. The data are presented as the means ± SDof three independent experiments. * Significant difference ($p < 0.05$) compared with other groups. ** Significant difference ($p < 0.01$) compared with other groups. *** Significant difference ($p < 0.001$) compared with other groups

essential link in autophagy, *ATG7* undoubtedly plays an important role in regulating apoptosis. Previous studies have found that *ATG7* knockout reduces cell viability and the proliferation rate in smooth muscle cells and confirmed that this effect is due to increased antioxidant gene expression and NRF2 nuclear translocation (Grootaert et al. 2015). Lee reported that ATG7 binds to p53 in a manner independent of its E1-like enzymatic activity, inhibiting the apoptosis of mouse embryonic fibroblasts and reducing the expression of proapoptotic genes (*Bax*, *Puma* and *Noxa*) (Lee et al. 2012). In contrast to earlier findings, the findings of the current study demonstrated that silencing *ATG7* induced a reduction in apoptosis in goat EECs compared with shN under LPS treatment. In accordance with the present results, Karthikkeyan demonstrated that high levels of *ATG7* inhibit the lysosomal-mediated degradation of autophagic vacuoles and promote apoptosis (Karthikkeyan et al. 2022). We speculate that ATG7-mediated EEC apoptosis may also be a similar mechanism under LPS treatment, but more studies need to be carried out to confirm this hypothesis.

In summary, this study showed that LPS induced increased *ATG7* expression in endometrial epithelial cells rather than endometrial stromal cells. Knocking down *ATG7* can reduce cell mortality and regulate the expression of apoptotic molecules in endometrial epithelial cells. Overexpression *ATG7* further aggravated the apoptosis of goat endometrial epithelial cells induced by LPS. This study indicated that *ATG7* may play a key regulatory role in the process of endometrial epithelial cell apoptosis in ruminants under inflammatory injury.

Methods

Experimental animal groups and treatments

Kunming female virgin mice of specific pathogen-free (SPF) grade at the age of approximately seven weeks were purchased from Hubei Provincial Center for Laboratory Animal Research, Wuhan, Hubei Province, China (approval number of the ethical proof of animal experiment: HZAUMO-2022-0058). The mice were housed in a temperature-controlled room (22 ± 2°C) with a 12 h light/12 h dark cycle and randomly acquired food and water. All experimental procedures were performed in strict accordance with the guidelines of the Animal Care and Use Committee of Huazhong Agricultural University. All experimental protocols involving animal subjects were approved by the Animal Care and Use Committee of Huazhong Agricultural University.

Mice (25–30 g) were randomly divided into two groups, with 10 mice in each group (n = 10), consisting of a control group and an LPS group. The method for constructing a mouse endometritis model was described previously (Liu, Wu et al. 2021). Twenty microliters of LPS (Sigma, St. Louis, MO, USA) at a concentration of 5 mg/mL was infused into the uterus to induce endometritis damage. Equal volumes of ddH_2O were administered into the uterus of the control group. At 24 h after LPS treatment, all mice were sacrificed (Fig. 1B).

Histological examination

The uteri were fixed with paraformaldehyde and then embedded in paraffin. The slides of uteri were dehydrated with gradient alcohol and subsequently stained with hematoxylin and eosin (H&E). The histological changes in the uteri were observed by an optical microscope (Olympus Shinjuku ku, Tokyo, Japan).

Immunofluorescence staining

The uteri were fixed with paraformaldehyde and then embedded in paraffin. The slides were dewaxed and rehydrated by subsequent immersion in xylene, ethanol (100%, 95%, 70% and 50%) and deionized H_2O. Antigen was then retrieved in citrate buffer. The slides of uteri were sequentially incubated with anti-ATG7 (ABclonal A0691, diluted 1:200) and Alexa-labeled secondary antibodies (Invitrogen, Life Technologies) at 37°C, and nuclei were counterstained with DAPI for 10 min. The localization and expression of ATG7 were observed in the uterus by a fluorescence microscope (Nikon, Inc., Melville, NY, USA).

Cell culture and cell transfection

Goat endometrial epithelial cells (EECs) are immortalized by transfection with human telomerase reverse transcriptase (Zhang, Wang et al. 2010). DMEM/F-12 medium containing 10% fetal bovine serum was used to culture EECs inoculated in a six-well plate. After treatment with LPS (2 μg/mL) for 6 h, 12 h or 24 h, EECs were collected for subsequent testing.

The *ATG7* shRNA (shATG7), negative control (shN) and *ATG7* overexpression vectors were constructed as previously reported (Yang et al. 2021; Yang et al. 2022). Supplementary Table S1 shows the sequences of shATG7 and shN. After lentivirus packaging, EECs were infected as previously reported (Chen, Lin et al. 2014).

RNA extraction and real-time quantitative PCR

After the total RNA was extracted, cDNA was synthesized according to the instructions (ABclonal Biotechnology, Wuhan, China). Supplementary Table 2 shows the primer sequences. Real-time quantitative PCR was performed using qPCR Mix (ABclonal Biotechnology, Wuhan, China) on a Roche LightCycler® 480 (Roche, USA). The *GAPDH* gene served as an internal control, and the expression levels of *ATG7*, *bax* and *bcl-2* were estimated using the $2^{-\triangle\triangle Ct}$ method. Each real-time

quantitative PCR experiment was performed with three independent samples.

Western blot analysis

Total protein was extracted using RIPA buffer. The EEC samples were separated by a 12% SDS–PAGE gel, and the proteins were transferred to PVDF membranes (Millipore; Bedford, MA, USA). After incubation with 10% nonfat milk in Tris-buffered saline (TBS, pH 7.4)/0.1% Tween (TBST), the PVDF membranes were incubated with anti-ATG7 antibody (ABclonal A0691, diluted 1:1000), anti-BCL2 antibody (ABclonal A19693, diluted 1:1000), anti-BAX antibody (ABclonal A19684, diluted 1:1000) and anti-ACTB antibody (ABclonal AC038, diluted 1:2000) for 2 h at 37°C. Then, HRP-labeled secondary antibody was incubated with the PVDF membranes. Finally, the grayscale values of the protein bands were analyzed using Quantity One software (Bio-Rad Laboratories, Hercules, CA, USA).

Statistical analysis

Unless otherwise specified, all data are expressed as the mean ± SD of three samples for each experimental point. All data were analyzed using SPSS V. 22 (IBM-SPSS, Inc., Chicago, USA). Significant differences between samples were analyzed using one-way ANOVA with the least significant difference (LSD), and the significance level was set as $p < 0.05$.

Abbreviations

ATG7	Autophagy-related 7
LPS	Lipopolysaccharide
EEC	Endometrial epithelial cells
H&E	Hematoxylin and eosin

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s44149-023-00079-7.

Additional file 1: Supplementary Table 1. Short hairpin interfering RNA (shRNA) inserts.

Additional file 2: Supplementary Table 2. qPCR Primers.

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Authors' contributions

Tingting Jiang and Jianguo Chen conceived and designed the experiments. Tingting Jiang and Xueting Yin carried out the experiments. Tingting Jiang and Jianguo Chen wrote the manuscript. All authors agreed to be responsible for the content of the work. The author(s) read and approved the final manuscript.

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Availability of data and materials

Data will be shared upon request by the readers.

Declarations

Ethics approval and consent to participate

All experimental protocols were approved by the Research Ethics Committee of College of Veterinary Medicine (HZAUMO-2022-0058) HZAUMO-2022-0058, Huazhong Agricultural University, Hubei, China.

Consent for publication

Not applicable.

Competing interests

The authors declare no conflict of interest.

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References

Beena, V., R.V.S. Pawaiya, K. Gururaj, D.D. Singh, A.K. Mishra, N.K. Gangwar, V.K. Gupta, R. Singh, A.K. Sharma, M. Karikalan, and A. Kumar. 2017. Molecular etiopathology of naturally occurring reproductive diseases in female goats. *Veterinary World* 10 (8): 964–972.

Chen, F., P.F. Lin, X. Li, J. Sun, Z. Zhang, E. Du, A. Wang, and Y.P. Jin. 2014. Construction and expression of lentiviral vectors encoding recombinant mouse CREBZF in NIH 3T3 cells. *Plasmid* 76: 24–31.

Collier, J.J., F. Suomi, M. Olahova, T.G. McWilliams, and R.W. Taylor. 2021a. Emerging roles of ATG7 in human health and disease. *EMBO Molecular Medicine* 13 (12): e14824.

Cui, L., Y. Zheng, H. Wang, J. Dong, J. Li, Q. Song, C. Qian, and J. Li. 2020. Cortisol inhibits the Escherichia coli-induced endometrial inflammatory response through NF-κB and MAPK pathways in postpartum goats. *Animal Reproduction Science* 215: 106333.

Grootaert, M.O., P.A. da Costa Martins, N. Bitsch, I. Pintelon, G.R. De Meyer, W. Martinet, and D.M. Schrijvers. 2015. Defective autophagy in vascular smooth muscle cells accelerates senescence and promotes neointima formation and atherogenesis. *Autophagy* 11 (11): 2014–2032.

He, X., W. Yuan, F. Liu, J. Feng and Y. X. Guo. 2021. Acylated ghrelin is protective against 6-OHDA-induced neurotoxicity by regulating autophagic flux. *Frontiers in Pharmacology* 27 (11): 586302. https://doi.org/10.3389/fphar.2020.586302.

Karthikkeyan, G., S.K. Behera, S.S. Upadhyay, R. Pervaje, T.S.K. Prasad, and P.K. Modi. 2022. Metabolomics analysis highlights Yashtimadhu (*Glycyrrhiza glabra* L.)-mediated neuroprotection in a rotenone-induced cellular model of Parkinson's disease by restoring the mTORC1-AMPK1 axis in autophagic regulation. *Phytotherapy Research* 36 (5): 2207–2222.

Lee, I.H., Y. Kawai, M.M. Fergusson, A.J. Rovira II., N. Bishop, L. Cao. Motoyama, and T. Finkel. 2012. Atg7 modulates p53 activity to regulate cell cycle and survival during metabolic stress. *Science* 336 (6078): 225–228.

Liao, Y.H., J.T. Wu, I.C. Hsieh, H.H. Lee, and P.H. Huang. 2023. ARMS-NF-kappaB signaling regulates intracellular ROS to induce autophagy-associated cell death upon oxidative stress. *iScience* 26 (2): 106005.

Liu, J., Z. Wu, S. Guo, T. Zhang, X. Ma, K. Jiang, X. Guo, and G. Deng. 2021. IFN-τ attenuates LPS-induced endometritis by restraining HMGB1/NF-κB activation in bEECs. *Inflammation* 44 (4): 1478–1489.

She, Y.Y., J.J. Lin, J.H. Su, T.S. Chang, and Y.J. Wu. 2022. 4-Carbomethoxyl-10-Epigyrosanoldie E extracted from cultured soft coral sinularia sandensis induced apoptosis and autophagy via ROS and mitochondrial dysfunction and ER stress in oral cancer cells. *Oxidative Medicine and Cellular Longevity* 2022: 3017807.

Wang, Q., D. Liang, P. Shen, Y. Yu, Y. Yan, and W. You. 2021. Hsa_circ_0092276 promotes doxorubicin resistance in breast cancer cells by regulating autophagy via miR-348/ATG7 axis. *Translational Oncology* 14 (8): 101045.

Wang, M., K. Liu, H. Bu, H. Cong, G. Dong, N. Xu, C. Li, Y. Zhao, F. Jiang, Y. Zhang, B. Yuan, R. Li, and J. Jiang. 2022. Purple sweet potato delphinidin-3-rutin

represses glioma proliferation by inducing miR-20b-5p/Atg7-dependent cytostatic autophagy. *Molecular Therapy-Oncolytics* 26: 314–329.
Wu, Z., Z. Xu, X. Zhou, H. Li, L. Zhao, Y. Lv, Y. Guo, G. Shen, Y. He, and P. Lei. 2022. sGRP78 enhances selective autophagy of monomeric TLR4 to regulate myeloid cell death. *Cell Death & Disease* 13 (7): 587.
Yang, D., R. Yin, Q. Lei, J. Zhu, S. Nan, N. Ma, H. Zhu, J. Chen, L. Han, M. Ding, and Y. Ding. 2022a. Baicalin alleviates endometrial inflammatory injury through regulation of tight junction proteins. *Food & Function* 13 (12): 6522–6533.
Yang, D., A. Liu, Y. Zhang, S. Nan, R. Yin, Q. Lei, H. Zhu, J. Chen, L. Han, M. Ding and Y. Ding. 2021. Essential role of CRIM1 on endometrial receptivity in goat. *International Journal of Molecular Sciences* 22 (10): e5323. https://doi.org/10.3390/ijms22105323.
Yin, B., T. Umar, X. Ma, Y. Chen, N. Chen, Z. Wu, and G. Deng. 2021. MiR-193a-3p targets LGR4 to promote the inflammatory response in endometritis. *International Immunopharmacology* 98: 107718.
Zhang, Y.Y., A.H. Wang, Q.X. Wu, H.X. Sheng, and Y.P. Jin. 2010. Establishment and characteristics of immortal goat endometrial epithelial cells and stromal cells with hTERT. *Journal of Animal and Veterinary Advances* 9 (21): 2738–2747.

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