# Prenatal polychlorinated biphenyl exposure promotes invasion of progeny ectopic endometrial stromal cells via epigenetic modification of EZH2

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# Abstract

Polychlorinated biphenyls(PCBs) are persistent environmental endocrine disruptor. This study aim to investigate the changes of the ectopic endometrium invasive ability and the possible mechanism after exposure to PCBs during pregnancy. In total, 12 female Sprague Dawley rats were intraperitoneally injected with Aroclor 1221 (1 mg/kg) or dimethyl sulfoxide (1 mg/kg) at 16 and 18 days of gestation. The endometriosis model was established by autogenous uterine abdominal wall implantation 2 months after birth. The degree of adhesion between the endometriosis and the greater omentum, as well as cell morphology and Transwell invasion patterns were used to evaluate the invasive ability of progeny ectopic endometrial stromal cells (ESCs). Moreover, the effect of the enhancer of zeste 2 polycomb repressive complex 2 subunit (EZH2)/ trimethylation of Histone 3 lysine 27 (H3K27me3) axis was examined using a highly selective EZH2 inhibitor, GSK126. After gestational PCBs exposure, the adhesion between the endometrium and the greater omentum was enhanced ($P<0.05$), and the length and number of microvilli were significantly increased ($P<0.01$). Exposure to PCBs during pregnancy increased the invasive ability of the ectopic ESCs ($P<0.01$), with upregulated expression levels of EZH2 and H3k27me3, which were abrogated by the EZH2 inhibitor GSK126. In conclusion, exposure to PCBs during pregnancy increased the invasive ability of the ectopic endometrium, which may be mediated by EZH2.


# Introduction

Endometriosis is a chronic benign gynecologic disease in which the growing endometrium appears outside the uterus, causing chronic pelvic pain, dysmenorrhea, infertility and dysmenorrhea (Altayyeb et al. 2020). There are various theories regarding the pathogenesis of endometriosis, such as retrograde menstruation, genetic factors, blood lymphatic spread, cell apoptosis, immune factors and environmental toxins (Lagana et al. 2017; Parazzini et al. 2013; Sofo et al. 2015). Although the classic ‘Retrograde menstruation’ theory is widely accepted, it does not account for all cases of endometriosis(Giudice and Kao 2004; McKinnon et al. 2018). With the development of industrialization, environmental problems are prominent, and the influence of environmental endocrine disruptors on the incidence of endometriosis has attracted increased attention (Gerkowicz et al. 2020; Martinez-Zamora et al. 2015; Matta et al. 2020; Wen et al. 2020). Accumulating evidence has suggested that exposure to certain environmental endocrine chemicals, including dioxins, bisphenol A, phthalates and polychlorinated biphenyls (PCBs), may increase the risk of endometriosis (Simonelli et al. 2017; Smarr et al. 2016). Furthermore, our previous study has confirmed that PCBs are associated with the migration and invasion of ectopic endometrial stromal cells through *in vitro* culture of ectopic endometrial stromal cells (ESCs) (Hu et al. 2018).

PCBs are synthetic organic compounds, which contain 209 homologs. Due to their physicochemistry stability, non-flammability and favorable electrical insulation properties, PCBs were widely used in capacitors, lubricants, adhesives, turbines and plasticizers (Andersen et al. 2020; Ravenscroft et al. 2018), Although PCBs have been banned globally since the 1970s, they are still released from industrial incinerators and landfills, or leaked from old capacitors and transformers (Adams et al. 2019; Ravenscroft et al. 2018). With characteristics such as the resistance to environmental degradation, dissolving in liquid

and bioaccumulation, PCBs can circulate in the environment via the food chain, water, soil and air (Klocke et al. 2019; Warenik-Bany et al. 2019). They also transfer from the mother to fetus via the placenta and lactation (Abraham et al. 1998). According to the hypothesis of the Developmental Origins of Health and diseases (DOHaD), gestation is considered to be a particularly sensitive time to external environment. Intrauterine exposure to an adverse environment will not only affect fetal development and lead to unfavorable pregnancy outcomes, but it will also have long-term impacts on disease susceptibility into late adulthood (Barker 1997; Godfrey and Barker 2001), including endometriosis (Wei et al. 2016).

Previous studies have reported that human diseases are associated with epigenetic regulation caused by early life exposure to PCBs (Casati et al. 2013; Caspersen et al. 2016; Gladen et al. 2000; Su et al. 2015; Tang-Peronard et al. 2011). Histone methylation is one of the important epigenetic mechanisms, and is mediated by interacting with steroid receptors or non-genomic signaling pathways (Casati et al. 2012; Casati et al. 2013; Casati et al. 2015). Histone methyltransferase enhancer of zeste homolog 2 (EZH2) is an enzyme-catalyzed subunit of polycomb repressive complex 2 (PRC2) that alters gene expression by trimethylation of Histone 3 lysine 27 (H3K27me3)(Jones et al. 2018), which inhibits chromatin structure remodeling by switch/sucrose non-fermentable (SWI/SNF), resulting in gene inactivation. EZH2 is associated with the invasion and progression of breast cancer, lymphoma, ovarian cancer and other malignant tumors, and is consider to be one of the new targeted therapeutic factors for malignant tumors (Booth et al. 2018; Chang et al. 2011; Italiano et al. 2018; McCabe et al. 2012). Although endometriosis is a benign disease, it has biological characteristics of invasion (Zhang et al. 2017). The invasion indices of peritoneal endometriosis cells is similar to that of metastatic bladder cancer cells, while normal endometrial cells are non-invasive (Gaetje et al. 1995).

Based on previous research, we suppose that gestational exposure to PCBs increases the invasive ability of the ectopic endometrium, thus leading to the occurrence and development of endometriosis via the EZH2/ H3K27me3 pathway.

# Material And Methods

*Animal model*. The present study was performed in the Experimental Research Laboratory of Zhejiang Chinese Medical University, and complied with the approval of the ethic committee (approval no.

2018102904). In total, 12 non-pregnant female and 12 male Sprague Dawley (SD) rats (age, 10–12 weeks; weight, 180–220 g) were purchased from SLAC Laboratory Animal Co., Ltd. Rats were housed in a single cage with an environmentally controlled temperature (10–24˚C) and humidity (50–70%) under a 12/12 h (light/dark) schedule. They were feed with standard pellet food and water. The experiment began ≥ 1 week after the rats arrived to acclimatize them to the new housing conditions.

The female rats were randomly assigned into either a PCB group (n = 6) or a control group (n = 6). Any female rat was housed with a random male rat. The successful mating (indicated by morning sperm positive vaginal smear) was termed embryonic day (E)0. On E16 and E18, the rats in PCB group and control group were intraperitoneal injected with 1 mg/kg Aroclor1221 (cat. no. C221N; AccuStandard; New

Haven, CT) or dimethyl sulfoxide (DMSO, cat. no. D4540; Sigma-Aldrich; Merck KGaA), respectively, as previously reported (Bell et al. 2016; Reilly et al. 2015; Steinberg et al. 2007; Steinberg et al. 2008; Walker et al. 2013).

Male pups were removed after delivery. The endometriosis model was performed 2 months postpartum as described by Saltan *et al* (Saltan et al. 2016). The rats were fixed in the supine position after general anesthesia with intraperitoneally injected 3% chloral hydrate (350 mg/kg). A 3-4-cm longitudinal incision was made in the middle of the lower abdomen. The left side of uterine cornu was excised and cut into 5x5 mm pieces. The endometrium layer was stripped from myometrium and autotransplanted at the abdominal walls with abundant blood supply (Fig. 1). In total, two pieces of endometrium were transplanted on each side. The abdominal cavity was closed with absorbable suture. A single intramuscular injection of 80,000 U penicillin was administered to prevent infection.

The rats were anesthetized, as previously mentioned, 1 month after the first operation and were surgically examined to visualize the adhesion score of endometriotic foci. The adhesion scored was blindly evaluated by two doctors as previously reported by Belluco *et al* (Belluco et al. 2001), which involved the extent of site involvement (0–4), type of adhesion (0–4) and the tenacity of adhesion (0–3). The endometriotic foci were cultured or immobilized by 10% formalin for further experiment. Then, rats were euthanized by carbon dioxide inhalation.

*Cell culture and treatment*. The ectopic endometrium was cut into pieces and then digested with 0.2% type I collagenase (Sigma-Aldrich; Merck KGaA) for 1 h on a horizontal concussion meter at 37˚C and 180 rpm. After centrifugation at 1,000 rpm for 5 min, 3–5 ml PBS was added and the cell suspension was filtered with 100 and 40 μm strainers. Cells were centrifuged at 1,000 rpm for 5 min at 37˚C, then an appropriate amount of DMEM/F12 medium was added and incubated in 5% CO2 at 37˚C in an incubator (Forma 3111; Thermo Fisher Scientific, Inc.). The culture medium was replaced at 24 h after primary culture and then every 2–3 days.

In PCB group, GSK126 (cat. no.5005800001, Sigma-Aldrich; Merck KGaA), a highly selective inhibitor of EZH2 via competitive combination with S-adenosine methionine (McCabe et al. 2012), was added to evaluate the role of EZH2. ESCs were inoculated into a 6-well plate with a density of $3.0x10^5$ per well, and GSK126 was added at the doses of 0, 2.5, 5, 10 and 20 μM for 48 h, where 5μM was the optimal inhibitory amount and was used in subsequent experiments. GSK126 was not added to the control group. The role of EZH2 in ESCs invasion was further determined by observing microvilli under transmission electron microscopy and transwell invasion assay.

*Immunofluorescence.* The purity of ESCs was determined via immunofluorescence, using Cytokeratin 7(cat. no. ab181598, Abcam) and Vimentin(cat. no. ab92547, Abcam) as markers of epithelial and stromal cells respectively. The primary cells were fixed with 4% paraformaldehyde at room temperature for 30 min, followed by incubation with 0.5% tritox-100 at room temperature for 15 min. Cells were then washed three times with PBS and sealed with 5% BSA(Amresco, USA) solution at 37˚C for 1 h.

Subsequently, anti-vimentin antibody or Cytokeratin 7 were added, incubated at 4˚C overnight and washed three times with PBS. FITC-labeled IgG(Amresco, USA) was added at room temperature for 1 h and cells were washed three times with PBS. Finally, 2 mg/l DAPI solution was added to re-stain the nucleus at room temperature for 10 min. Cells were washed twice with PBS and sealed with neutral resin, and the fluorescence signals of the cells were observed under a fluorescence microscope (Axiovert200; Zeiss AG).

*Transmission electron microscopy*. Transmission electron microscopy was performed as described previously (Chen et al. 2019). ESCs cells were fixed overnight with 2.5% glutaraldehyde at 4˚C and then fixed with 1% osmium acid for 1 h at room temperature. Cells were rinsed with water 2–3 times for 10 min. Then, 50, 70, 90 and 100% ethanol for 15 min and 100% acetone for 15–20 min were used for dehydration at room temperature. Cells were embedded at room temperature for 4 h and ultrathin sections at 70-nm were sliced. The sections were placed in copper grids and stained with uranyl acetate and lead citrate. A transmission electron microscope (FEI Tecnai T10, USA) was used to observe the number and length of microvilli. In total, five fields were randomly selected from each section. The length of microvilli was measured using Image J software (V1.51, National Institute of Health). The number of microvilli was counted as number/circumference (n/μm).

*Immunohistochemistry*. All tissues were fixed with 10% neutral formalin, paraffin-embedded and prepared into slices of 4-μm thickness. The process and scoring of immunohistochemistry were based on a previous report (Kumari et al. 2018). According to the antibody instructions, each slice was incubated with 50–100 μM primary antibody and the tissue solution (EZH2, 1:8,000, cat. no. ab191080; H3K27me3, 1:40, cat. no. ab6002; Abcam) overnight at 4˚C. Then, 50–100 μl Envision +/horseradish peroxidase (HRP) secondary antibody (DAKO; Agilent Technologies, Inc.) was added to each slice and incubated at room temperature for 30–60 min. The immunoreactivity was detected using diaminobenzidine. The slices were subsequently stained with hematoxylin, and observed under a light microscope (DM500; Leica Microsystems GmbH), and images were captured at a magnification of x40 or x200. The staining intensity of ESCs was scored as follows: 1 for absent or weak; 2 for moderate; and 3 for strong. The percentage positive cells was scored as: Percent of cells < 1, 0 point; percent of cells between 1–10, 1 point; percent of cells between 11–33, 2 points; percent of cells between 34–66, 3 points; and percent of cells between 67–100, 4 points. The Q score of all the slices was calculated by multiplying the staining intensity by the percentage positive of ECSs cells (Kumari et al. 2018).

*Western blotting*. Western blotting was performed as described previously (Chen et al. 2019). ESCs were washed in PBS prior to being lysed in RIPA lysis buffer (Beyotime Institute of Biotechnology). The proteins were electrophoresed via 12% SDS-PAGE and transferred to PVDF membranes(Millipore, Billerica, MA, USA). Membranes were blocked in 3% bovine serum albumin (BSA, Sigma-Aldrich; Merck KGaA) at 37˚C for 2 h and then incubated with primary antibodies (EZH2, 1:100; H3K27me3, 1:80) overnight at 4˚C. Then, membranes were incubated with horseradish peroxidase-conjugated secondary antibody (1:10,000, cat.no. ab236555, Abcam) for 1.5 h at room temperature. Enhanced chemiluminescence detection reagent (Sigma-Aldrich, St Louis, MO, USA) was used to detect the signal. Relative protein expression

levels were semi-quantified with β-actin expression as the reference gene using ImageJ software (V1.51, National Institute of Health).

*Reverse transcription-quantitative PCR (RT-qPCR)*. EZH2 gene expression was analyzed using RT-qPCR with the following sequences (Sangon Biotech Co., Ltd.) as previously reported (Chen et al. 2019): EZH2 forward, 5'-CTTACTGCTGGCACCGTCTG-3' and reverse, 5'-CTTTCCCTCTTCTGTCTGCTTCA-3'; and GAPDH forward, 5'- CTGGAGAAACCTGCCAAGTATG-3' and reverse, 5'- GGTGGAAGAATGGGAGTTGCT-3'. Total mRNA was extracted from ESCs using RNAiso Plus reagent TaKaRa RNAiso Plus (Takara Bio, Inc.) according to the manufacturer's instructions. Total RNA was reverse transcribed to cDNA using the PrimeScript™ RT reagent (Takara Bio, Inc.). qPCR was performed using a SYBR Premix Ex Taq™ □ kit (Takara Bio, Inc.) according to the manufacturer's instructions. The following thermocycling conditions were used: Amplification of 48 cycles of 95˚C for 10 sec, 60˚C for 30 sec. The cyclic threshold (Cq) value was calculated using the average of three wells for each sample. The relative expression levels of target genes were normalized against actin using the $2^{-\Delta\Delta Cq}$ method(Livak and Schmittgen 2001).

*Cell migration and invasion assays*. The migration and invasion of ESCs were evaluated as previously reported (Hu et al. 2018). Transwell chambers were 6.5-mm in diameter with an 8-μm pore size (Corning, Inc.). A density of $2x10^5$ cells with 200 μl serum-free DMEM/F12 was seeded on the upper chamber. The lower chamber was filled with 500 μl DMEM/F12 containing 10% FBS. After incubation for 24 h, the cells on the underside of the membrane were fixed and then stained with crystal violet solution. For the invasion assay, the upper chamber was pre-coated with 25 μl Matrigel (BD Biosciences), and incubated for 48 h. A total of five fields were randomly selected to count the number of cells under a light microscope (x100).

*Statistical analysis.* Statistical analyses were performed using SPSS 23.0 software (IBM Corp.). Data are presented as the mean ± SD. Student's t-test was used to analyze differences for paired data and one-way ANOVA was used to analyze multiple comparisons. $P < 0.05$ was considered to indicate a statistically significant difference.

# Results

*Identification of endometriosis and ESCs*. The PCB group had 23 female offsprings, while the control group had 25($P > 0.05$). One month after the endometrium transplantation, the endometriotic implants formed a cystic nodule with vascularization. Hematoxylin and eosin staining showed that the ectopic lesions formed one or several cysts, and the cyst wall was composed of epithelial cell layer, stromal cell layer and a small amount of fibrous connective tissue from the inside to the outside. After 2–4 generations of purification, the purity of ESCs was > 95%. It had a long spindle shape with an oval nucleus, and immunofluorescence detection identified that Vimentin was positive and Cytokeratin7 was negative (Fig. 2).The adhesion score of the control group was 4.6 ± 1.3. After PCB exposure during pregnancy, the adhesion score of ectopic endometrial lesions in the offspring was significantly increased to 7.1 ± 0.9 ($P < 0.05$).

*PCB promotes the invasion of offspring ectopic ESCs and GSK126 abrogates the effect*. There was no difference in the migratory ability of the ESCs from rat offspring. However, the invasive potential was significantly increased in PCB group compared with the control group. To investigate the effect of EZH2 on cell invasion, ESCs were treated with GSK126 for 48 h. In addition, after treatment with GSK126, it was proved that the down-regulation of EZH2 significantly reduced the invasion ability of ESCs(Fig. 3).

*PCB exposure increases the length and number of microvilli via EZH2*. Transmission electron microscope was used to assess the microvilli on the surface of ESCs. The microvilli were mainly slender cylindrical and interwoven with the surrounding microvilli, and their bases were connected to the cell membrane. The number of microvilli in the PCB exposed group was 1.07 ± 0.21 per perimeter(μm), and that in the control group was 0.67 ± 0.26 per perimeter(μm) ($P < 0.01$). Moreover, the microvilli length was 1.14 ± 0.58 μm in PCB group and 0.82 ± 0.32 μm in control group, which demonstrated a significant difference ($P < 0.01$).

After GSK126 treatment, the microvilli were smooth and the length was shortened. The number of microvilli was 0.43 ± 0.21 per perimeter(μm) and the length of microvilli was 0.67 ± 0.37 μm, which were significantly reduced compared with those of the PCB group (Fig. 4).

*The expression of EZH2 and H3K27me3 increased in offspring ectopic ESCs*. After exposure to PCBs during pregnancy, the expression levels of EZH2 and H3k27me3 in the ectopic ESCs of the offspring were significantly increased ($P < 0.01$). In the cell culture of the PCB group, after adding the inhibitor GSK126, Western blotting detected that the expression levels of EZH2 and its target molecule H3K27me3 were significantly decreased(Fig. 5).

# Discussion

Endometriosis is a common refractory gynecological disease. Although it is estrogen-dependent benign disease, its biological behavior is similar to that of malignant tumors (Zhang et al. 2017). The migration, adhesion and invasion of retrograde endometrial cells are important steps in the formation of ectopic lesions (Gaetje et al. 1995; Meng et al. 2019; Witz et al. 2001). Previous studies have reported that PCBs increase the invasion of ESCs, as well as the incidence of endometriosis (Hu et al. 2018; Ploteau et al. 2016; Ploteau et al. 2017; Roy et al. 2015). However, little is known regarding the relationship between gestational PCBs in offspring endometriosis, and the role of histone methylation is not fully understood. In the present study, it was found that prenatal exposure to PCBs significantly increase the invasive ability of offspring ectopic ESCs with high expression levels of EZH2 and H3K27me3, which serve an important role in promoting this proses.

An increase in the number and length of microvilli was the main morphological change observe in the present study. The relationship between cell morphology and cell invasiveness has been widely studied in tumors (Antonescu and Baren 2004; Aznavoorian et al. 1993). The ultrastructure of the cell surface can predict the contact, movement and adhesion of cells to other cells and the cell matrix, which serves an important role in the occurrence and development of tumors (Black 1980; Nicolson and Poste 1976). A decrease of tumor cell invasiveness is often accompanied by the loss of microvilli (Priester et al. 2013),

while more microvilli and pseudopodia on the cell surface indicates a higher invasive ability of tumor cells (Yan et al. 2013). In patients with endometriosis, the morphology of endometrial gland epithelial cells (EGECs) is irregular, the number and length of microvilli is increased, the organelles are abundant and EGECs possess proliferative, migratory and invasive abilities, which are associated with the formation of endometriosis cysts (Yu et al. 2018).

It has been reported that Aroclor1221 is a mildly chlorinated PCBs mixture, and its level is difficult to determine directly in the offspring after gestational exposure (Thomas et al. 1998). According to previous literature reports (Bell et al. 2016; Reilly et al. 2015; Steinberg et al. 2007; Steinberg et al. 2008; Walker et al. 2013), the dose of 1 mg/kg intraperitoneal injection of Aroclor1221 at E16 and E18 is close to the observed exposure of humans and wild animals in PCBs contaminated areas. PCBs may inhibit the activity of human estrogen sulfotransferase, an estrogen-degrading enzyme (Kester et al. 2000), thereby increasing the secretion of estrogen and the risk of hormone-dependent diseases. Accumulating evidence had suggested that higher PCBs exposure may increase the risk of endometriosis (Hu et al. 2018; Martinez-Zamora et al. 2015; Porpora et al. 2006; Wei et al. 2016). A reduced responsiveness to progesterone and overexpression of matrix metalloproteinases may be a key element of endometrial dysfunction associated with endometriosis (Osteen et al. 2005). With similar effects to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), dioxin-like-PCBs can combine with aryl hydrocarbon receptor(AhR) and form an activated heterodimerization complex with the aromatic hydrocarbon receptor nuclear translocator protein, leading to the overexpression of AhR in endometrioid cells (Okino and Whitlock 2000; Sherr and Monti 2013). Over activated AhR induced by PCBs regulates multiple downstream biological events, including the epigenetic silencer activity of histone methyltransferase EZH2 (Lee et al. 2020).

Epigenetics refers to the heritable non-gene sequence changes, including DNA methylation, post-translational histone modification and microRNA. However, these are dynamic and may be affected by environmental exposures (Holland 2017). Pregnancy is a critical period of fetal epigenetic remodeling, which is sensitive to environmental endocrine disruptors (Casati et al. 2015; Pozharny et al. 2010). Maternal health can determine susceptibility to certain diseases in adulthood (Kanherkar et al. 2014). Moreover, gestational PCBs exposure not only induces an aberrant DNA methylation pattern, but also affect the histone modification balance, leading to long term and transgenerational effects (Casati et al. 2015).

Histone methylation has been shown to serve an important role in the etiology of endometriosis, and EZH2 and H3K27me3 are epigenetic marks of endometriosis (Colon-Caraballo et al. 2015). Zhang *et al* (Zhang et al. 2017) revealed that EZH2 was associated with the invasion of the ectopic endometrium, and that the levels of EZH2 and H3K27me3 in the ectopic endometrium were significantly higher compared with those in normal endometrium. EZH2 and H3K27me3 directly induce epithelial-mesenchymal transition, thereby promoting the occurrence and development of endometriosis (Cao et al. 2008; Colon-Caraballo et al. 2015; Zhang et al. 2017). The present study also identified similar findings; after PCBs exposure during pregnancy, the expression levels of EZH2 and H3K27me3 in ectopic ESCs were significantly increased and the cell invasive ability was enhanced. However, after inhibition of EZH2

using GSK126, the expression levels of EZH2 and H3K27me3 were decreased, accompanied by a decreased cell invasive ability, suggesting that EZH2 serves an important role in ectopic endometrial invasion caused by PCBs exposure during pregnancy.

In conclusion, PCBs exposure during pregnancy serves a significant role in the development and progression of endometriosis in offspring. As an early stage of life development, women should consider the effects of environmental pollution during pregnancy with regards to potential harm to next generations. EZH2-mediated epigenetic regulation may serve an important role in this process. However, further research is required to identify the underlying molecular mechanisms activated by EZH2 and H3K27me3.

# Declarations

### Ethics approval and consent to participate

This study was approved by the Ethics Committee of Zhejiang Chinese Medical University (approval no. 2018102904).

### Patient consent for publication

Not applicable.

### Availability of data and materials

All data generated or analyzed during this study are included in this published article.

### Competing interests

The authors declare that they have no competing interests.


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### Authors' contributions

WYF performed the experiments□HCC wrote the manuscript, YCS performed the experiments, CP and CCLanalyzed the data. WRJ designed the study. All authors read and approved the final manuscript.


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# Figures

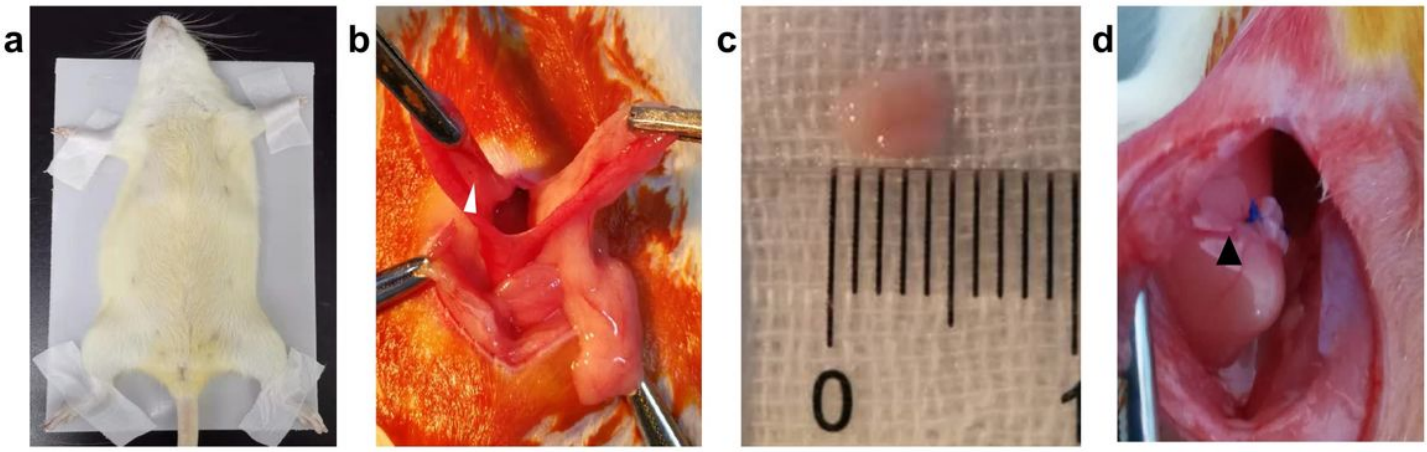
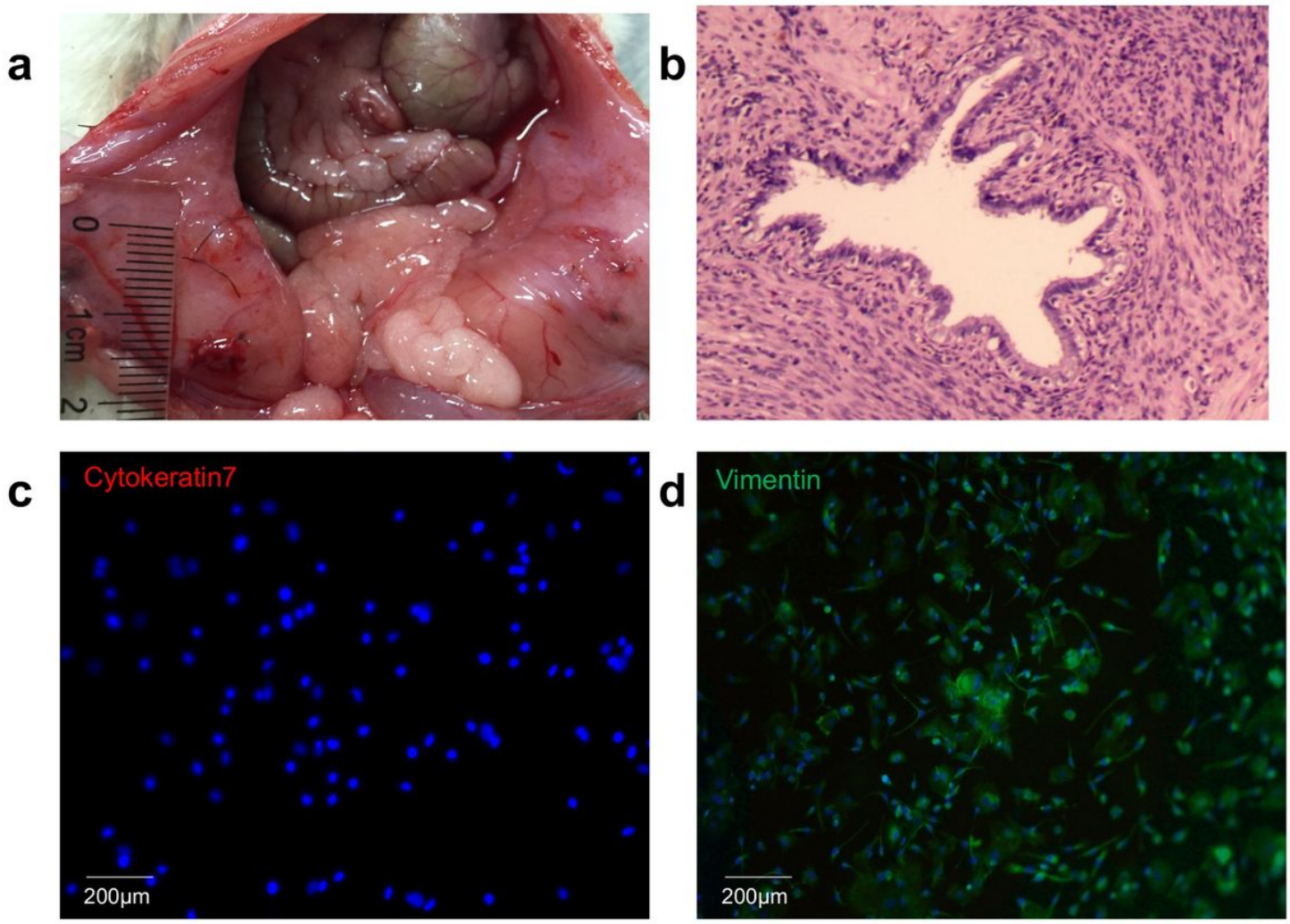


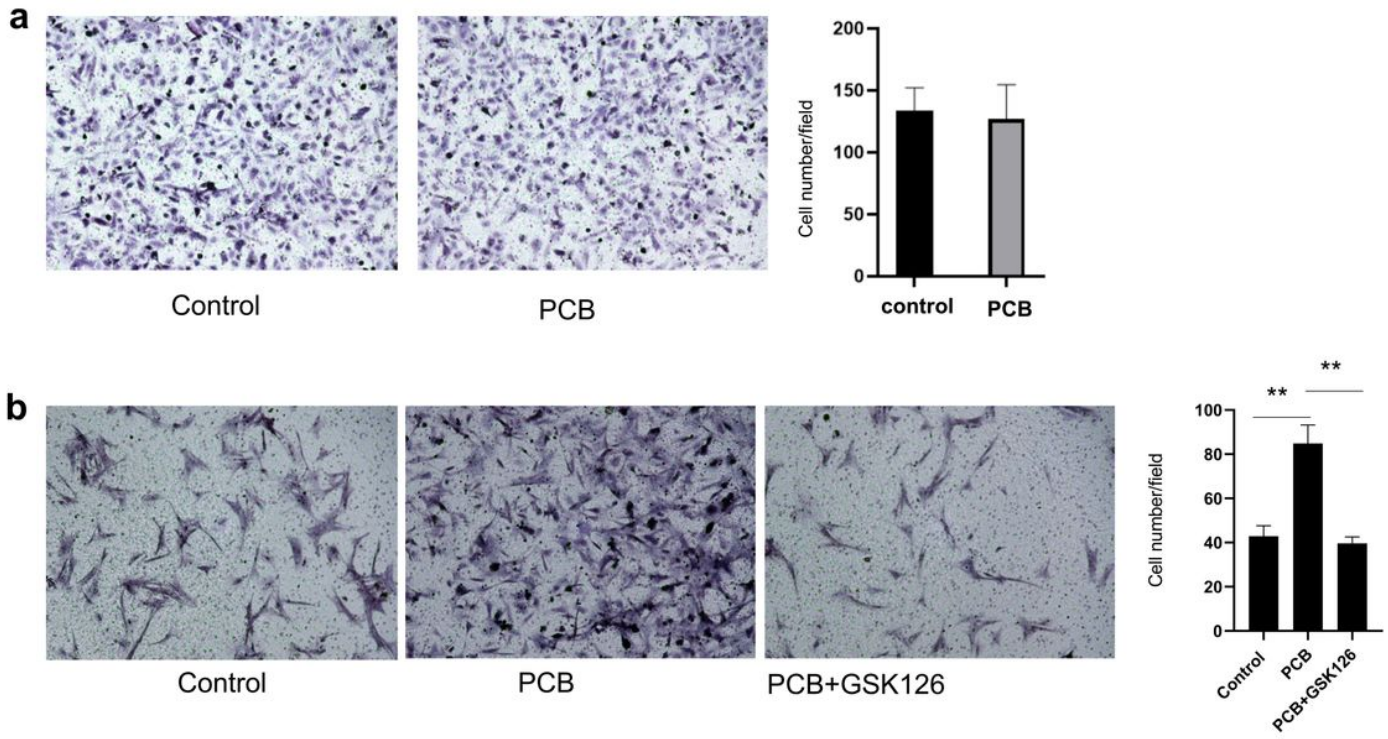

**Figure 1**

Rat model construction. (a) Adult female Sprague Dawley rats were fixed in supine position after anesthesia. (b) The left side of the uterine body was removed (white arrow), (c) cut into a size of ~5x5 mm and stripped of the myometrium. (d) Autologous transplantation was performed in the area with abundant blood vessels in the abdominal wall (black arrow) to establish the endometriosis model.



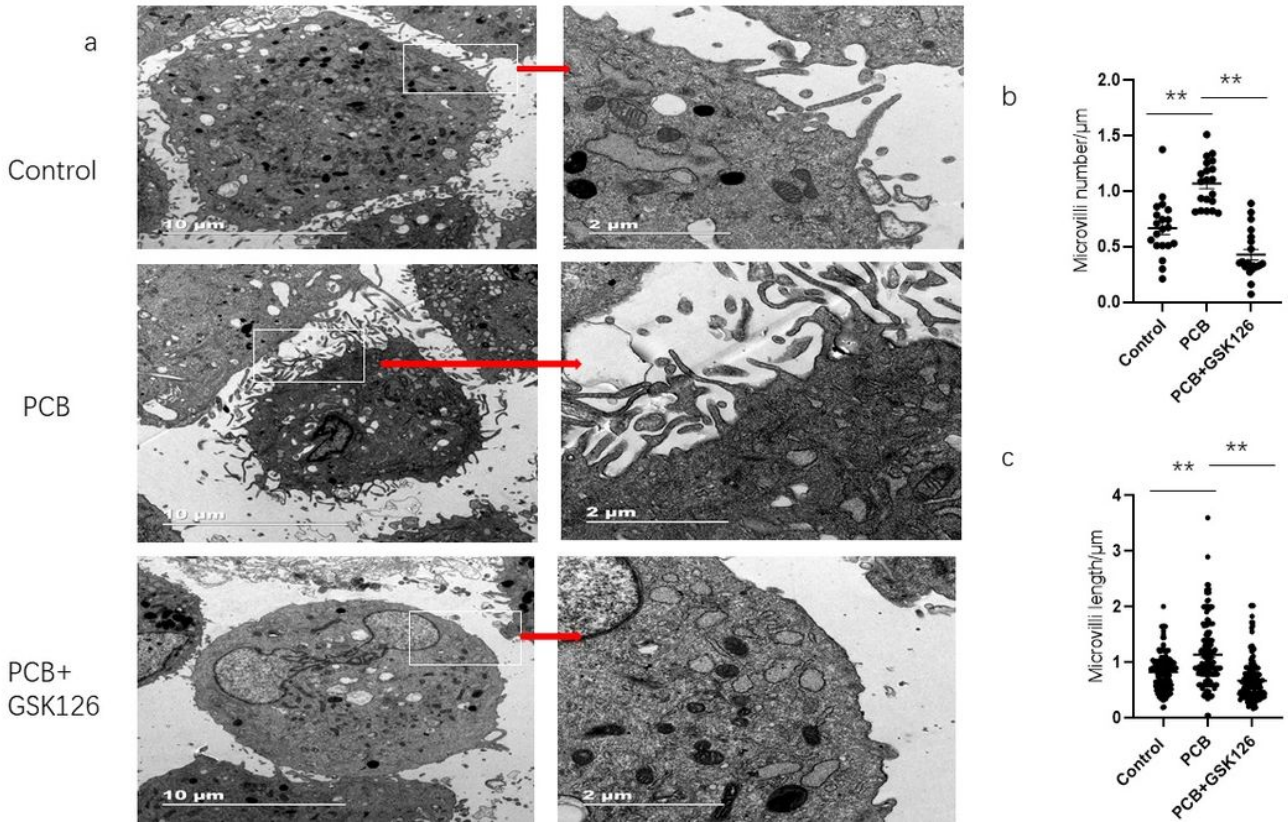

## Figure 2

Identification of ESCs. (a) General observation of ectopic endometrial lesions. (b) Hematoxylin and eosin stain of ectopic lesion (magnification, x200). (c) ESCs were negative for Cytokeratin7. (d) ESCs were positive for Vimentin. Magnification, x100; Scale bar, 200 μm. ESCs, endometrial stromal cells.



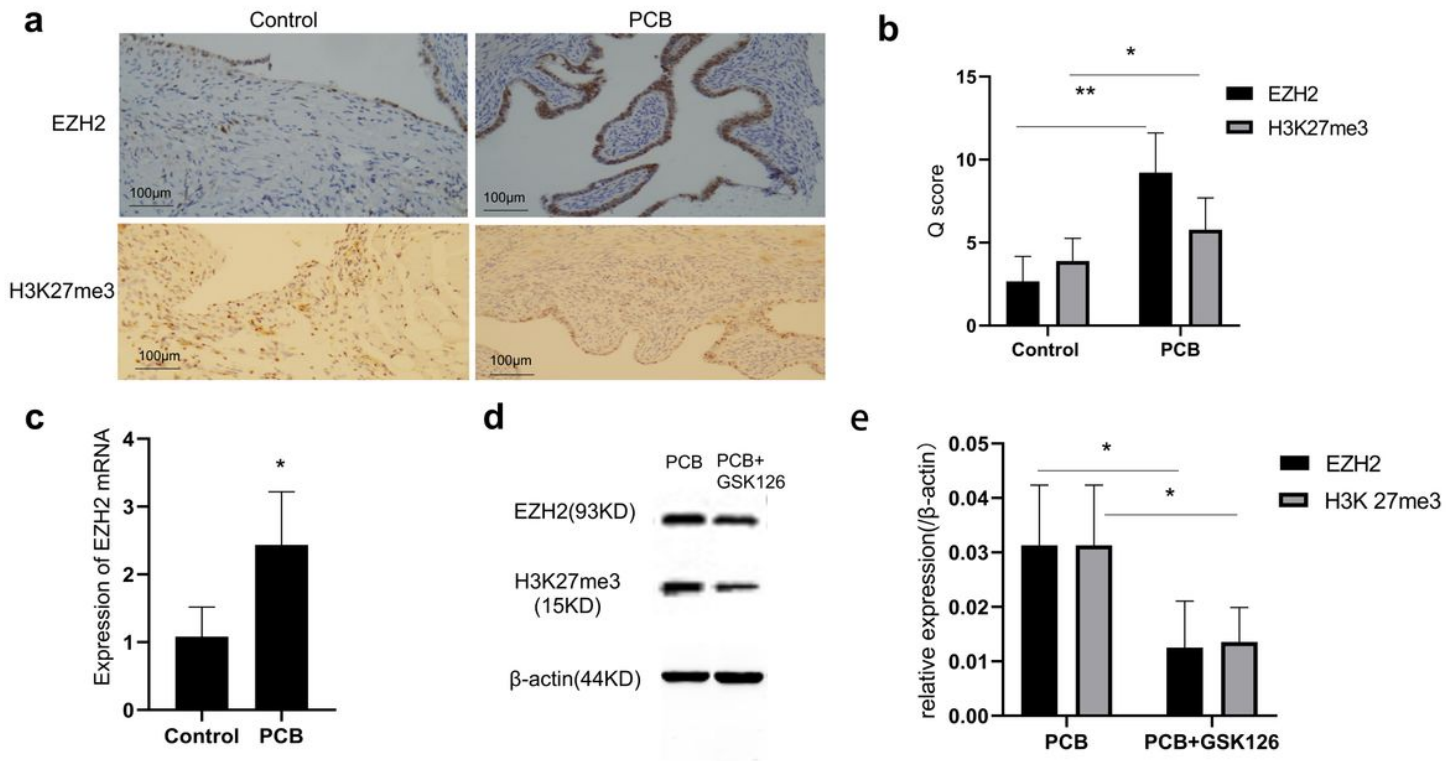

## Figure 3

Effects of gestational PCBs exposure on ectopic ESC migration and invasion. (a) No difference was found in the migratory ability of the ESCs of the offspring. (b) Prenatal PCBs exposure promoted the invasion of offspring ectopic ESCs. Furthermore, GSK126, an inhibitor of enhancer of zeste 2 polycomb repressive complex 2 subunit, significantly decreased the invasive ability induced by PCBs. Magnification, x100. **P<0.01. ESCs, endometrial stromal cells; PCBs, polychlorinated biphenyls.




## Figure 4

Ultrastructure of cell surface under transmission electron microscope. (a) Cell surface of ectopic ESCs from control group, PCB group and ectopic ESC in PCB group after EZH2 was inhibited by GSK126. (b) Analysis of microvilli number. (c) Analysis of microvilli length. a(left): Magnification, x4,800; a(right): Magnification, x18,500. **P<0.01. ESCs, endometrial stromal cells; PCBs, polychlorinated biphenyls;EZH2, enhancer of zeste 2 polycomb repressive complex 2 subunit.




## Figure 5

Expression levels of EZH2 and H3k27me3 in offspring ectopic ESCs. (a) Immunohistochemistry (magnification, x200; scale bar, 100 μm ) in tissue sections shows EZH2 and H3k27me3 expression levels were increased after gestational PCBs exposure. (b) Graph representing the Q score for EZH2 and H3H27me3 expression levels in offspring ectopic ESCs. (c) Graph depicting the mRNA expression of EZH2 in offspring ectopic ESCs. (d) Western blotting results demonstrated that (e) EZH2 and H3k27me3 expression levels were abrogated by GSK126. *P<0.05, **P<0.01. ESCs, endometrial stromal cells; PCBs, polychlorinated biphenyls; EZH2, enhancer of zeste 2 polycomb repressive complex 2 subunit; H3k27me3, trimethylation of Histone 3 lysine 27.