

Article

FABP3 Mediates Lipid Droplet Accumulation and Adhesive Capacity in Bovine Endometrial Epithelial Cells via PGE₂/PTGER4/PPAR Axis

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Simple Summary

To investigate the function of PGE₂ on endometrial function during the diestrus period and its mechanisms, bovine endometrial epithelial cells (bEECs) were treated with P₄ or P₄ + PGE₂. Results showed that PGE₂ treatment promoted the accumulation of lipid droplets and induced cytoskeletal reorganization; the PPAR signaling pathway was the key pathway, and its downstream target gene FABP3 was markedly up-regulated; FABP3 knockdown reduced the adhesion of bovine trophoblast cell (BTC) spheroids to bEECs and down-regulation of adhesion-related proteins in bEECs while increasing the density of microvilli and up-regulated the expression of epithelial markers. These findings provide new insights into the metabolic regulation of endometrial receptivity in ruminants.



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Abstract

Prostaglandin E₂ (PGE₂) has been implicated in multiple biological processes during pregnancy in ruminants. However, the regulatory effects of PGE₂ on endometrial function during the diestrus period and its underlying molecular mechanisms remain poorly understood. Herein, PGE₂ treatment promoted the accumulation of lipid droplets and induced cytoskeletal reorganization in bEECs. As a well-established inducer of lipid droplet formation, oleic acid (OA) treatment significantly increased the number of lipid droplets in bEECs, altered the distribution of F-actin and disrupted the expression patterns of key adhesion-related proteins. Transcriptomic analysis revealed that the PPAR signaling pathway was the key pathway that responded to PGE₂ treatment in bEECs, and its downstream target gene FABP3 was markedly up-regulated. Knockdown of FABP3 led to a reduced number of BTC spheroids and down-regulation of adhesion-related proteins in bEECs while increasing the density of microvilli and up-regulating the expression of epithelial markers. Prostaglandin E receptor 4 (PTGER4) was the primary receptor that responded to PGE₂ treatment, and PTGER4 knockdown or pharmacological inhibition with GW-627368 suppressed FABP3 expression in bEECs. Moreover, uterine samples from dairy cows at different stages of the estrous cycle showed that FABP3 expression was significantly elevated in the endometrium tissue during mid-diestrus compared to metestrus, with predominant localization in the luminal and superficial glandular epithelium. Collectively, these findings

indicate that FABP3 regulates lipid droplet accumulation and adhesion ability in bEECs via the PGE₂/PTGER4/PPAR signaling axis, providing new insights into the metabolic regulation of endometrial receptivity in ruminants.

Keywords: endometrial receptivity; estrous cycle; PGE₂; FABP3; lipid droplet

1. Introduction

Subfertility is a prevalent reproductive disorder in dairy cows, significantly compromising both productivity and economic returns [1,2]. Accumulating evidence indicates that prostaglandin (PG) levels in uterine lumen fluid and the expression of prostaglandin-related genes in the endometrium are closely linked to fertility [3,4]. As a key immunomodulatory and luteal protective factor in ruminants [5], PGE₂ level in uterine lumen fluid increases rapidly during early pregnancy and comparable luteal phase [6], which is associated with conceptus elongation in dairy cows [3]. Notably, PGE₂ predominantly exerts biological effects on the endometrium, owing to the high expression of its receptors (PTGER2 and PTGER4) in endometrial tissue and low expression level in the conceptus during the peri-implantation period of dairy heifers [6,7]. Meanwhile, PGs are important mediators of endometrial response to P₄ and IFNT in the ovine uterus [8,9]. In ruminants, available data support that P₄ from the ovary induces gene expression in the endometrium, which is further stimulated by factors (PGs, IFNT) to regulate the endometrial functions [1,10,11]. Despite these observations, the functional roles of PGE₂ in endometrial physiology during the diestrus period, as well as the underlying molecular mechanisms, remain largely unclear.

Previous studies have demonstrated that the intrauterine infusion of meloxicam during mid-diestrus in dairy heifers inhibits the synthesis and secretion of prostaglandins, which leads to the alternations of lipids in uterine luminal fluid [12,13]. In ruminants, lipid droplets (LDs) in endometrium are considered a major source of lipids within the uterine fluid [14–16]. As a cytoplasmic organelle, LDs serve as a reservoir for energy storage, which plays regulatory roles in cellular signaling and metabolic processes [17]. The abundance of LDs in the endometrium exhibits dynamic fluctuations throughout the estrous cycle, exhibiting low levels during metestrus and increasing accumulation during diestrus, which corresponds with corpus luteum development in sheep and cattle [18–20]. The source of LDs is predominantly localized in the luminal and superficial glandular epithelia, with minor presence in stromal cells and deep glandular epithelium [20]. Progesterone is a primary inducer of LD accumulation, as demonstrated by progesterone supplementation experiments in ovariectomized ewes [19]. In contrast, administration of 17β-estradiol in the same experimental model results in a reduction in endometrial LD content [19]. However, the molecular mechanisms governing LD dynamics in endometrial epithelial cells during the estrous cycle and their functions in endometrial physiology remain to be elucidated.

Several genes related to lipid metabolism have been implicated in reproductive performance of dairy cows [3,20]. Fatty acid-binding protein 3 (FABP3), a critical member of the fatty acid-binding protein family, plays a central role in the intracellular transport and metabolism of long-chain fatty acids [21]. In dairy cows, FABP3 is highly expressed in endometrium and significantly elevated in pregnant cows compared to non-pregnant dairy cows [1,22], with peak expression on days 15 and 18 of gestation. Notably, FABP3 expression is higher in the endometrium of high-fertility heifers compared to those with subfertility [1]. Moreover, the abundance of FABP3 protein in uterine fluid de-

creases when the synthesis and secretion of uterine prostaglandins are inhibited in dairy heifers [13]. Collectively, these findings suggest that FABP3 may play a regulatory role in the function of uterine endometrium and potentially influence pregnancy outcomes in dairy cows.

This study aimed to investigate the effects of PGE₂ on lipid droplet dynamics in endometrial epithelial cells, identify key responsive genes, and evaluate its functional roles in regulating endometrial cell function. The findings provide a theoretical basis for understanding the molecular mechanisms underlying endometrial receptivity and early embryonic development in ruminants.

2. Materials and Methods

2.1. Cell Culture and Treatment

In this study, bEECs were purchased from the American Type Culture Collection (ATCC[®] CRL-2398[™], Manassas, VA, USA) and cultured in DMEM/F-12 medium (HyClone, Logan, UT, USA) containing 10% fetal bovine serum (FBS, ZETA, New York, NY, USA) and incubated at 37 °C in a humidified 5% CO₂ incubator. When bEECs reached 50–60% confluence, the medium was replaced with fresh phenol red-free DMEM/F-12 and 0.1% bovine serum albumin (BSA) for 12 h to synchronize cells. The bEECs were then cultured for an additional 24 h in phenol red-free DMEM/F-12 medium supplemented with 10% FBS and P₄ (10^{−7} M; P0130, Sigma, Saint Louis, MO, USA) to mimic the uterine hormone environment of dairy cows during the luteal phase. Then, PGE₂ (10^{−8}–10^{−6} M; 14010, Cayman, Ann Arbor, MI, USA) was added to the medium, and the cells were cultured for 24 h. For inhibitor treatment, 5 and 10 μM PTGER2 inhibitor (TG-155; MedChemExpress, Newark, NJ, USA) or 5 and 10 μM PTGER4 inhibitor (GW627368; Selleck, Houston, TX, USA) were added to the medium 1 h prior to the PGE₂ treatment.

For the oleic acid (OA) experiments, three experimental conditions were used. In the 5% FBS group (CON group), the medium was replaced with phenol red-free DMEM/F-12 containing 5% FBS. In the 1% BSA group, the medium was replaced with phenol red-free DMEM/F-12 containing 1% BSA to reduce the accumulation of intracellular lipid droplets. In the 5% FBS + OA group, the medium was replaced with phenol red-free DMEM/F-12 containing 5% FBS and 200 μM OA to promote lipid droplet accumulation.

2.2. Bulk RNA Sequencing (RNA-Seq)

Total RNA was extracted from bEECs after treatment with P₄ or P₄ + PGE₂ using Trizol Reagent. Next, RNA was reverse-transcribed and amplified according to the manufacturer's instructions. Sequencing libraries were constructed on the Illumina Novaseq 6000 platform (Genedenovo, Guangzhou, China). Briefly, Eukaryotic mRNA was enriched using Oligo(dT) beads. Then the enriched mRNA was fragmented into short fragments using fragmentation buffer and reverse-transcribed into cDNA with NEBNext Ultra RNA Library Prep Kit for Illumina (NEB, Ipswich, MA, USA). The purified double-stranded cDNA fragments were end-repaired, with a base added, and ligated to Illumina sequencing adapters. The ligation reaction was purified using the AMPure XP Beads (1.0×); then, the cDNA library was amplified by polymerase chain reaction (PCR). The resulting cDNA library was sequenced on the Illumina Novaseq6000. Firstly, we removed reads containing sequencing adapters or reads of poor quality in raw data. Secondly, transcriptional reads were mapped to the genome (Ensembl_release110) using HISAT2.2.4. With a threshold of absolute log₂ fold change ≥0.58 and FDR < 0.05 [23,24], the differentially expressed genes (DEGs) were identified using DESeq2 (version 1.50.2). The RNA-seq analysis was performed by Genedenovo, using standard and consistent procedures. For functional

analysis, KEGG and Gene set enrichment analysis (GSEA) were performed using the cluster Profiler R package (version 4.3.0).

2.3. Cell Transfection

The oligonucleotide sequences targeting bovine FABP3, PTGER2, PTGER4 mRNA and a scrambled sequence were designed using online software (<https://rnaidesigner.thermofisher.com/rnaexpress/> accessed on 24 October 2023). The sequences of shFABP3, shPTGER2, shPTGER4 and shN are shown in Supplementary Table S1. The virus packaging and cell transfection were performed as previously described [25,26]. These shRNA fragments were cloned into the pCD513B-U6 vector using *EcoR* I and *BamH* I restriction enzymes. After screening with ampicillin, the correct vectors were co-transfected with the third-generation lentivirus package plasmids (encoding GAG, REV, and VSV-G protein) into HEK293 cells with TurboFect Transfection Reagent (R0533, Invitrogen, Carlsbad, CA, USA). The supernatant was collected and filtered using a 0.45 µm PVDF filter after transfection for 48 h and then used to infect bEECs. Cells with stable gene knockdown were selected with 0.2 µg/mL puromycin.

The cDNA encoding FABP3 was amplified and cloned into the lenti-CMV-MCS-flag-IRES-PUPO vector using *Nhe* I and *Xho* I restriction enzymes. The sequences of primers are shown in Supplementary Table S1. After screening with ampicillin, the correct vector was co-transfected with the second-generation lentivirus package plasmids (encoding GAG, Pol, REV, Tat and VSV-G protein) into HEK293 cells with TurboFect Transfection Reagent (R0533, Invitrogen). The supernatant was collected and filtered with a 0.45-µm PVDF filter after transfection for 48 h and used to infect bEECs. Cells with stable gene overexpression were selected with 0.2 µg/mL puromycin. Transfection with an empty vector was used as a control group.

2.4. Tissue Collection

Uteri from healthy cows, ipsilateral to the ovary containing a corpus luteum (CL), were immediately collected at a commercial slaughterhouse under veterinary supervision. The stage of the estrous cycle was determined according to the morphological observation of ovaries (follicles and CL) and uterus, following previous reports [27,28]. Endometrial tissues were obtained from dairy cows in metestrus (days 2–5) and diestrus (days 11–15) ($n = 3$ per stage) [29]. The endometrium was immediately separated from the harvested uterus and snap-frozen in liquid nitrogen, transported to the laboratory and stored at -80°C .

2.5. Progesterone Determination

To confirm the accuracy of the estrous cycle, progesterone (P_4) concentration in endometrial tissues was measured according to the report [29]. The concentration of P_4 extracted from endometrial tissues using petroleum ether was measured using an Enzyme-Linked Immunosorbent Assay (ELISA) (002401, MEIMIAN, Yancheng, China) following the manufacturer's instructions. The standard curve ranged from 0.1 to 100 ng/mL, with a sensitivity of 0.15 ng/mL. The intra- and inter-assay coefficients of variation were 8.7% and 11%, respectively.

2.6. Immunohistochemistry

Paraffin-embedded uterine tissues were deparaffinized, rehydrated, and then stained with the streptavidin–peroxidase method following the previous report [30]. Briefly, antigen retrieval was performed by boiling the sections in citrate antigen retrieval solution (Solarbio, Beijing, China) for 20 min. Subsequently, the universal SP staining kit (Maixin Biotechnologies, Fuzhou, China) was used according to the manufacturer's instructions.

After blocking, the sections were incubated overnight at 4 °C with primary antibodies, including anti-PGR antibody (1:200, 25871-1-AP, Proteintech, Wuhan, China) [31], anti-FABP3 antibody (1:200, 10676-1-AP, Proteintech) [32] and a negative control (1:1000, IgG, Abcam, Cambridge, UK). The sections were subsequently incubated with the second antibody at 37 °C for 2 h and incubated with streptavidin–biotin peroxidase at 25 °C for 40 min after washing three times with PBS. Visualization was performed using diaminobenzidine (DAB) as the chromogen, followed by light counterstaining with hematoxylin. Images were captured using a microscope (Nikon, Tokyo, Japan).

2.7. RNA Extraction and Real-Time Quantitative PCR

Total RNA was extracted from the bEECs using TRIzol reagent (TaKaRa, Tokyo, Japan) according to the manufacturer's protocols. The RNA (1 µg) was converted into complementary DNA (cDNA) using a PrimeScript™ RT reagent kit with gDNA Eraser (TaKaRa). Real-time quantitative PCR (qPCR) was performed using SYBR Green Master Mix (Vazyme, Nanjing, China) in a Bio-Rad CFX96 (Bio-Rad, Hercules, CA, USA), according to the manufacturer's protocol. Gene expression of mRNA was normalized to the *RPS9* gene. Relative expression levels were calculated with the $2^{-\Delta\Delta Ct}$ method. The primer sequences are shown in Supplementary Table S2.

2.8. Western Blot

The samples were lysed with RIPA buffer (Solarbio, Beijing, China), according to the manufacturer's protocol. The total protein concentration was measured using the BCA assay (Keygen Biotech, Nanjing, China). The total proteins (20 µg) were loaded onto an SDS-PAGE gel and then transferred to PVDF membranes (Millipore, Bedford, MA, USA). After blocking with 5% non-fat milk for 2 h, the membranes were incubated with primary antibodies at 4 °C overnight, including anti-PLIN2 antibody (1:1000, ab181452, Abcam) [33], anti-CDH1 antibody (1:1000, 3195, Cell Signaling Technology, Danvers, MA, USA) [34], anti-ZO-1 antibody (1:1000, 21773-1-AP, Proteintech, bovine), anti-β-catenin antibody (1:500, sc-53484, Santa Cruz, CA, USA) [35], anti-FABP3 antibody (1:1000, ab133585, Abcam), anti-OPN antibody (1:1000, 22952-1-AP, Proteintech) [36], anti-ITGB3 antibody (1:1000, CY5237, Abways, Shanghai, China), anti-PTGER2 antibody (1:1000, ab167171, Abcam), anti-PTGER4 antibody (1:1000, ab217966, Abcam), anti-β-Tubulin antibody (1:2000, 56739S, Cell signaling Technology) [37] and anti-GAPDH (1:2000, 60004-1-Ig, Proteintech) [38]. Subsequently, the membranes were incubated for 1 h with an HRP-labeled secondary antibody at room temperature.

2.9. Measurement of Cell Viability

The bEECs (5×10^3 cells/well) were seeded into a 96-well plate. Following 36 h of inhibitor treatment, cells were incubated with 10 µL reagent of the Cell Counting Kit-8 (C0037, Beyotime, Shanghai, China) for 2 h at 37 °C. The OD value was measured at 450 nm using a Multimode Microplate Reader (TECAN, Männedorf, Switzerland).

2.10. Cell Proliferation Assay

The bEECs with stable FABP3 knockdown or overexpression were harvested using trypsin after washing once with PBS. Cells were then centrifuged at 2000 rpm for 5 min and subsequently fixed in cold 70% ethanol overnight at 4 °C. After fixation, samples were stained using the Cell Cycle Detection Kit (KGA9101, KeyGEN biotech, Co., Ltd., Nanjing, China) according to the manufacturer's instructions. DNA content was measured using a BD FACSaria II SORP flow cytometer (Franklin Lakes, NJ, USA), and the number of cells in the G₁, S, and G₂ phases was calculated using Modfit 3.1 software.

The bEECs with FABP3 knockdown or overexpression were cultured in 24-well plate at a density of 5×10^4 cells/well and incubated with 10 μ M EdU (C0075S, Beyotime) for 6 h. After removing the medium and washing, cells were permeabilized with PBS containing 3% Triton X-100. Subsequently, staining was performed according to the manufacturer's instructions. Samples were observed by fluorescence microscope (Nikon).

2.11. Immunofluorescent Staining

The cell samples were fixed with 4% formaldehyde for 30 min, rinsed three times with PBS, and permeabilized with PBST (0.1% Triton X-100 in PBS) for 5 min. After blocking with 3% BSA for 1 h and washing three times, the samples were incubated with anti-FABP3 antibody (1:500, ab133585, Abcam) overnight at 4 °C; after washing three times with PBS, incubation with Alexa-labeled donkey anti-rabbit IgG (1:1000, A16028, Invitrogen) was performed at room temperature for 1 h. The nuclei were counterstained with 4',6-diamidino-2-phenylindole (DAPI) (C1002, Beyotime) for 5 min, and the cells were observed using laser-scanning confocal microscopy (Nikon, Tokyo, Japan).

2.12. Scanning Electron Microscope

The cell samples were fixed in a 3% glutaraldehyde-buffered solution for 30 min at room temperature. Afterward, the samples were rinsed three times with PBS and subsequently fixed in a 1% rhodium acid solution for 1 h at 4 °C. Following rinsing in demineralized water, the samples were dehydrated through a graded ethanol series up to 100% ethanol. They were then critical point-dried using liquid CO₂ (Leica EM CPD 300 Manual) and sputter-coated with a 7 nm gold/palladium layer (Leica EM ACE 200). All samples were examined using a JSM-IT700HR scanning electron microscope (JEOL Ltd., Tokyo, Japan).

2.13. In Vitro Implantation Assay

The BTCs were a gift from associate professor Xiangguo Wang of Beijing University of Agriculture [39]. In this study, BTCs were cultured in DMEM/F12 medium containing 10% FBS and incubated at 37 °C in a humidified 5% CO₂ incubator. The in vitro embryo implantation was simulated using a spheroid coculture assay, performed according to the previous study [30]. The cell suspension containing approximately 1.0×10^6 cells was stained with CellTracker Red CMFDA (0.5 μ M; Yeasen Biotech, Shanghai, China) and cultured on an orbital shaker rotating at 200 rpm for 10 h at 37 °C. The spheroids had a diameter of approximately 100–200 μ m and contained about 200–1000 cells each. Then, the BTC spheroids were delivered onto monolayer bEECs after PGE₂ treatment. The bEECs and BTC spheroids (approximately 100 spheroids per well) were cocultured for 2 h at 37 °C. After washing three times with PBS, the BTC spheroids were counted under a fluorescence microscope. The attachment rate is expressed as the percentage of seeded spheroids.

2.14. BODIPY Staining

The cell samples were fixed with 4% formaldehyde for 30 min, rinsed three times with PBS, each time for 5 min, and permeabilized by incubation with PBST (0.1% Triton X-100 in PBS) for 5 min. After washing three times with PBS, incubation with Alexa FluorTM 594 phalloidin (A12381, Invitrogen) was performed at room temperature for 20 min. Then, the samples were incubated with 10 μ g/mL BODIPY 493/503 (D3922, Invitrogen) for 30 min at room temperature after washing three times. Cell nuclei were counterstained with DAPI for 5 min, and the cells were observed using laser-scanning confocal microscopy (Nikon).

The cell samples were washed with precooled PBS and harvested with trypsin. Next, the cells were centrifuged at 2000 rpm for 5 min and then stained using 10 µg/mL BODIPY 493/503 for 30 min. After washing three times, the fluorescence intensity of samples was tested by flow cytometry. The fluorescence intensity was analyzed by Image Pro Plus 6.0 software.

2.15. Statistical Analysis

Results were presented as the means ± SEM and obtained from at least 3 independent experiments. Statistical analyses were performed with GraphPad Prism software (version 8.0) using unpaired Student's *t*-test for experiments with two groups or one-way analysis of variance (ANOVA) followed by Tukey's post hoc test between multiple groups. The results with statistically significant differences are indicated by asterisks ($p < 0.05$ denoted by *, $p < 0.01$ denoted by **, $p > 0.05$ denoted by ns).

3. Results

3.1. PGE₂ Treatment Induces Lipid Droplet Accumulation and Alternation of Cell Morphology in Bovine Endometrial Epithelial Cells

To explore the potential role of PGE₂ in endometrium function during diestrus, P₄ and PGE₂ were used to mimic hormonal changes, and detect lipid droplet accumulation in bEECs after treatment. Compared with the CON group, P₄ treatment had a limited effect on lipid droplet accumulation, while the combination of P₄ and PGE₂ treatment significantly promoted lipid droplet accumulation (Figure 1A). The fluorescence intensity of BODIPY 493/503 was analyzed by flow cytometry, and the results showed that PGE₂ treatment promoted lipid droplet accumulation (Figure 1B). Moreover, PGE₂ treatment significantly increased PLIN2 expression as a marker of lipid droplet (Figure 1C,D). Meanwhile, P₄ + PGE₂ treatment led to alterations in cell morphology, with partial cytoskeleton disorganization (F-actin). Since the 10^{−7} M PGE₂ concentration enhanced PLIN2 expression, this concentration was used for subsequent processing treatment and analysis. The above results indicated that PGE₂ treatment induced lipid droplet accumulation and alternation of cell morphology in bEECs.

3.2. The Number of Lipid Droplets Is Related to Cell Morphology in Bovine Endometrial Epithelial Cells

Previous studies show that there is a correlation between cytoskeletal remodeling and lipid droplets [40,41]. Hence, the lipid droplet levels of bEECs were detected under different culture conditions in this study. The content of lipid droplets in bEECs was reduced when cultured in serum-free medium added 1% BSA, while the number of lipid droplets was increased when cultured in medium added 200 µM oleic acid (OA). Cell morphology was observed by staining F-actin, a cytoskeletal component, with phalloidin. Compared with the control group (5% FBS), the cells in the 1% BSA group were flattened and closely arranged (Figure 2A,B), and PLIN2 expression was significantly down-regulated in the 1% BSA group (Figure 2C–E). After treatment with 5% FBS + OA, the cells were rounder in shape and loosely arranged (Figure 2A,B), and PLIN2 expression was significantly up-regulated compared with 5% FBS group (Figure 2C–E).

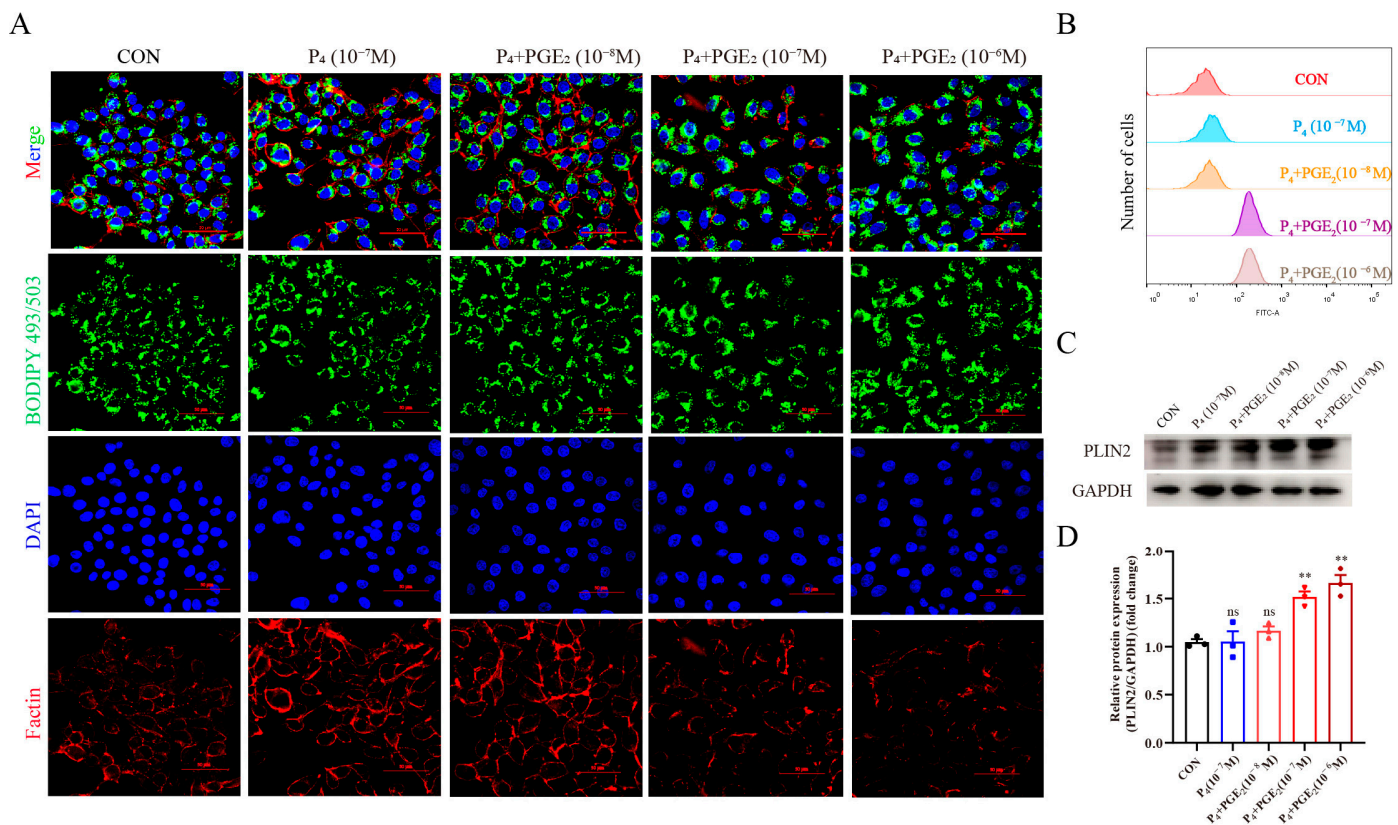


Figure 1. PGE₂ treatment induces lipid droplet accumulation and alternation of cell morphology of bEECs: (A) The representative images of lipid droplets and cytoskeleton staining in bEECs after treatment with P₄ and/or PGE₂. BODIPY 493/503 (green), F-actin (red) and DAPI (blue) marked lipid droplets, cytoskeleton and cell nuclei, respectively. Scale bar: 50 μ m. (B) The fluorescence intensity analysis of BODIPY 493/503 staining in bEECs after treatment with P₄ and/or PGE₂. Y-axis represents the number of cells detected at each fluorescence. (C,D) The representative images and analysis of PLIN2 expression in bEECs after treatment with P₄ and/or PGE₂, respectively. The data are means $\pm$ SEM of three independent experiments. Statistically significant differences are indicated by asterisks: ** $p < 0.01$, ns $p > 0.05$.

Epithelial cell tight junction protein (ZO-1) and adherens junction proteins (CDH1, β -catenin) were detected (Figure 2F–K). Results showed that alternations in cellular lipid metabolism significantly reduced CDH1 expression (Figure 2H,I). Compared with the control group (5% FBS), the protein level of ZO-1 was significantly up-regulated in the 1% BSA group but markedly decreased in the 5% FBS + OA group (Figure 2F,G). The protein level of β -catenin showed no significant changes in the 1% BSA group, while it was down-regulated in the 5% FBS + OA group (Figure 2J,K). Collectively, the number of lipid droplets was related to cell morphology in bEECs, and disruption of lipid droplets altered cell morphology.

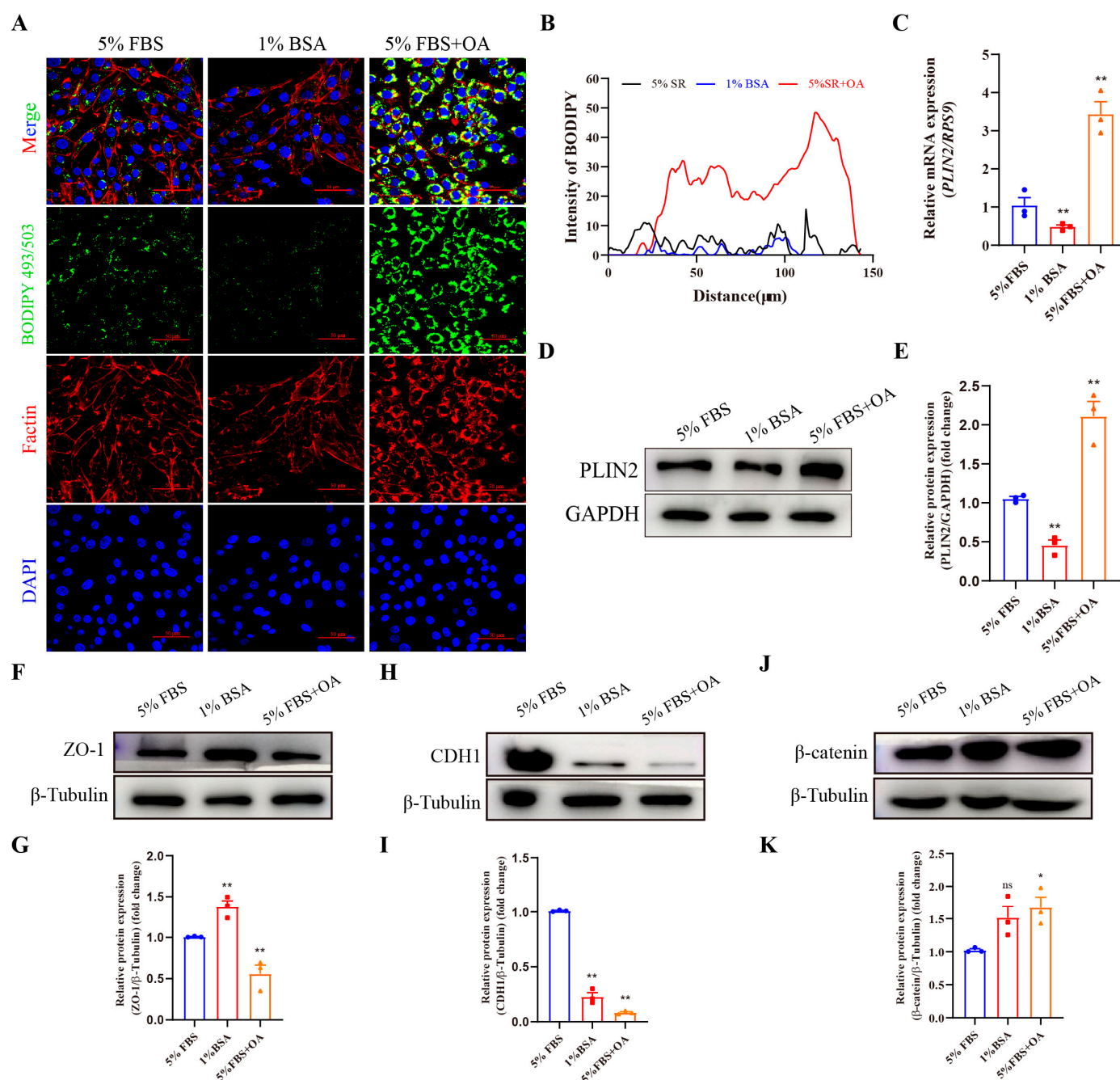


Figure 2. The number of lipid droplets in bEECs is related to the cell morphology: **(A)** The representative images of lipid droplets and cytoskeleton staining in bEECs after treatment with 5% FBS, 1% BSA or 5% FBS + OA. BODIPY 493/503, F-actin and DAPI marked lipid droplets, cytoskeleton and cell nuclei, respectively. Scale bar: 50 μ m. **(B)** The fluorescence intensity analysis of BODIPY 493/503 staining in bEECs after treatment with 5% FBS, 1% BSA or 5% FBS + OA. **(C)** The analysis of *PLIN2* expression in bEECs after treatment with 5% FBS, 1% BSA or 5% FBS + OA. **(D,E)** The representative images and analysis of *PLIN2* expression in bEECs after treatment with 5% FBS, 1% BSA or 5% FBS + OA, respectively. **(F,G)** The representative images and analysis of *ZO-1* expression in bEECs after treatment with 5% FBS, 1% BSA or 5% FBS + OA, respectively. **(H,I)** The representative images and analysis of *CDH1* expression in bEECs after treatment with 5% FBS, 1% BSA or 5% FBS + OA, respectively. **(J,K)** The representative images and analysis of β -catenin expression in bEECs after treatment with 5% FBS, 1% BSA or 5% FBS + OA, respectively. The data are means $\pm$ SEM of three independent experiments. Statistically significant differences are indicated by asterisks: * $p < 0.05$, ** $p < 0.01$, ns $p > 0.05$.

3.3. RNA-Seq Reveals PPAR Signaling Pathway and Its Key Molecules Respond to PGE₂ Treatment in Bovine Endometrial Epithelial Cells

To analyze the signaling response to PGE₂ treatment in bovine endometrial epithelial cells, cells from the P₄ and P₄ + PGE₂ treatment groups were collected and subjected to RNA-seq. The principal component analysis (PCA) is shown in Figure S1A, and a total of 230 DEGs were identified between the P₄ and P₄ + PGE₂ groups (Figure S1B). The volcano plot and heatmap of DEGs are shown in Figure S1C and S1D, respectively, and these DEGs were used for GO analyses and are shown in Figure S1E. The KEGG analysis of these DEGs is shown in Figure 3A, showing that PGE₂ treatment significantly enriched Ferroptosis, IL-17 signaling pathway, TNF signaling pathway and others. Notably, the PPAR signaling pathway ($p = 0.001$ and FDR = 0.042) was enriched after PGE₂ treatment (Figure 3A), which was associated with lipid droplet accumulation. The GSEA result showed that PGE₂ treatment enhanced the PPAR signaling pathway (Figure 3B). The mRNA expression of key genes (EHHADH, FABP3, ACSL4, ACSL6 and DBI) was significantly up-regulated after PGE₂ treatment (Figure 3C), and these alternations were further confirmed by q-PCR results (Figure 3D).

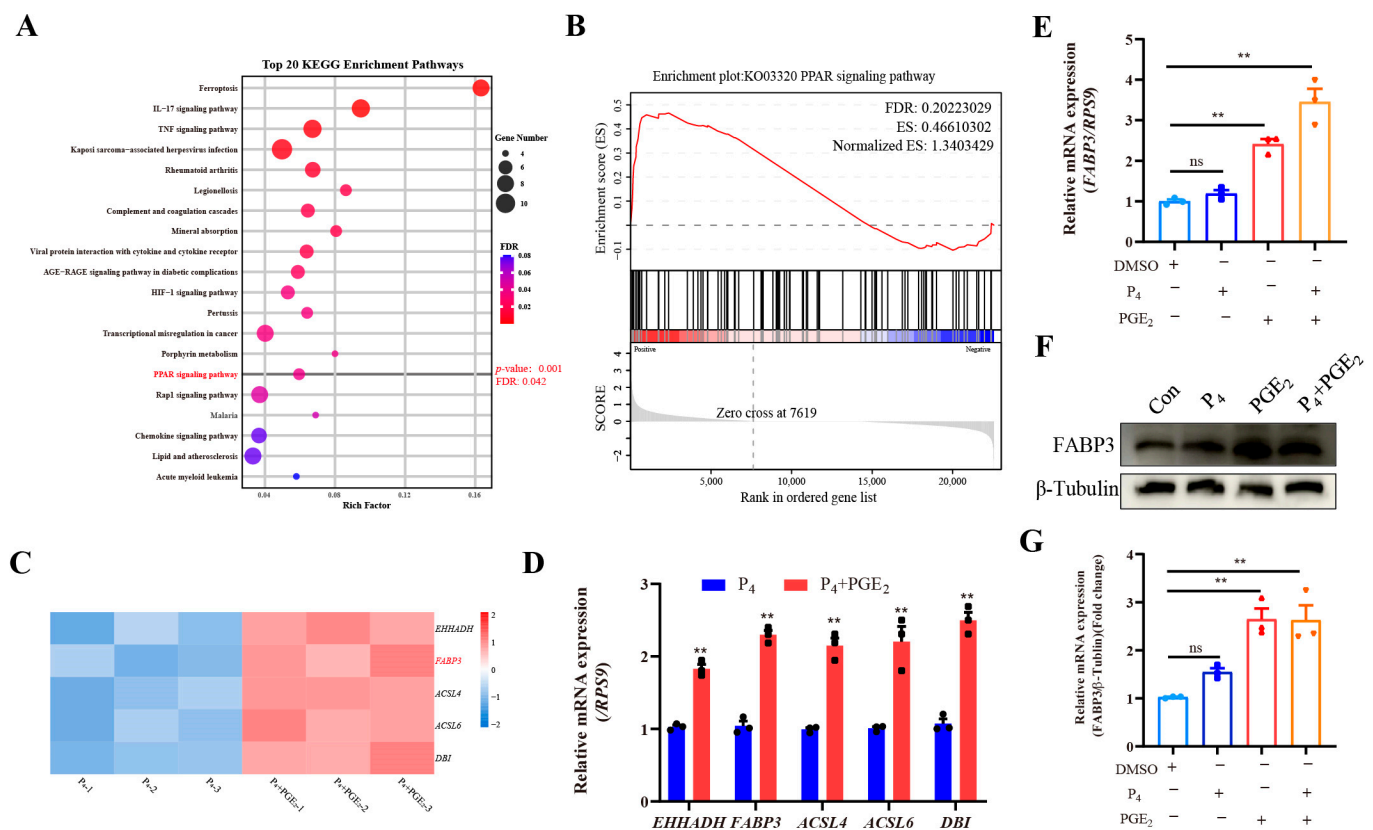


Figure 3. PGE₂ treatment promotes PPAR signaling pathway and FABP3 expression in bEECs: (A) The top 20 pathways from KEGG analysis between P₄ and P₄ + PGE₂ groups. (B) The GSEA of PPAR signaling pathway between P₄ and P₄ + PGE₂ groups. (C) The heatmap of key gene (*EHHADH*, *FABP3*, *ACSL4*, *ACSL6* and *DBI*) expression related to PPAR signaling pathway. (D) The analysis of key gene (*EHHADH*, *FABP3*, *ACSL4*, *ACSL6* and *DBI*) expression in bEECs between P₄ and P₄ + PGE₂ groups. (E) The analysis of *FABP3* expression in bEECs after P₄ and/or PGE₂ treatment. (F,G) The representative images and analysis of *FABP3* in bEECs after P₄ and/or PGE₂ treatment. The data are means ± SEM of three independent experiments. Statistically significant differences are indicated by asterisks: ** $p < 0.01$, ns $p > 0.05$.

According to the above analysis, FABP3 may be the key gene that responds to PGE₂ treatment in bEECs and regulates lipid droplet accumulation. Therefore, we further analyzed FABP3 expression after P₄ and/or PGE₂ treatment. The results of q-PCR and WB showed that P₄ treatment had a limited effect on FABP3 expression; PGE₂ treatment alone significantly increased the mRNA and protein expression of FABP3; and the combination treatment further enhanced this trend (Figure 3E–G). Collectively, the PPAR signaling pathway and FABP3 were the key molecules responding to PGE₂ treatment in bovine endometrial epithelial cells.

3.4. FABP3 Regulates the Proliferation of Bovine Endometrial Epithelial Cells

To explore the function of FABP3, stable knockdown or overexpression of FABP3 in bEECs was established. The efficiency of FABP3 knockdown or overexpression was verified by RT-qPCR, WB and IF (Figure S2). The results showed that shFABP3-2 and shFABP3-3 constructs had high interference efficiency and were used for subsequent experiments (Figure S2A–C). The results of IF staining showed that FABP3 was mainly located in the cytoplasm (Figure S2D). Compared with the CON group, FABP3 expression was significantly increased (Figure S2E) in FABP3 overexpression (FABP3 OE) group. The FLAG tag was inserted during the construction of the lentiviral overexpression vector, so the FLAG tag was expressed simultaneously with FABP3 (Figure S2E–H).

To study the function of FABP3 in bEECs, the effect of FABP3 on cell proliferation was first detected. The results of CCK8 indicated that the knockdown or overexpression of FABP3 significantly altered the cell cycle (Figure 4A,F). Concretely, the knockdown of FABP3 increased the proportion of cells in S phase and decreased the cell proportion in G1 phase, while there was no significant difference in G2 phase (Figure 4B,C). The EdU staining results showed that the proportion of positive cells in the shFABP3-3 group was significantly greater than in the shN group (Figure 4D,E). In contrast, after overexpression of FABP3, the proportion of cells in the G1 phase increased (Figure 4G,H), the proportion of cells in the G2 phase decreased (Figure 4G,H), and there was no significant change in cells in the S phase (Figure 4G,H). Moreover, the EdU staining results showed that there was no significant difference in the proportion of positive cells between the CON and FABP3 overexpression groups (Figure 4I,J). The above results showed that FABP3 regulated the proliferation of bEECs, and disruption of FABP3 expression altered the cell cycle.

3.5. FABP3 Regulates the Adhesion Ability of Bovine Endometrial Epithelial Cells

To further understand the impact of FABP3 on the dynamic remodeling of endometrium, we detected the effects of FABP3 interference or overexpression in bEECs on cell adhesion ability, the number of microvilli and the related protein expression. The attachment of BTC spheroids in the shFABP3 group was lower than that in the shN group (Figure 5A,B). The adhesion ability of endometrial epithelial cells is related to the number of surface microvilli. The results of scanning electron microscopy further indicated that FABP3 interference led to an increase in microvilli on the surface of bEECs (Figure 5C). The expression of adhesion-related proteins (OPN and ITGB3) was significantly decreased in the shFABP3 group compared to the shN group (Figure 5D–F). Interference with FABP3 in bEECs led to the up-regulation of ZO-1 (Figure 5G,H) and CDH1 (Figure 5G,I) while down-regulating β -catenin expression (Figure 5G,J). Nevertheless, overexpression of FABP3 in bEECs had no significant effect on the adhesion ability (Figure S3A,B) and the microvilli on the surface (Figure S3C). The expression of adhesion-related proteins (OPN and ITGB3) did not change significantly (Figure S3D–F). However, the expression level of cell remodeling-related proteins (ZO-1 and CDH1) was significantly decreased in the FABP3 overexpression group compared with the CON group (Figure S3G–I). Additionally, the expression level

of β -catenin protein was significantly decreased (Figure S3G,J). Collectively, FABP3 was involved in regulating the adhesion ability of bEECs.

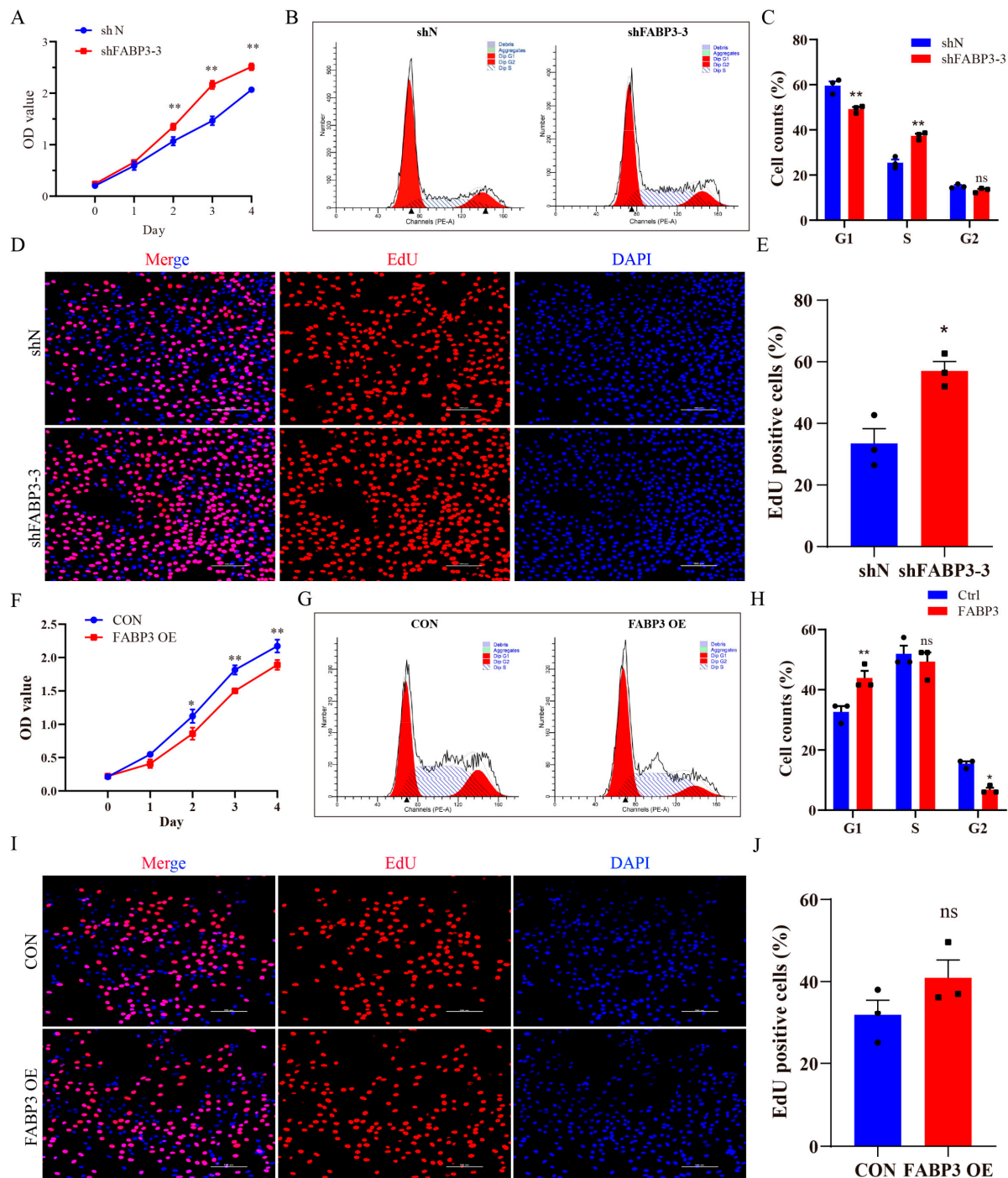


Figure 4. FABP3 expression regulates the cell proliferation and cycle in bEECs: (A) The analysis of cell proliferation in bEECs after FABP3 interference by CCK8. (B,C) The detection and analysis of cell cycle in bEECs after FABP3 interference by flow cytometer. (D,E) The representative images and analysis of EdU staining in bEECs between shN and shFABP3-3 groups. Scale bar: 100 μ m. (F) The analysis of cell proliferation in bEECs after FABP3 overexpression by CCK8. (G,H) The detection and analysis of cell cycle in bEECs after FABP3 overexpression with by flow cytometer. (I,J) The representative images and analysis of EdU staining in bEECs between CON and FABP3-overexpression groups. Edu (red), DAPI (blue), Scale bar: 100 μ m. The data are means $\pm$ SEM of three independent experiments. Statistically significant differences are indicated by asterisks: * $p < 0.05$, ** $p < 0.01$, ns $p > 0.05$.

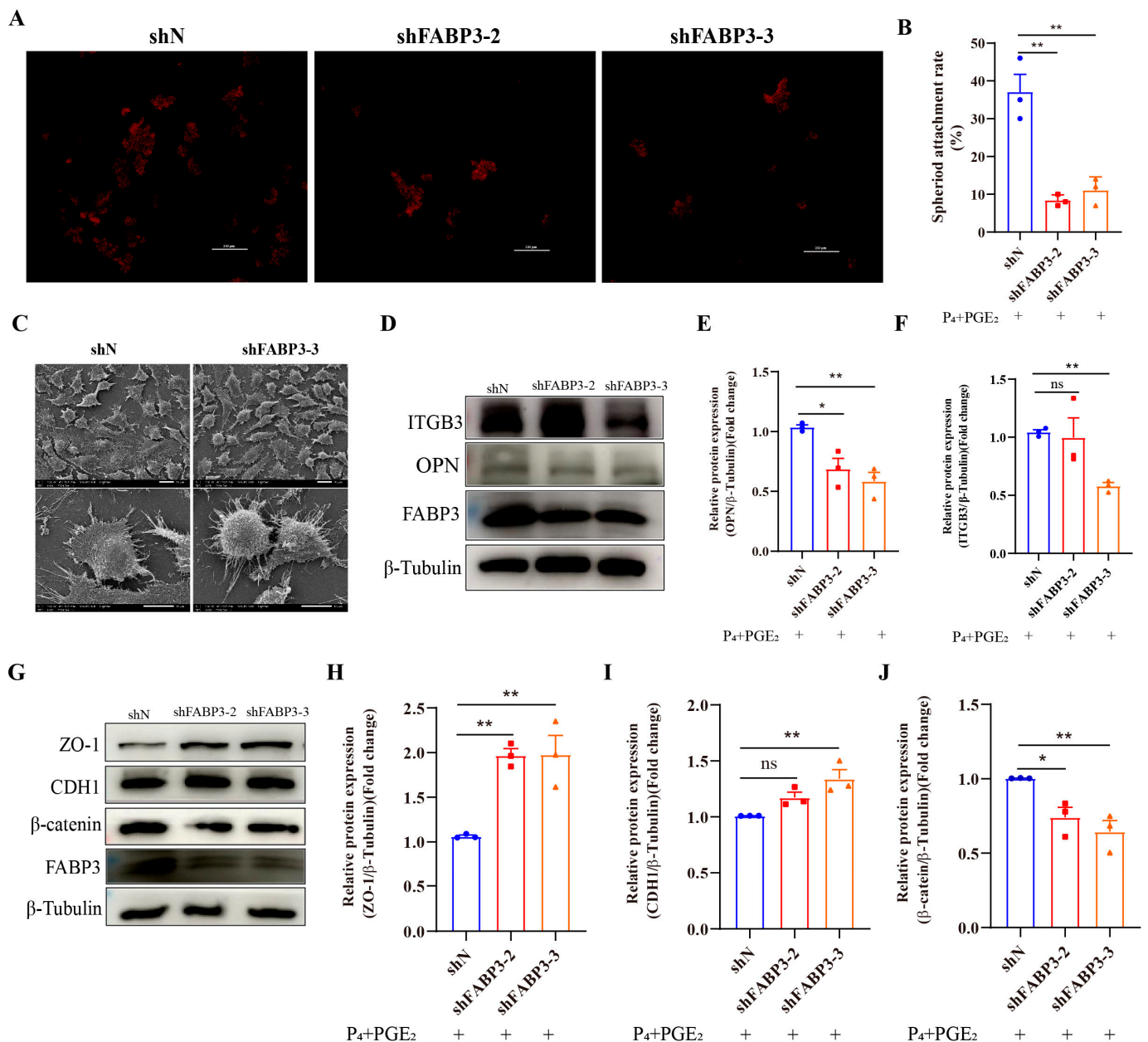


Figure 5. The expression of FABP3 regulates endometrial epithelial cell remodeling: (A,B) The representative images and analysis of BTCs spheroid adhesion rate in bEECs after FABP3 knockdown, respectively. BTCs: Bovine placental trophoblast cells. Scale bar: 200 μ m. (C) Representative images of scanning electron microscope in the microvilli structure on the surface of bEECs after FABP3 knockdown. Scale bar: 20 μ m (Others), 10 μ m (Enlarge). (D–F) The representative images and analysis of the protein expression related to cell adhesion (OPN and ITGB3) in bEECs between shN and shFABP3 groups, respectively. (G–J) The representative images and analysis of the protein expression (ZO-1, CDH1 and β -catenin) in bEECs between shN and shFABP3 groups, respectively. The data are means $\pm$ SEM of three independent experiments. Statistically significant differences are indicated by asterisks: * $p < 0.05$, ** $p < 0.01$, ns $p > 0.05$.

3.6. PGE₂ Binds to PTGER4 to Regulate FABP3 Expression of Bovine Endometrial Epithelial Cells

The PGE₂ mainly exerts its biological functions through its receptors, so we detected the expression of PTGERs. Results showed that PGE₂ treatment significantly up-regulated *PTGER2* and *PTGER4* expression of bEECs (Figure 6A), and the combination treatment of P₄ and PGE₂ also significantly up-regulated *PTGER2* and *PTGER4* expression of bEECs (Figure 6A). The WB results further confirmed the up-regulation of *PTGER2* and *PTGER4*

expression (Figure 6B–D). Subsequently, stable knockdown of PTGER2 and PTGER4 was constructed in bEECs (Figure S4A–D). Results showed that knockdown of PTGER2 has no significant effect on FABP3 expression after treatment (Figure 6E,F), while knockdown of PTGER4 significantly inhibited FABP3 expression after treatment (Figure 6G,H). Moreover, PTGER2 (TG-155) and PTGER4 (GW-627368) inhibitors were used to treat bEECs. TG-155 or GW-627368 (5 μ M) treatment had no significant effect on cell viability (Figure S4E,F) and *FABP3* expression (Figure S4G). However, 10 μ M GW-627368 treatment inhibited the up-regulation of *FABP3* by PGE₂ treatment (Figure 6I). Collectively, PGE₂ mainly binds to PTGER4 to regulate *FABP3* expression in bEECs.

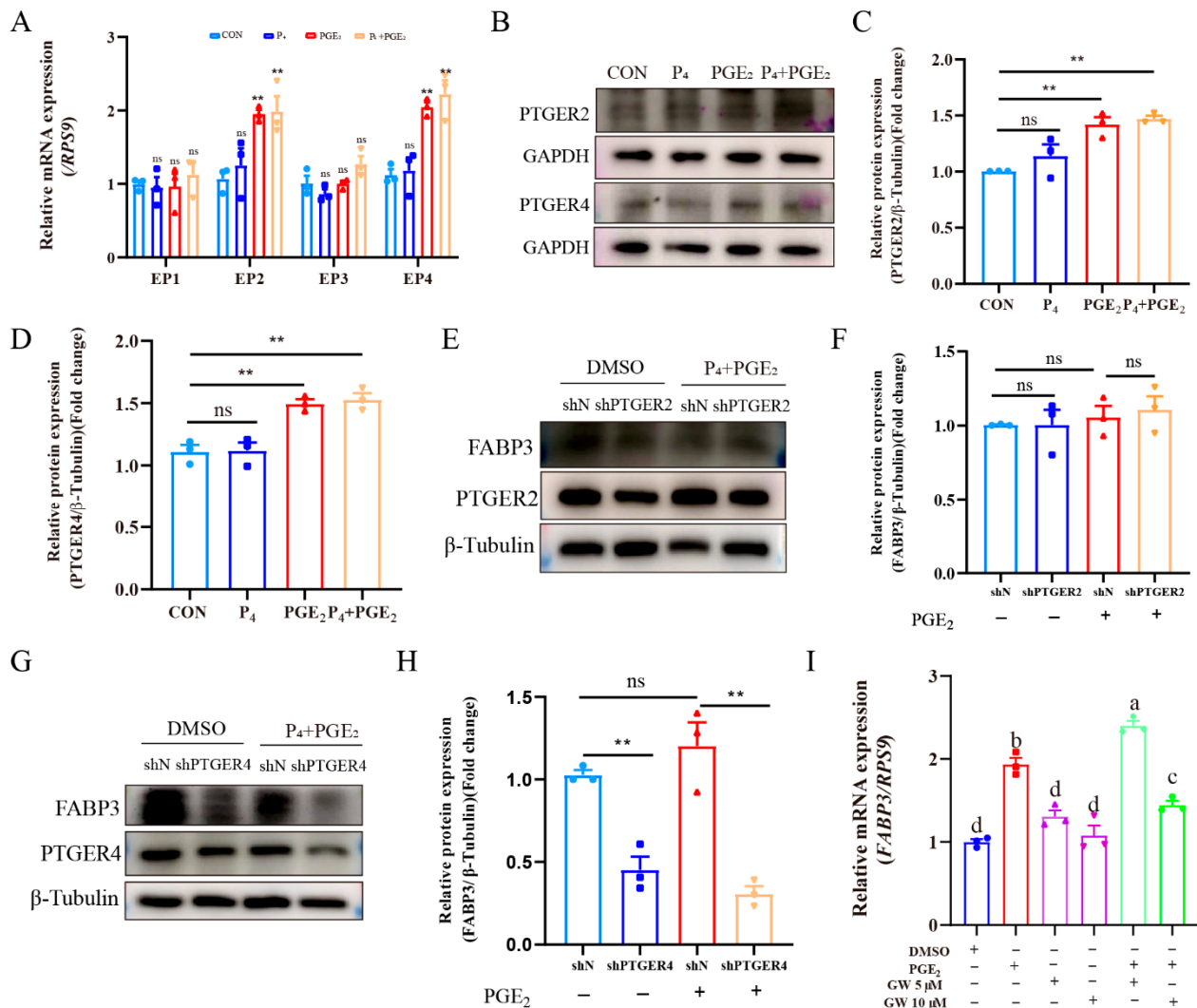


Figure 6. PGE₂ binds to PTGER4 to regulate FABP3 expression in bEECs: (A) The difference in PTGER (*PTGER1*, *PTGER2*, *PTGER3* and *PTGER4*) expression in bEECs after treatment with P₄ and/or PGE₂. (B–D) The representative images and analysis of PTGER2 and PTGER4 expression in bEECs after treatment with P₄ and/or PGE₂. (E,F) The representative images and analysis of PTGER2 expression in bEECs between shN and shPTGER2 groups after treatment with DMSO or P₄ + PGE₂. (G,H) The representative images and analysis of PTGER4 expression in bEECs between shN and shPTGER4 groups after treatment with DMSO or P₄ + PGE₂. (I) The analysis of FABP3 expression in bEECs after treatment with DMSO, PGE₂ or the inhibitor of PTGER4 (GW627368). The data are means ± SEM of three independent experiments. Statistically significant differences are indicated by asterisks: ** $p < 0.01$, ns $p > 0.05$. Bars with different letters are significantly different ($p < 0.05$).

3.7. Immunolocalization and Expression of FABP3 in the Endometrial Tissue of Dairy Cows at Different Stages of Estrus Cycle

To determine FABP3 expression in the endometrium of dairy cows at different stages of the estrous cycle, uterine samples were collected during metestrus (days 2–5) and mid-diestrus (days 11–15). The uterine tissue samples were further screened for P₄ content and PGR expression in the endometrial tissues to confirm the accuracy of the estrous cycle stages. Compared to the tissues in the metestrus phase, both progesterone levels and PGR expression were reduced in the endometrium during the mid-diestrus period (Figure 7A–C), while FABP3 expression in endometrial tissue was elevated in mid-diestrus phase (Figure 7D). Moreover, FABP3 was mainly expressed in endometrial LE and sGE, while it was expressed at low levels in the deeper glandular epithelial cells and the myometrium. Its abundance was increased in the endometrium during mid-diestrus compared to the metestrus (Figure 7E,F). Collectively, FABP3 was elevated in the endometrial tissue of dairy cows during mid-diestrus compared to metestrus, with predominant localization in the luminal and superficial glandular epithelium.

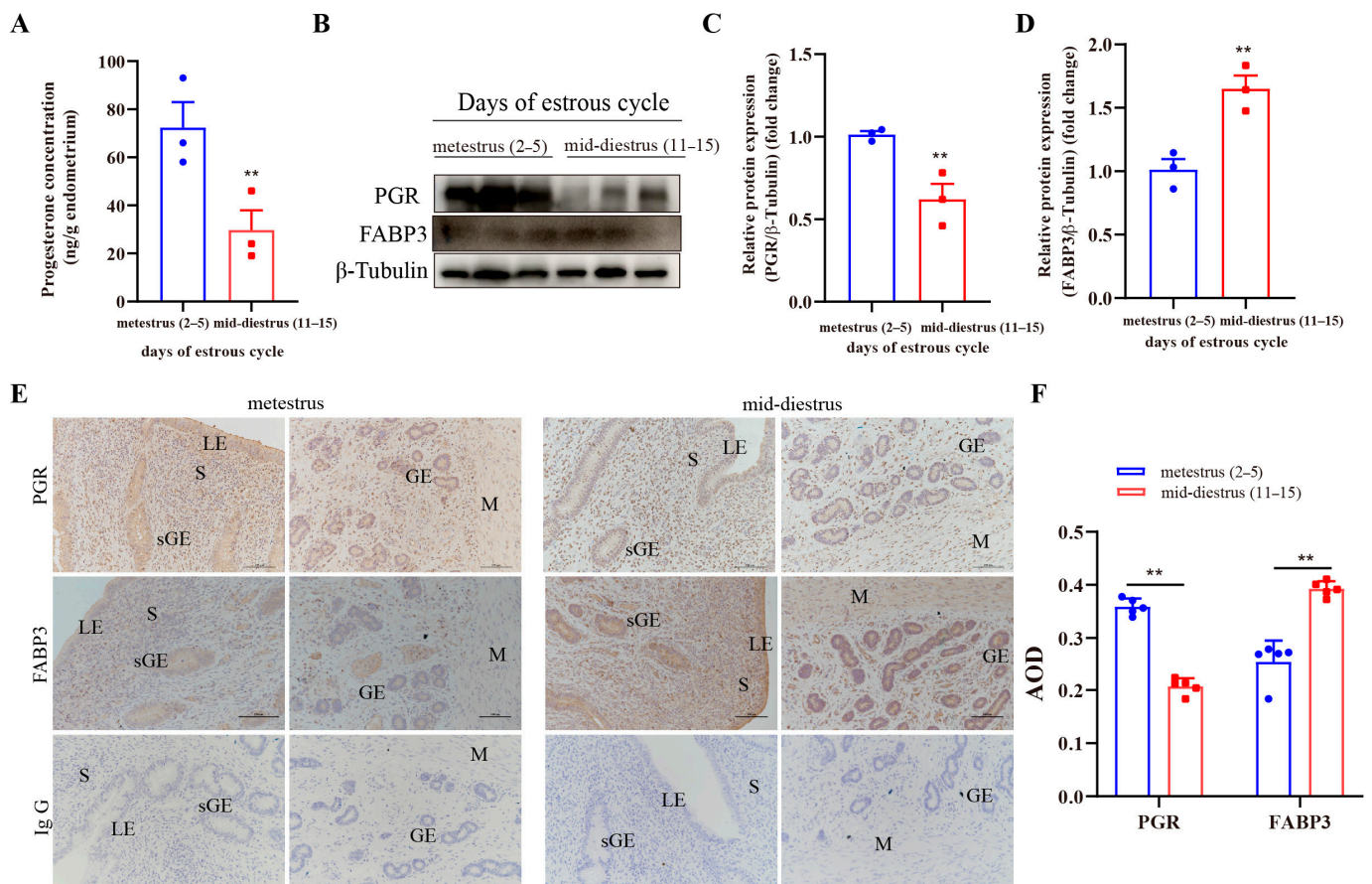


Figure 7. The expression of PGR and FABP3 in endometrial tissues of dairy cows at different stages of the estrus cycle: **(A)** The analysis of progesterone concentration in endometrial tissues of dairy cows at different stages of the estrus cycle. **(B–D)** The representative images and analysis of PGR and FABP3 expression in endometrial tissues of dairy cows at different stages of the estrus cycle, respectively. **(E,F)** The representative images and quantitative analysis of PGR and FABP3 staining in endometrial tissues of dairy cows at different stages of the estrus cycle, respectively. LEs: luminal epithelial cells; sGE: superficial glandular epithelium; GEs: glandular epithelial cells; S: stromal cells; M: myometrium. Scale bar = 100 μ m. AOD = Average Optical Density. The data are means $\pm$ SEM of three independent experiments. Statistically significant differences are indicated by asterisks: ** $p < 0.01$.

4. Discussion

Previous studies find that the number of lipid droplets in the endometrial epithelial cells of cattle [20,42] and sheep [43] significantly varies at different stages of the estrous cycle. The number of lipid droplets in the endometrial epithelium is relatively low during the metestrus period and gradually increases during diestrus [16]. The content of PGE₂ in the uterine lumen fluid of dairy cows also rapidly increases during the diestrus and pre-implantation period [3,6]. During the diestrus in dairy heifers, the inhibition of prostaglandin synthesis and secretion in the endometrium affects lipid content in uterine fluid [12,13]. In this study, PGE₂ treatment promoted the accumulation of lipid droplets in bEECs after P₄ pre-treatment. These results suggest that PGE₂ collaborates with P₄ to regulate the accumulation of lipid droplets in endometrium.

In ruminants, dynamic remodeling of endometrium is an essential prerequisite for successful embryonic implantation and represents a pivotal factor influencing the differential fertility in dairy cows [1], involving morphological changes in endometrial epithelial cells. The remodeling of junctional complexes and membrane-associated cytoskeleton is vital for epithelial transformation [44,45]. In dairy cows, the accumulation of endometrial lipid droplets during the luteal phase aligns with the timing of dynamic endometrial remodeling. In this study, the accumulation of lipid droplets promoted the remodeling of cytoskeleton (F-actin) by adjusting the culture conditions of bEECs; the expression of tight junction protein (ZO-1) and epithelial marker protein E-cadherin (CDH1) decreased, while it increased the expression of β -catenin protein. In contrast, reducing lipid droplet content by serum starvation led to the up-regulation of ZO-1 expression. Although serum starvation effectively reduced lipid droplets in cells, it has limitations, including a decrease in cell viability. As a key component of the tight junction, ZO-1 establishes a crucial connection between the transmembrane protein occludin and actin cytoskeleton [46,47]. The CDH1 engages with intracellular β -catenin (CTNNB1) and anchors the complex to the actin cytoskeleton [48]. This association is critical for preserving intercellular adhesion, and the onset of embryo implantation is marked by dynamic alterations of the expression and localization of these proteins [49,50]. These results further indicate that the dynamic changes in lipid droplets in endometrial epithelial cells are closely related to cell remodeling.

Herein, the RNA-seq analysis showed that the PPAR signaling pathway, which is involved in lipid metabolism in endometrial epithelial cells, was activated after PGE₂ treatment. Peroxisome proliferator-activated receptors (PPARs) are members of the nuclear hormone receptor superfamily, including PPAR α , PPAR β/δ , and PPAR γ [51]. They form a specific heterodimer complex with retinoid X receptor (RXR) and bind to a specific DNA sequence located in the promoter region of target genes, known as PPAR response elements (PPREs) [52]. PPARs are regarded as important regulators of lipid metabolism [53,54], and the PPAR signaling pathway plays a significant role in placental formation, embryonic development and endometrial receptivity formation in mammals [51,55,56]. Previous studies find that PPAR δ plays a significant role in regulating the formation and transport of lipid droplets in goat mammary epithelial cells, and FABP3 is the core gene downstream of PPAR δ [3,57]. This study found that PGE₂ treatment significantly induced FABP3 expression in endometrial epithelial cells under P₄ pretreatment. In ruminants, many genes related to pregnancy in the endometrium firstly require the stimulation of progesterone and are induced by other signaling molecules [10,58]. These findings suggest that FABP3 expression may be involved in P₄ induction and PGE₂ stimulation in endometrial epithelial cells.

Consistent with the existing research [1,3,22], FABP3 is highly expressed in the endometrium during mid-diestrus in dairy cows and is mainly expressed in the endometrial luminal epithelium and superficial glandular epithelium. The expression of FABP3 varies in the endometrium of dairy cows with different fertility levels [1]. Moreover, FABP3 is highly

expressed in the endometrium of dairy cows with high fertility, suggesting that it plays a role in regulating the functional changes in endometrium during early pregnancy [1]. This study found that FABP3 knockdown in bEECs increased the number of microvilli, reduced the number of trophoblast cell spheres, and decreased the expression of adhesion proteins (ITGB3 and OPN). However, there was a limited effect of FABP3 overexpression on cell adhesion and microvilli, which may be attributed to compensatory mechanisms activated in response to FABP3 modulation. Previous studies have shown that FABP3 overexpression is oncogenic [59]. Cancer cells undergo the epithelial-to-mesenchymal transition (EMT) process, resulting in a decrease in the expression of ZO-1 and CDH1 [60]. In this study, overexpression of FABP3 also resulted in a decrease in the levels of ZO-1 and CDH1, suggesting that excessive exogenous FABP3 overexpression may promote cellular carcinogenesis.

The endometrium undergoes extensive remodeling during the pre-implantation period to establish endometrial receptivity in response to pregnancy signals [61,62]. The receptivity of endometrium can be partially explained by the distribution and functional capacity of the microvilli in endometrial epithelial cells [63]. Compared with day 0 of the estrous cycle, the number of microvilli in endometrium of dairy cows on the 14th day was significantly lower [64]. The reduction in microvilli in endometrial epithelial cells is mainly involved in the formation of pinopodes and promoting embryo adhesion. Integrins and OPN are potential mediators for blastocysts to attach to the endometrium and initiate implantation [65]. Bovine trophoblast cells have the characteristics of migration and fusion with uterine epithelial cells, and are composed of five α -subunits (ITGA2B, ITGA3, ITGA5, ITGA8 and ITGAV) and two β -subunits (ITGB1 and ITGB3) [66]. However, FABP3 overexpression had no significant effects on the adhesion of microvilli and trophoblast cell spheres in endometrial epithelial cells as well as the expression of ITGB3 and OPN, while it down-regulated the expression of ZO-1 and CDH1, suggesting that FABP3 may participate in regulating the dynamic remodeling of endometrium in dairy cows.

In ruminants, PGE₂ is involved in multiple processes of early pregnancy, including conceptus elongation, maternal pregnancy recognition and embryo implantation. It mainly exerts multiple physiological functions by binding to different E-type prostaglandin receptors (PTGER1, PTGER2, PTGER3 and PTGER4) [67]. In this study, two types of receptors (PTGER2 and PTGER4) are mainly expressed in endometrial epithelial cells of dairy cows. This result is consistent with previous studies showing that the endometrium of cattle mainly expresses two receptors (PTGER2 and PTGER4), with temporal and cell-specific expression in the endometrium during the early stage of pregnancy or the estrus cycle [68]. Herein, PGE₂ mainly regulates the expression of FABP3 in bEECs by binding to PTGER4, by interference with PTGER2, PTGER4, or pretreatment with PTGER4 inhibitor GW627368. However, the potential synergistic effect of PTGER2 cannot be ruled out. In previous studies, PTGER4 has been found to be a primary regulatory factor for PGE₂ secretion and function [69,70]. In cattle, PTGER4 is more highly expressed in endometrial explants than PTGER2 [71], and its expression pattern is similar to that of PGE₂ secretion [7]. Therefore, PTGER4 may be a primary receptor involved in PGE₂-mediated signaling in endometrial cells. Moreover, FABP3 is elevated in the endometrial tissue of dairy cows during mid-diestrus compared to metestrus, with predominant localization in the luminal and superficial glandular epithelium. Collectively, this study demonstrates that FABP3 mediates lipid droplet accumulation and regulates the adhesive capacity of bovine endometrial epithelial cells through the PGE₂/PTGER4/PPAR signaling axis (Figure 8). These results suggest that FABP3 serves as a functional link between lipid metabolic activity and implantation competence, and may represent a useful molecular indicator or target for improving reproductive performance in dairy cows. This study provides preliminary insights based on in vitro models, and further studies should aim to validate these findings in physiologi-

cally relevant systems, such as organ cultures or in vivo intervention experiments, which provide a more comprehensive understanding of hormonal regulation of lipid metabolism and cell morphology in reproduction.

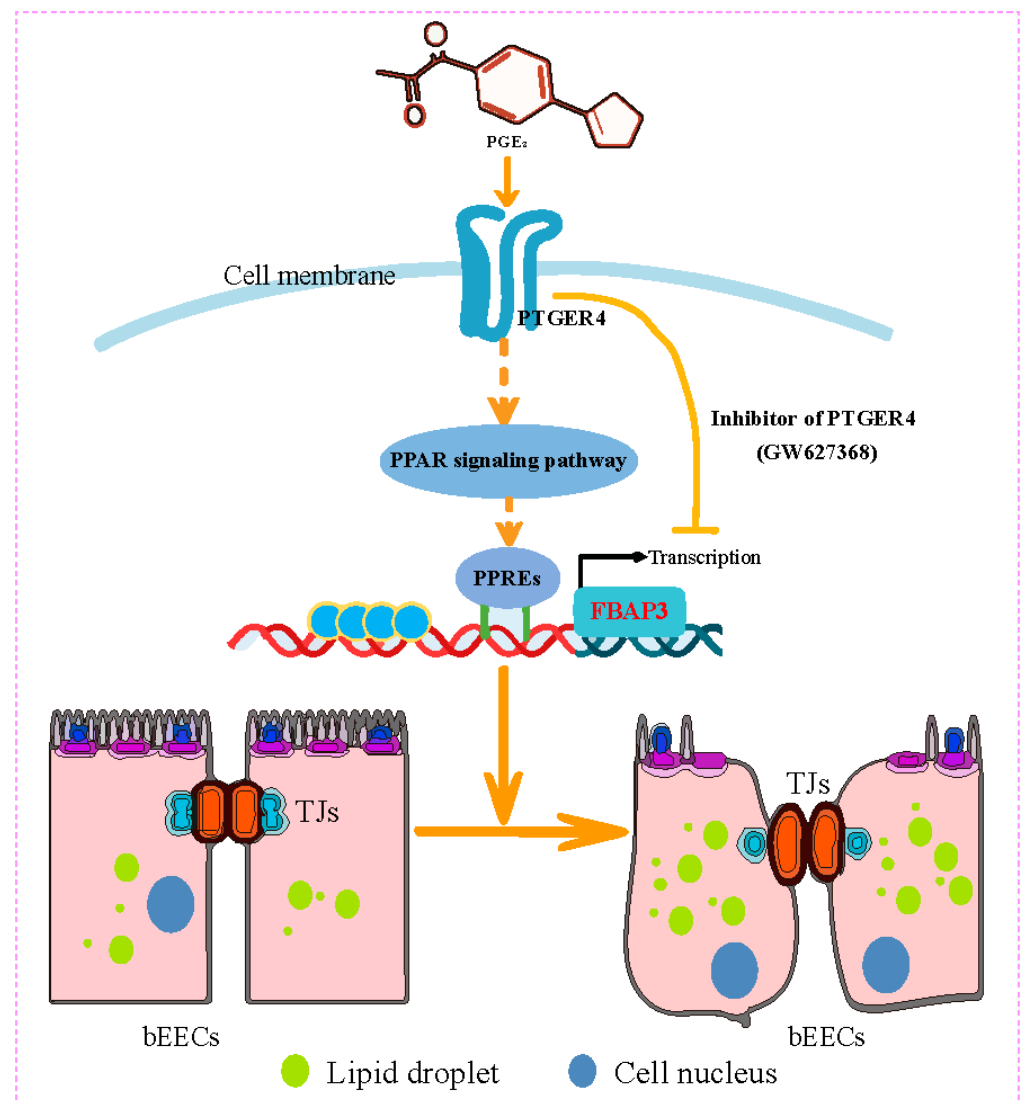


Figure 8. The mechanistic scheme of this study. A schematic model showing that FABP3 mediates lipid droplet accumulation and adhesive capacity in bovine endometrial epithelial cells via PGE₂/PTGER4/PPAR axis. TJs: tight junctions; bEECs: bovine endometrial epithelial cells.

5. Conclusions

In summary, this study identifies that FABP3 is a downstream effector of PGE₂ signaling to mediate lipid droplet accumulation and regulate the adhesive capacity of bovine endometrial epithelial cells through the PGE₂/PTGER4/PPAR signaling axis (Figure 8), which provides new insights into the metabolic regulation of endometrial receptivity.

Supplementary Materials: The following supporting information can be downloaded at <https://www.mdpi.com/article/10.3390/ani15233417/s1>, Figure S1: The analysis of RNA-seq between P₄ and P₄ + PGE₂ groups. Figure S2: The efficiency validation of FABP3 knockdown or overexpression in bEECs. Figure S3: The effect of cell remodeling in bEECs after FABP3 overexpression. Figure S4: The analysis of interference efficiency and treatment with inhibitors of PTGER2 and PTGER4. Table S1: Short hairpin interfering RNA (shRNA) inserts. Table S2: Primer pairs used for real-time quantitative PCR.

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Institutional Review Board Statement: All experiments in this study were conducted in accordance with the Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching and were approved by the Ethics Committee on the Use and Care of Animals at Northwest A&F University (No. 2021100903), 9 October 2021.

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Data Availability Statement: The original contributions presented in this study are included in the article/Supplementary Materials. Further inquiries can be directed to the corresponding authors.

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References

1. Moraes, J.G.N.; Behura, S.K.; Geary, T.W.; Hansen, P.J.; Neibergs, H.L.; Spencer, T.E. Uterine influences on conceptus development in fertility-classified animals. *Proc. Natl. Acad. Sci. USA* **2018**, *115*, E1749–E1758. [\[CrossRef\]](#)
2. Ichikawa, R.; Kimura, K.; Nakamura, S.; Ohkura, S.; Matsuyama, S. Effects of intrauterine extracellular vesicle microRNAs on embryonic gene expression in low-fertility cows. *FASEB J.* **2024**, *38*, e70116. [\[CrossRef\]](#)
3. Moraes, J.G.N.; Behura, S.K.; Geary, T.W.; Spencer, T.E. Analysis of the uterine lumen in fertility-classified heifers: I. Glucose, prostaglandins, and lipids. *Biol. Reprod.* **2020**, *102*, 456–474. [\[CrossRef\]](#)
4. Juengel, J.L.; Mosaad, E.M.O.; Mitchell, M.D.; Phyn, C.V.C.; French, M.C.; Meenken, E.D.; Burke, C.R.; Meier, S. Relationships between prostaglandin concentrations, a single nucleotide polymorphism in HSD17B12, and reproductive performance in dairy cows. *J. Dairy Sci.* **2022**, *105*, 4643–4652. [\[CrossRef\]](#) [\[PubMed\]](#)
5. Arosh, J.A.; Banu, S.K.; McCracken, J.A. Novel concepts on the role of prostaglandins on luteal maintenance and maternal recognition and establishment of pregnancy in ruminants. *J. Dairy Sci.* **2016**, *99*, 5926–5940. [\[CrossRef\]](#) [\[PubMed\]](#)
6. Ulbrich, S.E.; Schulke, K.; Groebner, A.E.; Reichenbach, H.D.; Angioni, C.; Geisslinger, G.; Meyer, H.H. Quantitative characterization of prostaglandins in the uterus of early pregnant cattle. *Reproduction* **2009**, *138*, 371–382. [\[CrossRef\]](#)
7. Arosh, J.A.; Banu, S.K.; Chapdelaine, P.; Emond, V.; Kim, J.J.; MacLaren, L.A.; Fortier, M.A. Molecular cloning and characterization of bovine prostaglandin E2 receptors EP2 and EP4: Expression and regulation in endometrium and myometrium during the estrous cycle and early pregnancy. *Endocrinology* **2003**, *144*, 3076–3091. [\[CrossRef\]](#) [\[PubMed\]](#)
8. Dorniak, P.; Bazer, F.W.; Spencer, T.E. Prostaglandins regulate conceptus elongation and mediate effects of interferon tau on the ovine uterine endometrium. *Biol. Reprod.* **2011**, *84*, 1119–1127. [\[CrossRef\]](#)
9. Dorniak, P.; Bazer, F.W.; Wu, G.; Spencer, T.E. Conceptus-derived prostaglandins regulate endometrial function in sheep. *Biol. Reprod.* **2012**, *87*, 1–7. [\[CrossRef\]](#)
10. Brooks, K.; Burns, G.; Spencer, T.E. Conceptus elongation in ruminants: Roles of progesterone, prostaglandin, interferon tau and cortisol. *J. Anim. Sci. Biotechnol.* **2014**, *5*, 53. [\[CrossRef\]](#)
11. Wu, W.X.; Coksaygan, T.; Chakrabarty, K.; Collins, V.; Rose, J.C.; Nathanielsz, P.W. Sufficient progesterone-priming prior to estradiol stimulation is required for optimal induction of the cervical prostaglandin system in pregnant sheep at 0.7 gestations. *Biol. Reprod.* **2005**, *73*, 343–350. [\[CrossRef\]](#) [\[PubMed\]](#)
12. Zhang, B.; Han, Y.; Cheng, M.; Yan, L.; Gao, K.; Zhou, D.; Wang, A.; Lin, P.; Jin, Y. Metabolomic effects of intrauterine meloxicam perfusion on histotroph in dairy heifers during diestrus. *Front. Vet. Sci.* **2025**, *12*, 1528530. [\[CrossRef\]](#) [\[PubMed\]](#)
13. Zhang, B.; Han, Y.; Wang, S.; Cheng, M.; Yan, L.; Zhou, D.; Wang, A.; Lin, P.; Jin, Y. The Impact of Uterus-Derived Prostaglandins on the Composition of Uterine Fluid During the Period of Conceptus Elongation in Dairy Heifers. *Int. J. Mol. Sci.* **2025**, *26*, 1792. [\[CrossRef\]](#) [\[PubMed\]](#)

14. Simintiras, C.A.; Sánchez, J.M.; McDonald, M.; Lonergan, P. The biochemistry surrounding bovine conceptus elongation†. *Biol. Reprod.* **2019**, *101*, 328–337. [[CrossRef](#)]
15. Simintiras, C.A.; Sánchez, J.M.; McDonald, M.; Lonergan, P. Progesterone alters the bovine uterine fluid lipidome during the period of elongation. *Reproduction* **2019**, *157*, 399–411. [[CrossRef](#)]
16. Ribeiro, E.S.; Santos, J.E.; Thatcher, W.W. Role of lipids on elongation of the preimplantation conceptus in ruminants. *Reproduction* **2016**, *152*, R115–R126. [[CrossRef](#)]
17. Olzmann, J.A.; Carvalho, P. Dynamics and functions of lipid droplets. *Nat. Rev. Mol. Cell Biol.* **2019**, *20*, 137–155. [[CrossRef](#)]
18. Wordinger, R.J.; Dickey, J.F.; Ellicott, A.R. Histochemical evaluation of the lipid droplet content of bovine oviductal and endometrial epithelial cells. *J. Reprod. Fertil.* **1977**, *49*, 113–114. [[CrossRef](#)]
19. Brinsfield, T.H.; Hawk, H.W. Control by progesterone of the concentration of lipid droplets in epithelial cells of the sheep endometrium. *J. Anim. Sci.* **1973**, *36*, 919–922. [[CrossRef](#)]
20. King, K.; Ticiani, E.; Sprícigo, J.F.W.; Carvalho, M.R.; Mion, B.; Bertolini, M.; Contreras, G.A.; Ribeiro, E.S. Dynamics of lipid droplets in the endometrium and fatty acids and oxylipins in the uterine lumen, blood, and milk of lactating cows during diestrus. *J. Dairy Sci.* **2021**, *104*, 3676–3692. [[CrossRef](#)]
21. Michler, S.; Schöffmann, F.A.; Robaa, D.; Volmer, J.; Hinderberger, D. Fatty acid binding to the human transport proteins FABP3, FABP4, and FABP5 from a Ligand's perspective. *J. Biol. Chem.* **2024**, *300*, 107396. [[CrossRef](#)]
22. Bauersachs, S.; Ulbrich, S.E.; Reichenbach, H.D.; Reichenbach, M.; Büttner, M.; Meyer, H.H.; Spencer, T.E.; Minten, M.; Sax, G.; Winter, G.; et al. Comparison of the effects of early pregnancy with human interferon, alpha 2 (IFNA2), on gene expression in bovine endometrium. *Biol. Reprod.* **2012**, *86*, 46. [[CrossRef](#)]
23. O'Keeffe, G.; Hammel, S.; Owens, R.A.; Keane, T.M.; Fitzpatrick, D.A.; Jones, G.W.; Doyle, S. RNA-seq reveals the pan-transcriptomic impact of attenuating the gliotoxin self-protection mechanism in *Aspergillus fumigatus*. *BMC Genom.* **2014**, *15*, 894. [[CrossRef](#)]
24. Yi, C.; Liu, J.; Deng, W.; Luo, C.; Qi, J.; Chen, M.; Xu, H. Macrophage elastase (MMP12) critically contributes to the development of subretinal fibrosis. *J. Neuroinflamm.* **2022**, *19*, 78. [[CrossRef](#)] [[PubMed](#)]
25. Chen, F.; Lin, P.F.; Li, X.; Sun, J.; Zhang, Z.; Du, E.; Wang, A.; Jin, Y.P. Construction and expression of lentiviral vectors encoding recombinant mouse CREBZF in NIH 3T3 cells. *Plasmid* **2014**, *76*, 24–31. [[CrossRef](#)]
26. Yang, D.; Jiang, T.; Lin, P.; Chen, H.; Wang, L.; Wang, N.; Zhao, F.; Wang, A.; Jin, Y. Knock-down of apoptosis inducing factor gene protects endoplasmic reticulum stress-mediated goat granulosa cell apoptosis. *Theriogenology* **2017**, *88*, 89–97. [[CrossRef](#)]
27. Ireland, J.J.; Murphee, R.L.; Coulson, P.B. Accuracy of predicting stages of bovine estrous cycle by gross appearance of the corpus luteum. *J. Dairy Sci.* **1980**, *63*, 155–160. [[CrossRef](#)] [[PubMed](#)]
28. Fields, M.J.; Fields, P.A. Morphological characteristics of the bovine corpus luteum during the estrous cycle and pregnancy. *Theriogenology* **1996**, *45*, 1295–1325. [[CrossRef](#)]
29. Kowalik, M.K.; Dobrzyn, K.; Rekawiecki, R.; Kotwica, J. Expression of membrane progesterin receptors (mPRs) α , β and γ in the bovine uterus during the oestrous cycle and pregnancy. *Theriogenology* **2019**, *140*, 171–179. [[CrossRef](#)] [[PubMed](#)]
30. Zhang, B.; Wang, Z.; Gao, K.; Fu, R.; Chen, H.; Lin, P.; Wang, A.; Jin, Y. MSX1 Regulates Goat Endometrial Function by Altering the Plasma Membrane Transformation of Endometrial Epithelium Cells during Early Pregnancy. *Int. J. Mol. Sci.* **2023**, *24*, 4121. [[CrossRef](#)]
31. Gao, Z.; Shao, D.; Zhao, C.; Liu, H.; Zhao, X.; Wei, Q.; Ma, B. The High Level of RANKL Improves I κ B/p65/Cyclin D1 Expression and Decreases p-Stat5 Expression in Firm Udder of Dairy Goats. *Int. J. Mol. Sci.* **2023**, *24*, 8841. [[CrossRef](#)] [[PubMed](#)]
32. Zhang, W.; Wang, S.; Yang, L.; Gao, L.; Ning, C.; Xu, M.; Deng, S.; Gan, S. Profile of miRNAs induced during sheep fat tail development and roles of four key miRNAs in proliferation and differentiation of sheep preadipocytes. *Front. Vet. Sci.* **2024**, *11*, 1491160. [[CrossRef](#)] [[PubMed](#)]
33. Li, P.; Mei, C.; Raza, S.H.A.; Cheng, G.; Ning, Y.; Zhang, L.; Zan, L. Arginine (315) is required for the PLIN2-CGI-58 interface and plays a functional role in regulating nascent LDs formation in bovine adipocytes. *Genomics* **2024**, *116*, 110817. [[CrossRef](#)]
34. Sun, M.; Song, P.; Zhao, Y.; Li, B.; Wang, P.; Cong, Z.; Hua, S. Mechanisms of LPS-induced epithelial mesenchymal transition in bEECs. *Theriogenology* **2024**, *216*, 30–41. [[CrossRef](#)] [[PubMed](#)]
35. Liu, J.; Qiu, R.; Liu, R.; Song, P.; Lin, P.; Chen, H.; Zhou, D.; Wang, A.; Jin, Y. YPEL3 Negatively Regulates Endometrial Function via the Wnt/ β -Catenin Pathways during Early Pregnancy in Goats. *Animals* **2022**, *12*, 2973. [[CrossRef](#)]
36. Maity, S.; Ambatipudi, K. Quantitative proteomics of milk whey reveals breed and season specific variation in protein abundance in Holstein Friesian cow and Murrah buffalo. *Res. Vet. Sci.* **2019**, *125*, 244–252. [[CrossRef](#)]
37. Zhou, M.; Barkema, H.W.; Gao, J.; Yang, J.; Wang, Y.; Kastelic, J.P.; Khan, S.; Liu, G.; Han, B. MicroRNA miR-223 modulates NLRP3 and Keap1, mitigating lipopolysaccharide-induced inflammation and oxidative stress in bovine mammary epithelial cells and murine mammary glands. *Vet. Res.* **2023**, *54*, 78. [[CrossRef](#)]

38. Yang, W.; Jin, M.; Wang, Y.; Zhao, H.; Wang, X.; Guo, Y.; Li, C.; Xiao, B.; Zhang, H.; Kiran, F.; et al. NR1D1 activation alleviates inflammatory response through inhibition of IL-6 expression in bovine endometrial epithelial cells. *Int. J. Biol. Macromol.* **2024**, *283*, 137642. [\[CrossRef\]](#)
39. Yuan, N.; Xiao, L.; Chen, J.; Liu, B.; Ren, S.; Sheng, X.; Qi, X.; Wang, Y.; Chen, C.; Guo, K.; et al. CREG1 promotes bovine placental trophoblast cells exosome release by targeting IGF2R and participates in regulating organoid differentiation via exosomes transport. *Int. J. Biol. Macromol.* **2024**, *274*, 133298. [\[CrossRef\]](#)
40. Giedt, M.S.; Thomalla, J.M.; White, R.P.; Johnson, M.R.; Lai, Z.W.; Tootle, T.L.; Welte, M.A. Adipose triglyceride lipase promotes prostaglandin-dependent actin remodeling by regulating substrate release from lipid droplets. *Development* **2023**, *150*, dev201516. [\[CrossRef\]](#)
41. Liang, Y.; Zhu, Z.; Lu, Y.; Ma, C.; Li, J.; Yu, K.; Wu, J.; Che, X.; Liu, X.; Huang, X.; et al. Cytoskeleton regulates lipid droplet fusion and lipid storage by controlling lipid droplet movement. *Biochim. Biophys. Acta Mol. Cell Biol. Lipids* **2025**, *1870*, 159610. [\[CrossRef\]](#) [\[PubMed\]](#)
42. Skjerven, O. Phosphatase, fat, and carbohydrate content of normal bovine endometrium; biopsy studies of cyclic variations. *Fertil. Steril.* **1956**, *7*, 31–43. [\[CrossRef\]](#)
43. Boshier, D.P.; Fairclough, R.J.; Holloway, H. Assessment of sheep blastocyst effects on neutral lipids in the uterine caruncular epithelium. *J. Reprod. Fertil.* **1987**, *79*, 569–573. [\[CrossRef\]](#)
44. Liang, J.; Li, K.; Chen, K.; Liang, J.; Qin, T.; He, J.; Shi, S.; Tan, Q.; Wang, Z. Regulation of ARHGAP19 in the endometrial epithelium: A possible role in the establishment of uterine receptivity. *Reprod. Biol. Endocrinol.* **2021**, *19*, 2. [\[CrossRef\]](#)
45. Owusu-Akyaw, A.; Krishnamoorthy, K.; Goldsmith, L.T.; Morelli, S.S. The role of mesenchymal-epithelial transition in endometrial function. *Hum. Reprod. Update* **2019**, *25*, 114–133. [\[CrossRef\]](#)
46. Fanning, A.S.; Jameson, B.J.; Jesaitis, L.A.; Anderson, J.M. The tight junction protein ZO-1 establishes a link between the transmembrane protein occludin and the actin cytoskeleton. *J. Biol. Chem.* **1998**, *273*, 29745–29753. [\[CrossRef\]](#)
47. Belardi, B.; Hamkins-Indik, T.; Harris, A.R.; Kim, J.; Xu, K.; Fletcher, D.A. A Weak Link with Actin Organizes Tight Junctions to Control Epithelial Permeability. *Dev. Cell* **2020**, *54*, 792–804.e7. [\[CrossRef\]](#) [\[PubMed\]](#)
48. Shapiro, L.; Weis, W.I. Structure and biochemistry of cadherins and catenins. *Cold Spring Harb. Perspect. Biol.* **2009**, *1*, a003053. [\[CrossRef\]](#) [\[PubMed\]](#)
49. Jalali, B.M.; Lukasik, K.; Witek, K.; Baclawska, A.; Skarzynski, D.J. Changes in the expression and distribution of junction and polarity proteins in the porcine endometrium during early pregnancy period. *Theriogenology* **2020**, *142*, 196–206. [\[CrossRef\]](#)
50. Zhang, B.; Song, C.; Zhou, B.; Zhang, J.; Dong, W.; Zhang, Y.; Zhao, X.; Zhang, Q. CTNNB1 and CDH1 Regulate Trophoblast Cell Adhesion and Junction Formation in Yak Placental Tissue at Different Gestational Stages. *Animals* **2025**, *15*, 876. [\[CrossRef\]](#)
51. Zhao, L.; Zheng, X.; Liu, J.; Zheng, R.; Yang, R.; Wang, Y.; Sun, L. PPAR signaling pathway in the first trimester placenta from in vitro fertilization and embryo transfer. *Biomed. Pharmacother.* **2019**, *118*, 109251. [\[CrossRef\]](#)
52. Berger, J.; Moller, D.E. The mechanisms of action of PPARs. *Annu. Rev. Med.* **2002**, *53*, 409–435. [\[CrossRef\]](#)
53. Li, Y.; Pan, Y.; Zhao, X.; Wu, S.; Li, F.; Wang, Y.; Liu, B.; Zhang, Y.; Gao, X.; Wang, Y.; et al. Peroxisome proliferator-activated receptors: A key link between lipid metabolism and cancer progression. *Clin. Nutr.* **2024**, *43*, 332–345. [\[CrossRef\]](#)
54. Zhang, W.; Raza, S.H.A.; Li, B.; Yang, W.; Khan, R.; Aloufi, B.H.; Zhang, G.; Zuo, F.; Zan, L. LncBNIP3 Inhibits Bovine Intramuscular Preadipocyte Differentiation via the PI3K-Akt and PPAR Signaling Pathways. *J. Agric. Food Chem.* **2024**, *72*, 24260–24271. [\[CrossRef\]](#)
55. Chen, Y.; Huang, B.; Liang, H.; Ji, H.; Wang, Z.; Song, X.; Zhu, H.; Song, S.; Yuan, W.; Wu, Q.; et al. Gestational organophosphate esters (OPEs) exposure in association with placental DNA methylation levels of peroxisome proliferator-activated receptors (PPARs) signaling pathway-related genes. *Sci. Total Environ.* **2024**, *947*, 174569. [\[CrossRef\]](#)
56. Wang, H.; Xie, H.; Sun, X.; Tranguch, S.; Zhang, H.; Jia, X.; Wang, D.; Das, S.K.; Desvergne, B.; Wahli, W.; et al. Stage-specific integration of maternal and embryonic peroxisome proliferator-activated receptor delta signaling is critical to pregnancy success. *J. Biol. Chem.* **2007**, *282*, 37770–37782. [\[CrossRef\]](#)
57. Shi, H.B.; Zhang, C.H.; Xu, Z.A.; Lou, G.G.; Liu, J.X.; Luo, J.; Loo, J.J. Peroxisome proliferator-activated receptor delta regulates lipid droplet formation and transport in goat mammary epithelial cells. *J. Dairy Sci.* **2018**, *101*, 2641–2649. [\[CrossRef\]](#) [\[PubMed\]](#)
58. Hansen, T.R.; Sinedino, L.D.P.; Spencer, T.E. Paracrine and endocrine actions of interferon tau (IFNT). *Reproduction* **2017**, *154*, F45–F59. [\[CrossRef\]](#) [\[PubMed\]](#)
59. Warren, W.; Osborn, M.; Yates, A.; O’Sullivan, S. The emerging role of fatty acid-binding protein 3 (FABP3) in cancer. *Drug Discov. Today* **2025**, *30*, 104504. [\[CrossRef\]](#) [\[PubMed\]](#)
60. Ramesh, V.; Brabletz, T.; Ceppi, P. Targeting EMT in Cancer with Repurposed Metabolic Inhibitors. *Trends Cancer* **2020**, *6*, 942–950. [\[CrossRef\]](#)
61. Wei, D.; Su, Y.; Leung, P.C.K.; Li, Y.; Chen, Z.J. Roles of bone morphogenetic proteins in endometrial remodeling during the human menstrual cycle and pregnancy. *Hum. Reprod. Update* **2024**, *30*, 215–237. [\[CrossRef\]](#)

62. Bauersachs, S.; Mitko, K.; Ulbrich, S.E.; Blum, H.; Wolf, E. Transcriptome studies of bovine endometrium reveal molecular profiles characteristic for specific stages of estrous cycle and early pregnancy. *Exp. Clin. Endocrinol. Diabetes* **2008**, *116*, 371–384. [[CrossRef](#)]
63. Quinn, K.E.; Matson, B.C.; Wetendorf, M.; Caron, K.M. Pinopodes: Recent advancements, current perspectives, and future directions. *Mol. Cell. Endocrinol.* **2020**, *501*, 110644. [[CrossRef](#)]
64. Kumro, F.G.; O’Neil, E.V.; Ciernia, L.A.; Moraes, J.G.N.; Spencer, T.E.; Lucy, M.C. Scanning electron microscopy of the surface epithelium of the bovine endometrium. *J. Dairy Sci.* **2020**, *103*, 12083–12090. [[CrossRef](#)] [[PubMed](#)]
65. Seo, H.; Frank, J.W.; Burghardt, R.C.; Bazer, F.W.; Johnson, G.A. Integrins and OPN localize to adhesion complexes during placentation in sheep. *Reproduction* **2020**, *160*, 521–532. [[CrossRef](#)] [[PubMed](#)]
66. Imakawa, K.; Bai, R.; Kusama, K. Integration of molecules to construct the processes of conceptus implantation to the maternal endometrium. *J. Anim. Sci.* **2018**, *96*, 3009–3021. [[CrossRef](#)] [[PubMed](#)]
67. Narumiya, S.; FitzGerald, G.A. Genetic and pharmacological analysis of prostanoid receptor function. *J. Clin. Investig.* **2001**, *108*, 25–30. [[CrossRef](#)]
68. Arosh, J.A.; Banu, S.K.; Chapdelaine, P.; Fortier, M.A. Temporal and tissue-specific expression of prostaglandin receptors EP2, EP3, EP4, FP, and cyclooxygenases 1 and 2 in uterus and fetal membranes during bovine pregnancy. *Endocrinology* **2004**, *145*, 407–417. [[CrossRef](#)]
69. Nishimura, T.; Zhao, X.; Gan, H.; Koyasu, S.; Remold, H.G. The prostaglandin E2 receptor EP4 is integral to a positive feedback loop for prostaglandin E2 production in human macrophages infected with Mycobacterium tuberculosis. *FASEB J.* **2013**, *27*, 3827–3836. [[CrossRef](#)]
70. Ohmura, T.; Tian, Y.; Sarich, N.; Ke, Y.; Meliton, A.; Shah, A.S.; Andreasson, K.; Birukov, K.G.; Birukova, A.A. Regulation of lung endothelial permeability and inflammatory responses by prostaglandin A2: Role of EP4 receptor. *Mol. Biol. Cell* **2017**, *28*, 1622–1635. [[CrossRef](#)]
71. Li, Q.; Zhang, S.; Mao, W.; Fu, C.; Shen, Y.; Wang, Y.; Liu, B.; Cao, J. 17 β -estradiol regulates prostaglandin E₂ and F_{2 α} synthesis and function in endometrial explants of cattle. *Anim. Reprod. Sci.* **2020**, *216*, 106466. [[CrossRef](#)] [[PubMed](#)]

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