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Monoamine oxidases activity maintains endometrial monoamine homeostasis and participates in embryo implantation and development

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Abstract

Background Monoamine oxidases (MAOs) is an enzyme that catalyzes the deamination of monoamines. The current research on this enzyme is focused on its role in neuropsychiatric, neurodevelopmental, and neurodegenerative diseases. Indeed, MAOs with two isoforms, namely, A and B, are located on the outer mitochondrial membrane and are widely distributed in the central nervous system and peripheral tissues. Several reports have described periodic changes in the levels of this enzyme in the human endometrial tissue.

Results The novel role of MAOs in endometrial receptivity establishment and embryonic development by maintaining monoamine homeostasis was investigated in this study. MAOs activity was observed to be enhanced during the first trimester in both humans and mice under normal conditions. However, under pathological conditions, MAOs activity was reduced and was linked to early pregnancy failure. During the secretory phase, the endometrial stromal cells differentiated into decidual cells with a stronger metabolism of monoamines by MAOs. Excessive monoamine levels cause monoamine imbalance in decidual cells, which results in the activation of the AKT signal, decreased FOXO1 expression, and decidual dysfunction.

Conclusions The findings suggest that endometrial receptivity depends on the maintenance of monoamine homeostasis via MAOs activity and that this enzyme participates in embryo implantation and development.

Keywords Endometrial receptivity, Decidual cell, Embryo implantation, Recurrent implantation failure, Early pregnancy failure

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Background

A proper crosstalk between the blastocyst and the receptive endometrium supports the establishment of a successful pregnancy. Major advancements in effective embryo selection have led to a decreased incidence of chromosomal abnormalities in cases of pregnancy loss [1, 2], which emphasizes the significance of the uterine environment. Particularly, decidualization, a process that involves the rapid proliferation and differentiation of human endometrial stromal cells (HESCs), the secretory transformation of uterine glands, and vascular remodeling [3, 4], is necessary to maintain early pregnancy. This complex network encompasses transcription factors, endocrine factors, cytokines, and their respective signaling pathways and is believed to regulate endometrial remodeling [5]. However, the exact mechanism of implantation failure and endometrial receptivity needs to be investigated further.

Maternal anxiety in early pregnancy is one of the critical factors that affect the success of pregnancy. Monoamine oxidases (MAOs) is an iron-containing enzyme that exists as two isozymes, A and B (MAOA and MAOB, respectively), which regulate motion and adaptive stress by metabolizing monoamines. Several investigations have reported that abnormal monoamine metabolism contributes to the common pathogenesis of early pregnancy loss in women experiencing psychological anxiety [6, 7]. Stress-derived monoamines, such as norepinephrine (NE) and epinephrine (Epi), inhibit decidualization by disrupting adrenergic receptor signals [8–10]. Both in vitro and in vivo studies have shown that transient adrenoceptor activation abolishes normal preimplantation uterine contractility and adversely affects on-site intrauterine embryo location via the downregulation of LPA3 [11, 12]. The activity of MAOs, which are central regulatory molecules in monoamine neurotransmitters, changes periodically throughout the menstrual cycle [13]. Increased expression of MAOA has been reported in the endometrium during the receptive phase of spontaneous menstrual cycles in fertile women [14]. Attenuated MAOA stimulates the inappropriate proliferation of endometrial epithelial cells via the downregulation of FOXO1 during the window of implantation (WOI) [15]. Furthermore, MAOB interacts with progesterone during decidualization and aggregates predominantly with proteins involved in biosynthesis, intracellular transport, and mitochondrial activity [16].

This study identified that MAOs are key molecules associated with endometrial receptivity. Patients with repeated implantation failure (RIF) and early pregnancy failure (EPF) exhibited complications related to the decline of MAOs levels in the endometrium. In addition, MAOs levels were diminished in stress-induced mouse endometrium. Moreover, attenuation of MAOs decreased the decidualization of HESCs via the downregulation of FOXO1 by activating AKT signaling and impaired the establishment of endometrial receptivity, which is one of the reasons for RIF. Collectively, our data established the vital role of MAOs in maintaining the endometrial homeostasis of monoamines, which enhance endometrial receptivity.

Results

Endometrial MAOs activity in patients with RIF and EPF

Previous studies have shown that mental stress during early pregnancy could alter the functional status of endometrial receptivity. In patients with RIF, elevated serum adrenaline disrupts endometrial adrenergic receptor signaling, thereby affecting endometrial function. NE and Epi belong to monoamines, which play key roles in decidualization, implantation, immune modulation, and inflammation [6]. Non-neuronal monoamine transporters (NET, VMAT1, VMAT2, EMT, and PMAT) participate in the clearance of monoamines from extracellular compartments [17, 18]. In addition, PMAT, EMT, and OCT2 transporters are expressed in the endometrial stroma and can potentially regulate the reuptake of monoamines in general and histamine in particular [17]. *TH*, *COMT*, *MAOA*, *MAOB*, and *PNMT* are involved in the synthesis and metabolism of monoamines. Based on this observation, the results of previous RNA sequencing for observing the transcription levels of synthetase, a metabolic enzyme, and transporter of monoamines in the endometrium with RIF, were reanalyzed (Fig. 1A). The findings indicated that MAOs expression was reduced in patients with RIF (Fig. 1A). MAOs are important molecules that regulate neurotransmitter metabolism in the nervous system. Subsequently, the levels of MAOs proteins were tested in EPF tissues and the receptive endometrium of RIF. The results signified that the protein levels of both MAOA and MAOB were decreased (Fig. 1B–E). Interestingly, these tissues exhibited correspondingly increased levels of Epi and NE monoamines (Fig. 1F–I). Therefore, we hypothesize that endometrial MAOs activity balances monoamine levels involved in endometrial function and early pregnancy.

Dynamic expression pattern of MAOs in the peri-implantation uterus of mice

To investigate the effect of MAOs activity on different stages of early pregnancy in mice, the expression patterns of MAOs were examined in the uterus of mice on days 1, 4, 5, 6, and 8 of pregnancy (Fig. 2A–C). Furthermore, dynamic protein levels of MAOs in utero during implantation of mice were also detected. The results showed that the protein levels of MAOs in mouse decidual cells

increased during implantation (Fig. 2D and Additional file 1: Fig. S1). Our previous studies had shown that Epi levels were increased in the uterus of a stress-induced mouse model [10]. Similarly, MAOs expression was downregulated in the uterine endometrium of mouse models with elevated endogenous and exogenous Epi levels (Fig. 2D–F). To exclude the influence of embryonic development on decidua, a mouse model of artificially induced decidualization was designed (Fig. 2G). At 72 h of oil stimulation, impaired decidualization following Epi exposure was observed (Additional file 1: Fig. S2). In pseudopregnant mice, MAOs downregulation in the mouse endometrium triggered by elevated monoamine levels was not associated with embryonic development (Fig. 2G–H). These results signified that the expressions of both *MAOA* and *MAOB* were significantly increased in the glandular and luminal epithelial cells and stromal cells in the receptive endometrium. In addition, the transcriptional activities of MAOA and MAOB were enhanced with the process of decidualization of endometrial stromal cells. Hence, MAOs might be involved in uterine receptivity establishment and embryo implantation and development.

Attenuated MAOs activity interferes with embryo implantation and early embryonic development in mice

The involvement of MAOs activity in endometrial receptivity and early embryonic development was evaluated. Phenelzine is a nonselective MAOs-inhibiting antidepressant with anxiolytic properties [19]. To validate the biological functional link between MAOs and early pregnancy, phenelzine was applied to interfere with MAOs activity at different stages of conception in mice. As shown in Fig. 3A–C, MAOs activity intervention affected embryo implantation. The arrow indicates that the embryo implantation site was not obvious due to MAOs attenuation, indicating abnormal implantation (Fig. 3C). Mouse embryos complete implantation on the night of day 4 [20]. A post-implantation MAOs activity interference model (Fig. 3D and Additional file 1: Fig. S3) was designed. Inhibition of MAOs activity after implantation resulted in restricted embryonic development in the mice (Fig. 3D–E). The arrow signifies the growth-restricted implantation sites on day 8 in the inhibitor-injected mouse models (Fig. 3E). As expected, embryos in the trilaminar disc stage were observed in the control litters; however, embryos were delayed in dams treated with the MAOs inhibitor on day 8 (Fig. 3F–G). These findings confirm that MAOs activity is related to EPF.

The expression and localization of MAOs in the human endometrium

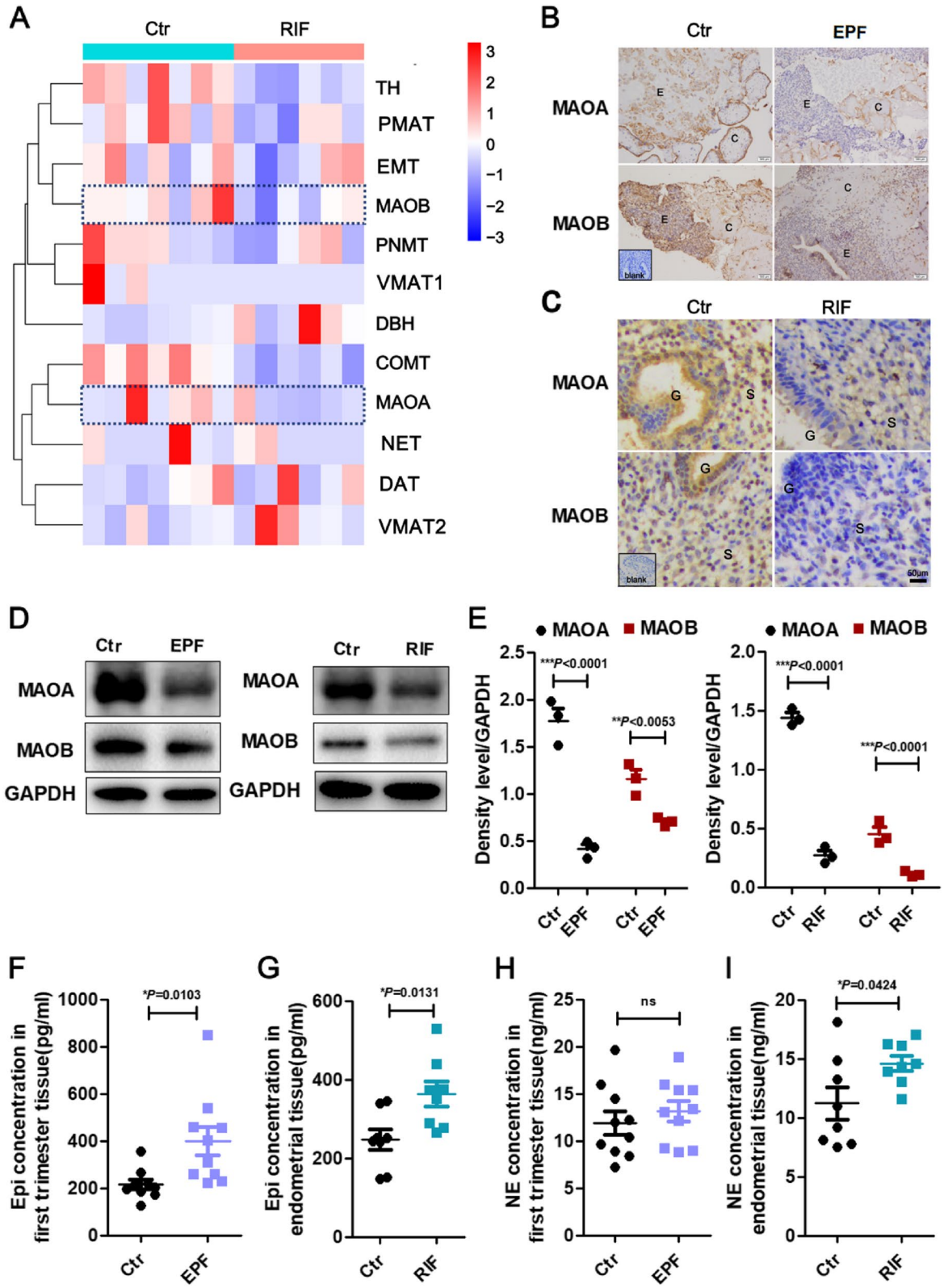
Based on the above evidence, disruption of MAOs activity and imbalance in monoamine homeostasis have been linked to EPF. Consistent with previous studies, this research confirmed that MAOs were highly expressed in the receptive phase of the human endometrium (Fig. 4A–D). Similarly, MAOs were highly expressed in human endometrial stromal and epithelial cells (Fig. 4A–D). Researchers have noted that attenuated MAOA impairs the cessation of endometrial epithelial cell proliferation and endometrial receptivity [15]. Generally, endometrial stromal cells contribute immensely to the establishment of an endometrial receptive state and early embryonic development. Therefore, an HESC-induced differentiation model was constructed in vitro to evaluate the expression of MAOs during the decidual differentiation of HESCs (Fig. 4E–F). Notably, the expression of MAOs increased with the differentiation of HESCs in vitro (Fig. 4E–H). This provides a good model to further examine the molecular mechanism by which MAOs activity balances monoamine homeostasis to regulate endometrial function.

The ability of MAOs to balance monoamine levels in decidual differentiated stromal cells in vitro

As MAOs are known to be located on the outer membrane of the mitochondria, it is necessary to confirm that differentiated HESCs can balance monoamines in the external environment. *VMAT1*, *VMAT2*, *PMAT*, *DAT*, *EMT*, and *NET* are considered to be monoamine transporters with a certain transport capability. The

(See figure on next page.)

Fig. 1 MAOs activity and monoamine levels in the endometrial tissues with repeated implantation failure and early pregnancy failure. **A** Heatmap of upregulated and downregulated monoamine synthesis and transport genes in the putative receptive windows in RIF when compared with control (upregulated genes in red and downregulated genes in blue). **B** The levels and localization of MAOA and MAOB proteins in early pregnancy failure (EPF) were determined by immunohistochemistry. E, endometrium; C, villous; scale bars, 50 μm. **C** The levels and localization of MAOA and MAOB proteins in the mid-secretory phase of endometrial tissues with RIF were determined by immunohistochemistry. S, stroma; G, glandular epithelium. Scale bars, 50 μm. **D** Western blotting for the MAOA and MAOB protein levels in EPF tissues ($n=10$, with equal mixed) and the mid-secretory phase of endometrial tissues with RIF ($n=8$, with equal mixed). RIF, repeated implantation failure; Ctr, control. **E** Relative fold change in density in **D**. **F**, **G** Comparison of epinephrine level in EPF tissues ($n=10$) and mid-secretory phase of endometrial tissues with RIF ($n=8$). **H**, **I** Comparison of norepinephrine in EPF tissues ($n=10$) and mid-secretory phase of endometrial tissues with RIF ($n=8$). Epi, epinephrine; NE, norepinephrine



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transcription levels of these monoamine transporters in the human endometrium and deciduating HESCs were examined. The results showed that the transcription of monoamine transporters was significantly upregulated in both receptive endometrium (Fig. 5A) and decidual differentiated stromal cells (Fig. 5B). This observation appears to indicate that deciduated stromal cells have a stronger ability to transport and metabolize monoamines. To confirm this hypothesis, appropriate concentrations of Epi and NE were added to the differentiated culture system. When the culture medium and intracellular levels of Epi and NE were measured, differentiated HESCs possessed the ability to metabolize monoamines and were regulated by MAOs activity (Fig. 5C–F). Compared with undifferentiated HESCs, decidual cells exhibited a robust ability to metabolize monoamines.

MAOs inhibitors could alleviate the damage caused by high levels of Epi to decidual cells

To further examine the association between human endometrial MAOs activity and EPF, the decidualization process of HESCs during exposure to different concentrations of Epi was simulated. Our previous studies have shown that appropriate concentrations of Epi can promote the decidual differentiation of stromal cells in vitro [9, 10]. Prolactin (PRL) and insulin-like growth factor-binding protein 1 (IGFBP1) are well-known decidualization markers of HESC [21]. To elucidate the role of MAOs activity in the decidualization of HESCs, the levels of PRL and IGFBP1 were measured in HESCs treated with different concentrations of Epi. The findings suggested that high levels of Epi impaired the decidual differentiation of stromal cells, which was mitigated by inhibiting MAOs activity (Fig. 6A–B). FOXO1 is an important downstream molecule of MAOA. Attenuated MAOA could impair endometrial receptivity in women with adenomyosis via the downregulation of FOXO1 [15]. In this study, 10 μM of Epi decreased the levels of FOXO1 in decidual cells with the activation of AKT signaling (Fig. 6C–D). Activation of AKT signaling via downstream FOXO1 has been shown to inhibit decidual differentiation [15, 22]. Based on the results of 5 μM concentration, the decidual cells were treated with 10 μM phenelzine, which revealed that the inhibition of MAOs reversed the activation of AKT signaling (Fig. 6C–D). FOXO1 protein levels and nuclear localization in decidual cells were also salvaged (Fig. 6E–F). Consequently, we hypothesized that MAOs might play a role in endometrial receptivity establishment by balancing monoamine homeostasis. In addition, surprisingly, the inhibition of MAOs activity did not affect the physiological effects of Epi at appropriate concentrations (Fig. 6). This finding denotes that Epi also acts via other pathways such as ADRA2C in HESCs, which were reported by us previously. Although the present findings are somewhat consistent with past results, further experiments are needed to confirm these speculations.

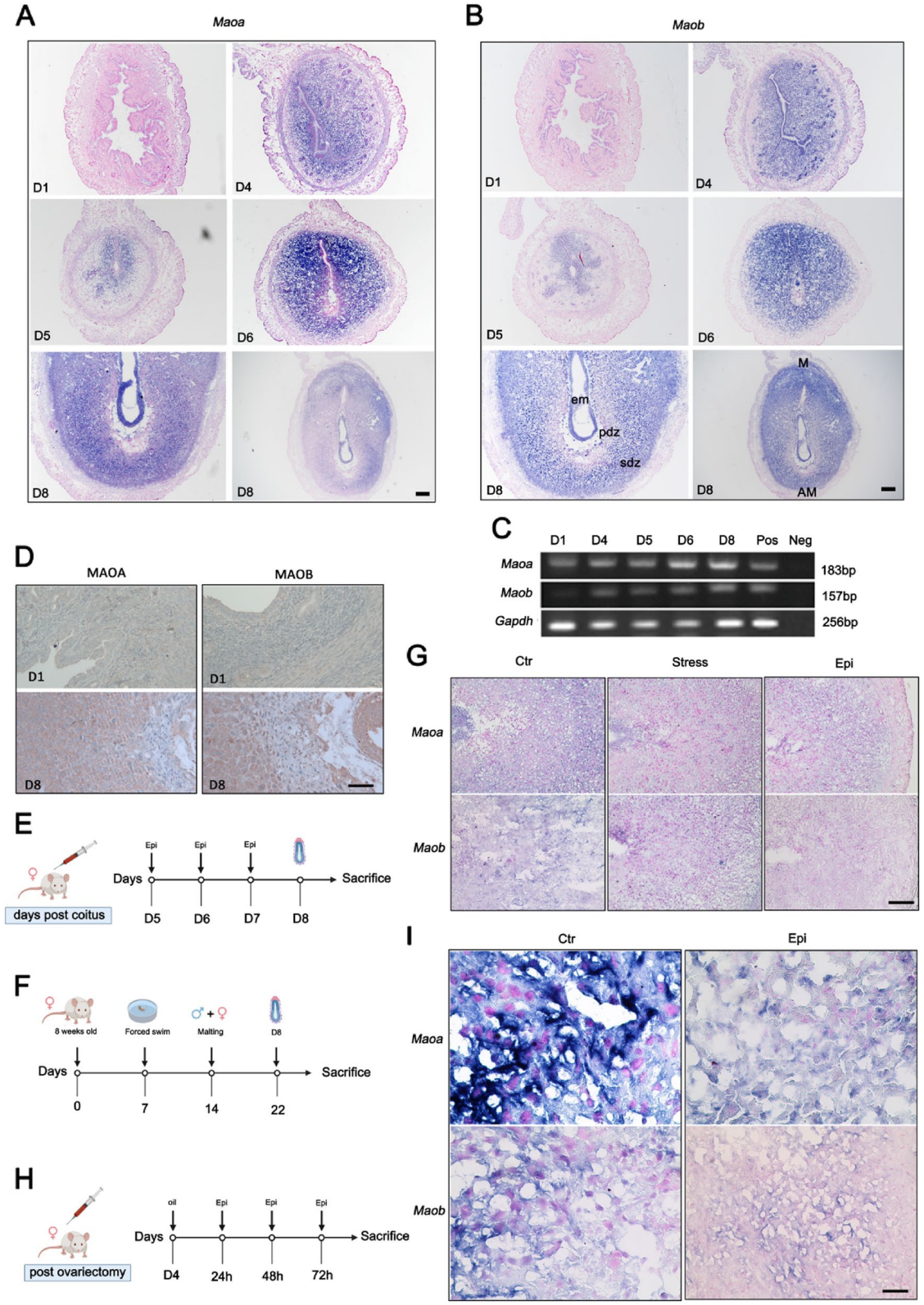
Discussion

The endometrial receptive state is the basis of maternal–fetal interaction and provides a guarantee for subsequent implantation and development of the embryo. Defective endometrial receptivity is considered to be one of the main causes of repeated pregnancy failure. Studies have shown that several factors are involved in regulating the establishment of endometrial receptivity [23]. Our previous research noted that an imbalance in monoamine levels in the body caused by maternal stress impairs endometrial function and leads to miscarriage [10]. In this study, the pertinent role of MAOs in endometrial receptivity by regulating endometrial monoamine homeostasis has been clarified.

MAOs are mitochondria-bound enzymes that catalyze the oxidative deamination of monoamine neurotransmitters. MAOs are postulated to function principally in nervous system regulation. These enzymes are involved in the production of oxidative stress and the catabolism of dopamine, especially in the subcortical regions of the brain, and are thought to participate in the pathophysiology of schizophrenia [24]. Imbalanced monoamine homeostasis is linked to an increased risk of neurosecretion disorders, immune regulation abnormalities, and tumorigenesis [25, 26]. Furthermore, monoamines play a

(See figure on next page.)

Fig. 2 Relationship between the expression of MAOs in mouse uterus and stress processing or Epi exposure. **A**, **B** In situ hybridization revealed the expression pattern of *Maoa* and *Maob* in the uteri on D1, D4, D5, D6, and D8 (scale bar = 500 μm). em, embryo; pdz, primary decidual zone; sdz, secondary decidual zone; M, mesometrial pole; AM, antimesometrial pole. Scale bar = 500 μm. **C** The expression profile of *Maoa* and *Maob* in mouse uterus on D1, D4, D5, D6, and D8. Pos, positive control; Neg, negative control. **D** The protein levels of MAOA and MAOB in mouse uterus on D1 and D8. Scale bar = 50 μm. **E** Schematic diagram of the experimental design for exogenous epinephrine exposure. **F** Schematic diagram of the experimental design of stressed mouse model. **G** In situ hybridization revealed the expression pattern of *Maoa* and *Maob* at the D8 uteri on the stressed mouse and exogenous epinephrine exposed mouse model versus control. Scale bar = 100 μm; em, embryo; de, decidua; blank, negative control. **H** Schematic design of pseudopregnant mice exposed to exogenous epinephrine. Epi, epinephrine. **I** In situ hybridization revealed the alternation expression of *Maoa* and *Maob* at the uteri on 72-h exogenous epinephrine exposed pseudopregnancy mouse model versus control. Scale bar = 50 μm



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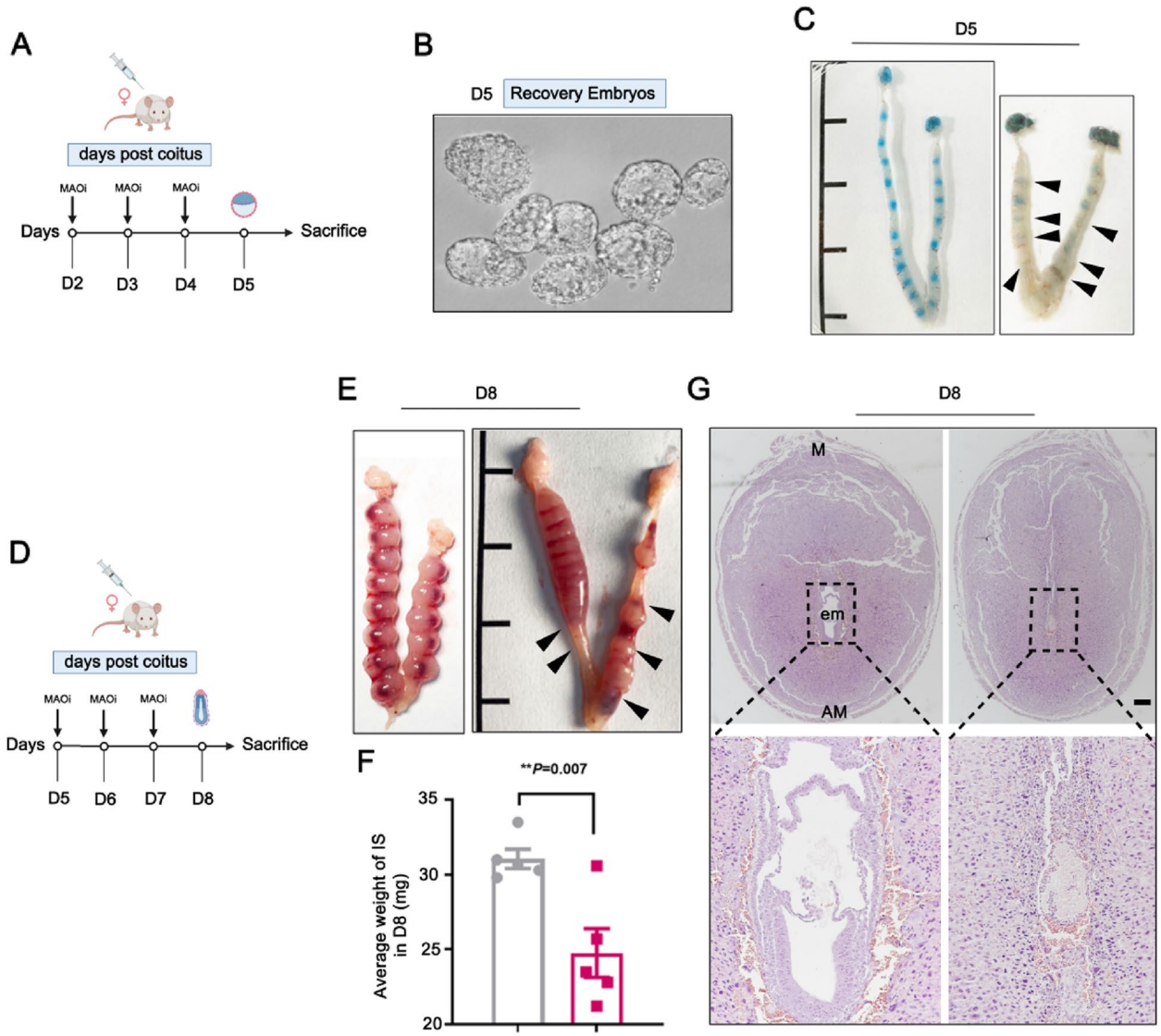

Fig. 3 The intervention of MAOs activity in mouse uterus before and after embryo implantation affected embryo implantation and embryo development. **A** Schematic diagram of the experimental design for MAOs activity intervention treated with phenelzine (50 mg/kg per day) before embryo implantation. **B** Collection of unsuccessfully implanted embryos. **C** Morphologically normal implantation in control mice compared with mice treated with phenelzine (50 mg/kg/day) as determined by blue dye injection on day 5. Arrows indicate that the planting sites were not obvious. **D** Schematic depiction of the experimental design for intervention of uterine MAOs activity in embryonic development after embryo implantation. The body weight of mice from control and mice treated with phenelzine (50 mg/kg/day) on day 8 of implantation. **E** At D8, representative images of intrauterine embryos in mice treated with phenelzine (50 mg/kg/day) were compared with controls. Arrows indicate the planting sites with growth restrictions. **F** The weight of the implantation sites and representative uteri from control and mice treated with phenelzine (50 mg/kg/day) on day 8 of implantation. IS, implantation site. **G** Gross morphology of D8 implantation sites. An embryo of the trilaminar disc stage in the control (left panel) and an embryo of the bilaminar disc stage in the mice treated with phenelzine (50 mg/kg/day) (right panel) (scale bar = 500 μm). em, embryo; M, mesometrial pole; AM, antimesometrial pole

physiological role in the endometrium, embryo implantation, placental development, and maternal–fetal interactions [6, 27]. Both MAOA and MAOB were upregulated in the uterus during the peri-implantation period and could play a role in mouse embryo implantation and endometrial receptivity [28]. A study has reported the presence of a strong association between endometrial receptivity and MAOA expression in the endometrial epithelium. Deficient expression of MAOA in the endometrium is linked to implantation failure [14]. Similarly, this study identified that women with RIF had reduced MAOs expression in the receptive endometrium

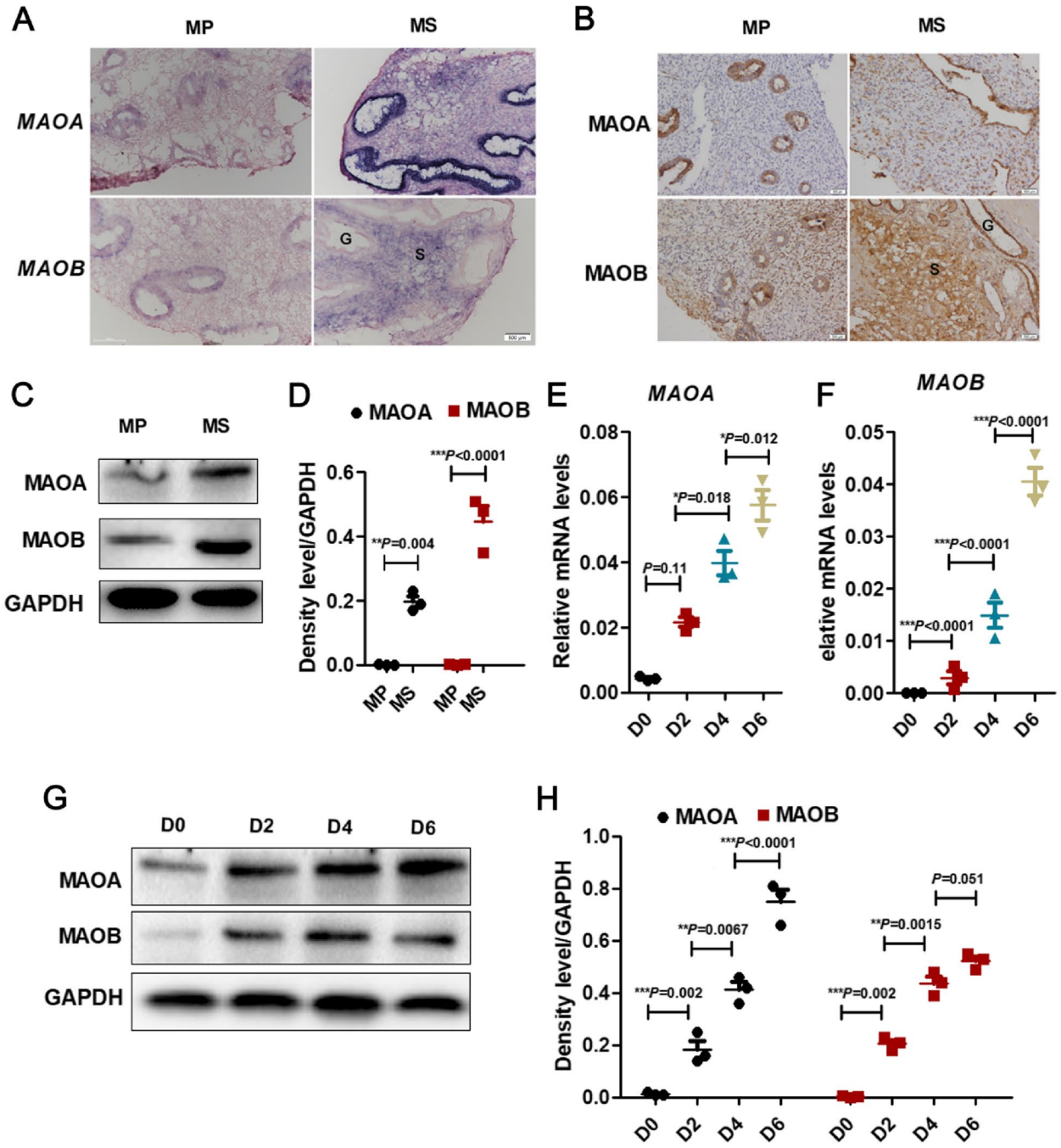

Fig. 4 Expression patterns of MAOs in human endometrium and endometrial stromal cells during decidualization. **A** In situ hybridization revealed the expression pattern of *MAOA* and *MAOB* in the mid-proliferative stage and mid-secretory phase of human endometrium. Scale bar = 500 μm. MP, mid-proliferative phase (day 8 of the menstrual cycle); MS, mid-secretory phase (day LH + 7); S, stroma; G, glandular epithelium. **B** The levels and localization of MAOA and MAOB proteins in the mid-proliferative stage and mid-secretory phase of human endometrium were determined by immunohistochemistry. Scale bar = 500 μm. **C** Western blotting for MAOA and MAOB protein levels in the mid-proliferative stage and mid-secretory phase of human endometrium. **D** Relative fold change of density levels for **C**. **E**, **F** Quantitative RT-PCR analysis of *MAOA* and *MAOB* mRNA level in HESCs during decidualization. **G** Western blotting for MAOA and MAOB protein levels in HESCs during decidualization. D0, HESCs cultured in vitro before differentiation; D2, D4, and D6, HESCs induced differentiation in vitro. **H** Relative fold change of the density levels for **G**

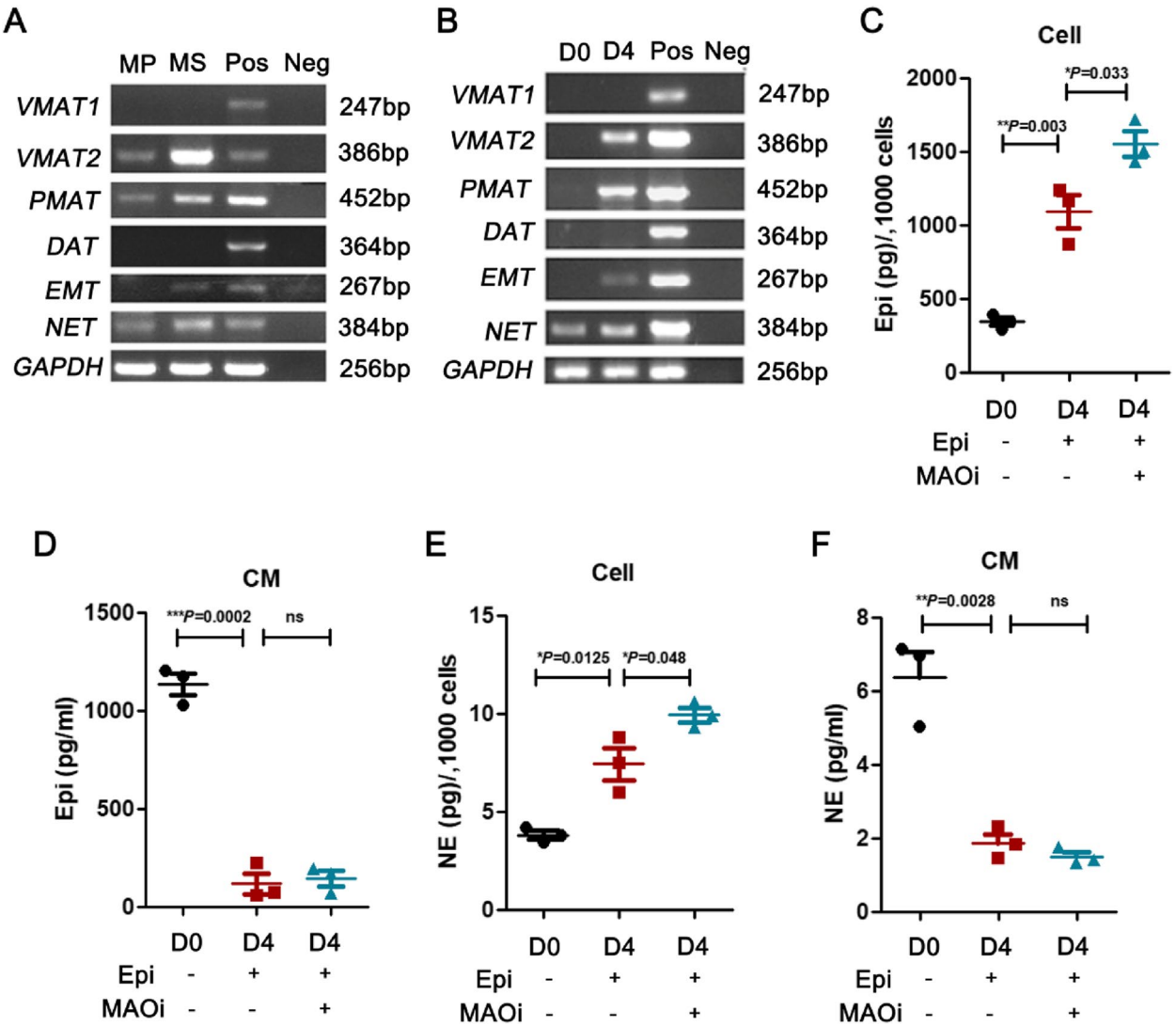

Fig. 5 Ability of MAOs to metabolize monoamines during decidualization of human endometrial stromal cells. **A** Quantitative RT-PCR analysis of monoamine transporters in the mid-proliferative stage and mid-secretory phase of human endometrium. **B** Quantitative RT-PCR analysis of monoamine transporters during decidualization of human endometrial stromal cells. **C** The epinephrine levels in the culture medium during HESC decidualization. **D** The epinephrine levels in HESCs during decidualization. **E** Norepinephrine levels in the culture medium during HESC decidualization. **F** Norepinephrine levels in HESCs during decidualization. D0, HESCs cultured in vitro before differentiation; D4, HESCs induced differentiation in vitro for 4 days; CM, culture medium; Epi, epinephrine; NE, norepinephrine

(Fig. 1A–B). In addition, the expression of MAOs was significantly decreased in the decidua of early abortion tissues (Fig. 1C). Our data showed a substantial reduction in MAOs protein immunoimprinting score in the endometrium with early pregnancy loss. Furthermore, we observed that MAOs were highly present in both the endometrial epithelia and stroma, and that the expression was lost in both the compartments of RIF and EPF patients. The role of MAOs in epithelial cells deserves further investigation. Besides the cell-intrinsic function of stromal expression MAOs for decidualization, the epithelial MAOs may also be involved in this process through the epithelial-stromal interaction. Moreover, the placenta is a non-neuronal organ that can transport and metabolize monoamines [29, 30]. The monoamine system in the placenta is a target of various psychoactive drugs and can be disrupted in several pregnancy-related pathologies.

Animal models have been extensively employed to study monoamine homeostasis as a mechanism involved in fetal programming. In this study too, the expression levels of MAOs were significantly increased

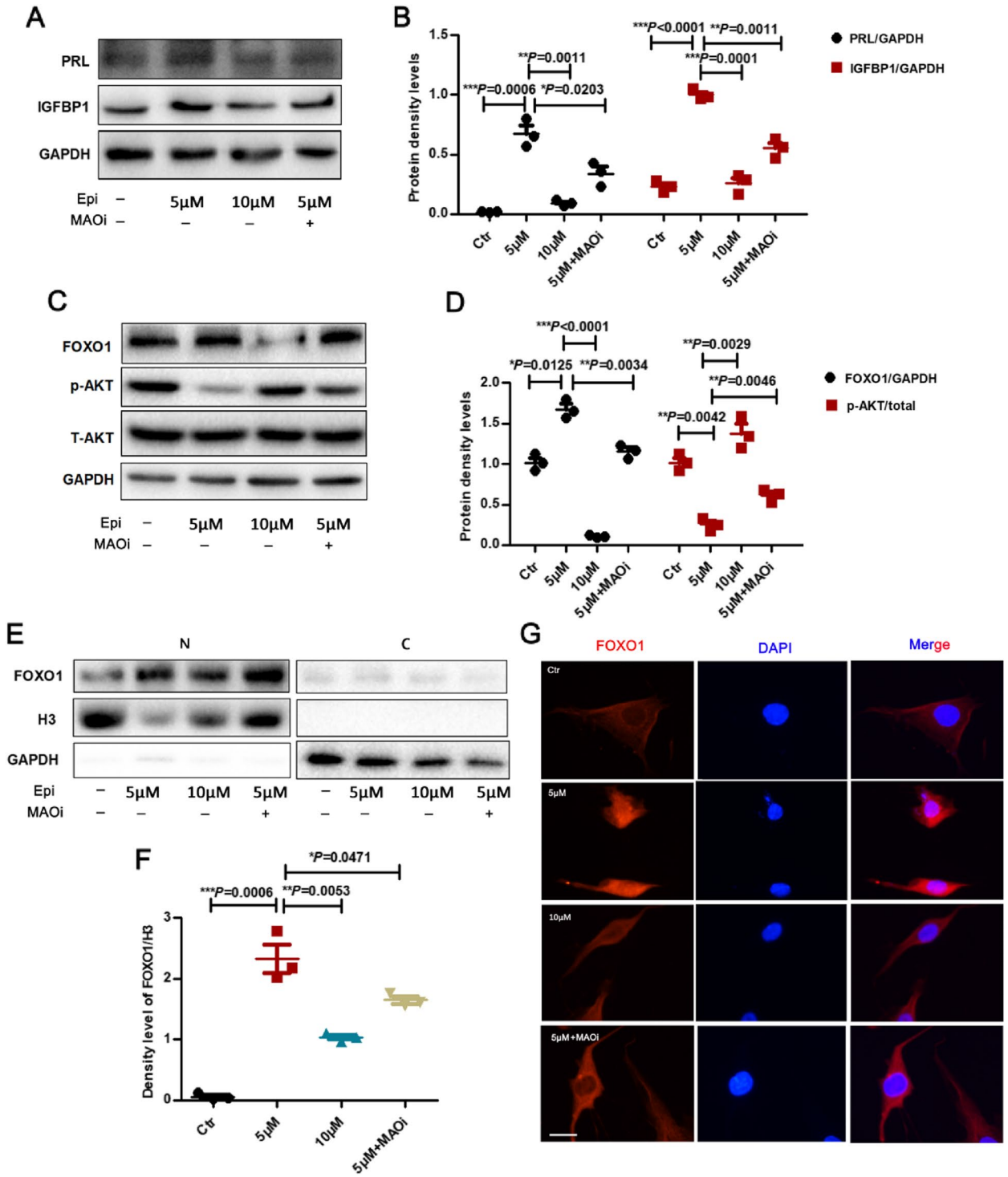

Fig. 6 The activity of MAOs could maintain the balance of monoamines and affect decidual function through the AKT/FOXO1 pathway. **A** Western blotting analysis of PRL and IGFBP-1 protein levels affected by epinephrine concentration and MAOs activity, with GAPDH as a loading control. **B** Relative fold change in the density levels for **A**. **C** Western blotting analysis of FOXO1 and total protein and active forms AKT signaling pathways in D4 decidualized cells affected by epinephrine concentration and MAOs activity, with GAPDH as a loading control. **D** Relative fold change in the density levels for **C**. **E** Western blotting analysis of FOXO1 in the nucleus and cytoplasm fraction. N, nucleus; C, cytoplasm. **F** Relative fold change in density levels for **E**. **G** The expression and localization of FOXO1 by cellular immunofluorescence assay. Scale bar = 20 μM

in endometrial stromal cells during the receptive window of the mouse uterus (Fig. 2A–B). A study has reported that MAOA is involved in the pathological process of endometrial receptivity by regulating endometrial 5-HT homeostasis and epithelial cell proliferation [15]. In the present research, the expression levels of MAOs in endometrial stromal cells were significantly increased in both human and mouse uterus during the receptive window (Fig. 4A–B). To confirm the significance of MAOs activity in early pregnancy, different animal models were used, which revealed that this activity could affect embryo implantation and early embryo development (Fig. 3A–E). In addition, endometrial MAOs activity is affected by the level of monoamine exposure. Interestingly, the ability of decidual cells to transport and metabolize monoamines increased during the receptive phase of the endometrium (Fig. 5). Decidual differentiated stromal cells were affected by the level of monoamine exposure, which led to decidual dysfunction via the AKT/FOXO1 pathway (Fig. 6). These data indicate that MAOs play an important physiological role in endometrial receptivity establishment. MAOs might regulate endometrial receptivity function and early pregnancy by maintaining monoamine homeostasis (Fig. 7).

Conclusions

Collectively, the findings from this study provide insights into the maternal monoamine dynamics between the endometrium and embryo implantation circulation. MAOs play a crucial role in human endometrial receptivity. The role of these enzymes is not only limited to the regulation of neurotransmitter metabolism but also covers pathologic processes involved in endometrial monoamine homeostasis and the regulation of HESC decidualization. These processes, in turn, contribute to impaired endometrial receptivity. In-depth analysis of MAOs activity and monoamine homeostasis in the endometrium are therefore vital for understanding the mechanisms underlying embryonic implantation.

Methods

Tissue collection

This study involved the collection of human abortion tissues and endometrial biopsies, as approved by the Second Affiliated Hospital of Fujian Medical University.

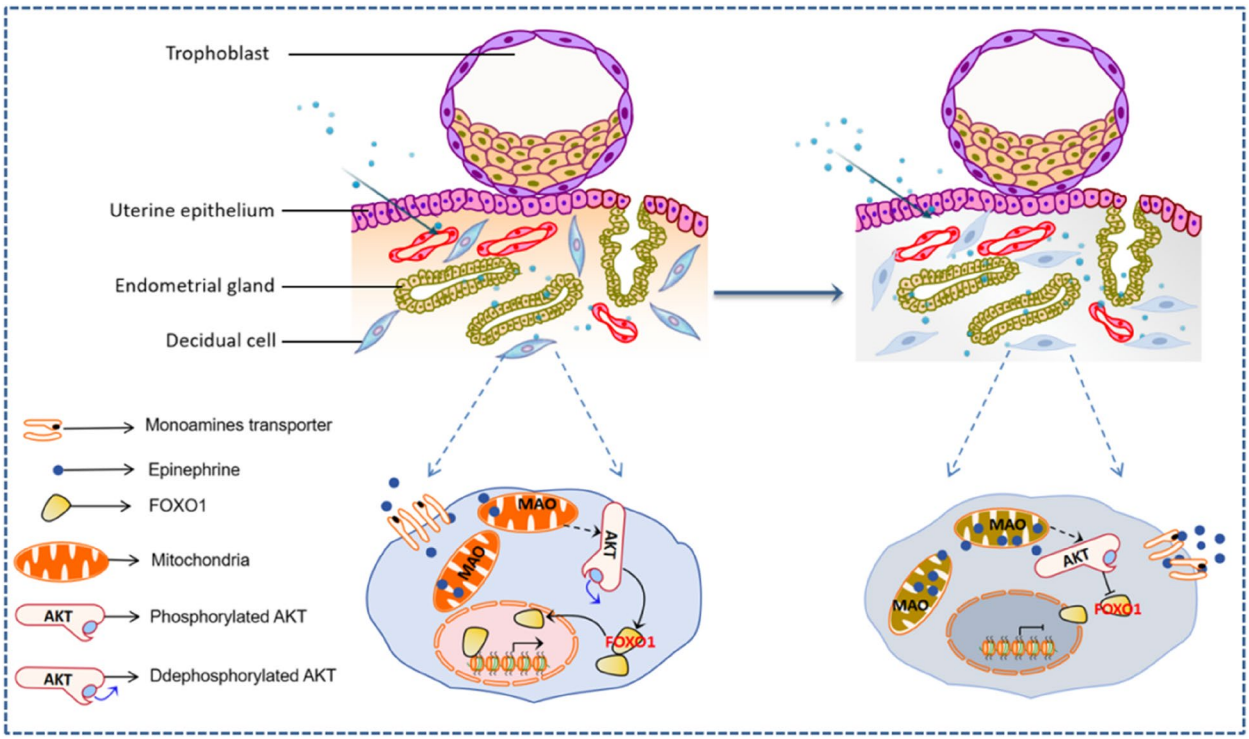

Fig. 7 Graphical abstract. Endometrial decidual cells have a robust ability to balance monoamine homeostasis. A certain level of monoamines is beneficial to the decidual differentiation of human endometrial stromal cells, regulating the receptivity stage of endometrial function. Excessive monoamine levels may interfere with the decidual differentiation of human endometrial stromal cells and thereby affect embryo implantation by activating the AKT-signaling pathway, followed by decreased FOXO1. Mental stress can increase the endogenous level of epinephrine as well as create an imbalance of monoamines in the internal environment, which, in turn, affects the functional state of endometrial receptivity and early pregnancy

Informed consent was obtained from all participants included in the study. Endometrial samples were collected from women with regular menstrual cycles (range: 28–35 days) and without significant intrauterine or ovarian abnormalities on the ultrasonogram. The timing of the proliferative phase sample was calculated according to the patient's cycle day and that of the luteal phase sample was determined based on luteinizing hormone (LH) surge and ovulation. After 7 days of LH surge, only endometrial tissues with no apparent pathology, as assessed by a pathologist, were retained for further experiments. Of these, eight patients who did not exhibit uterine adhesions, inflammation, fibroids, or polyps during hysteroscopy at the mid-proliferative stage were regarded as control subjects. In addition, eight subjects did not report any reproductive disorders, and the cause of infertility was tubal obstruction or male azoospermia. Another six subjects reported repeated failure of embryo implantation. No subject received exogenous steroid therapy during the first 3 months. The basic characteristics of the subjects are listed in Additional file 2: Table S1. A part of the tissues of each group was mixed with equal amounts and frozen at − 80 °C for subsequent western blotting detection. The remaining samples were frozen at − 80 °C for further experiments.

Chorionic villus samples were collected from women who underwent abortion procedures at the Department of Gynecology and Obstetrics in the Second Affiliated Hospital of Fujian Medical University from May 2020 to July 2021. Early pregnancy tissues from 8 weeks of gestation were collected. The gestational age of each early aborted tissue was determined based on the last menstrual period. There were 10 cases of normal pregnancy and 10 cases of early fetal arrest. Early pregnancy tissues were pathologically identified. The exclusion criteria were chromosomal abnormalities, endocrine diseases, infections and anatomical abnormalities of the genital tract, immunological diseases, trauma, signs of other concurrent medical complications, and the ingestion of any chemical agent before sample collection. Samples with aneuploidy were also excluded. The fundamental characteristics of the subjects are presented in Additional file 2: Table S2. A part of the tissues of each group was mixed with equal amounts and frozen at − 80 °C for subsequent western blotting detection. The remaining samples were frozen at − 80 °C for further experiments.

Analysis of differentially expressed monoamine synthesis and transport genes in the receptivity endometrial tissues

The mRNA extraction, purification, and analysis of endometrial tissue were performed as described [10]. RNA sequencing data used in this study are accessible with the following link: https://www.ncbi.nlm.nih.gov/sra/PRJNA838733. The differential monoamine synthesis and transport genes mRNA expression between the RIF and control endometrial receptivity was evaluated using the edgeR package in R/Bioconductor (version 3.26.5; http://www.bioconductor.org/packages/release/bioc/html/edgeR.html). We used a Bioconductor package complex Heatmap to generate the heatmaps.

Mouse models

All mouse experiments were approved by Ethics Committee of the Animal Center of Fujian Medical University for animal studies and Experimental Animals (license no IACUC FJMU 2023-Y-0039) compliant with EU directives on the protection of animals used for scientific purposes. All experimental mice were housed in a 12-h light/dark cycle (light on 07:00 A.M.–7:00 P.M.) with free access to water and food at a temperature of 23 °C. The mice were randomly assigned to groups at the time of purchase. None of the datasets was excluded from the analysis. The animals were examined daily for any health issues by qualified personnel, and the health statuses of all animals were normal. The sample size, sex, and age of all animals used are specified in the respective text and/or figure legends. Female mice were mated with fertile WT males to induce pregnancy (vaginal plug = day 1 of pregnancy). To examine implantation, pregnant dams were killed on day 5 (0900 h). Implantation sites were visualized via the intravenous injection of Chicago blue dye solution, and the number of implantation sites, demarcated by distinct blue bands, was recorded. The design schemes of stress, exogenous Epi injection, and artificially induced decidualization mouse models were based on previous studies [10]. Phenelzine was selected (CSNpharm, Chicago, USA) to inhibit MAOs activity. For the MAOs intervention experiment, the average phenelzine dose was 50 mg/kg body weight/day. Mice were anesthetized with 0.1 ml/10 g of 10% chloral hydrate solution and sacrificed by carbon dioxide asphyxia.

Immortalized HESC culture and treatments

The immortalized human endometrial stromal cell line was purchased from the American Type Culture Collection (ATCCRCRL-4003TM) and cultured according to the manufacturer's instructions. HESCs were cultured in phenol red-free DMEM/F-12 (Sigma-Aldrich, USA) containing 10% (vol/vol) charcoal-stripped fetal bovine serum (CS-FBS) (Gibco, Grand Island, USA), 3.1 g/L glucose41, and 1 mM sodium pyruvate41. The medium was supplemented with 1.5 g/L sodium bicarbonate41, 50 mg/mL penicillin–streptomycin (Gibco), 1% insulin–transferrin–selenium (Gibco), and 500 ng/mL puromycin. Cell cycle synchronization of HESCs was achieved via serum starvation, and the cells were treated with a

medium containing 0.5% CS-FBS for 12 h. Subsequently, the culture medium was replaced with another medium for further analysis.

To induce stromal cell decidualization in vitro, the cells were treated with a medium containing 2% CS-FBS, 1 mM medroxyprogesterone 17-acetate (MPA; Sigma), and 0.25 mM dibutyryl cAMP (db-cAMP; Sigma), as previously described. The medium was refreshed every 48 h, and the cultures were maintained for up to 6 days. To examine the role of the Epi, different concentrations (0.0, 5.0, and 10.0 μM) of Epi were added to the HESC in vitro differentiation system and the optimal concentration was selected for further analyses. Varying concentrations of Epi were added simultaneously with the induction of decidualization. To determine the effects of differing concentrations of Epi and the regulatory role of MAOs activity during the decidualization of stromal cells, various concentrations of Epi (0.0, 5.0, and 10.0 μM) were added to the in vitro differentiation system of HESCs. Furthermore, 10.0 μM phenelzine, which is a specific inhibitor of MAOs, was added to analyze the effects of MAOs activity and exogenous Epi concentration on the decidualization function of stromal cells.

Polymerase chain reaction (PCR) analysis

Total RNA was extracted from cultured HESCs using TRIzol (Invitrogen, USA) according to the manufacturer's protocol. The same input RNA was used for all experiments. The RNA concentration range before reverse transcription to cDNA in different experiments was 1–3 μg. Qualitative analysis of gene expression by agarose gel electrophoresis. The quantitative expression levels of different genes were validated using real-time RT-PCR analysis with the ABI 7500 sequence detector system according to the manufacturer's instructions (Applied Biosystems, Waltham, MA, USA). All primers used for the real-time PCR are listed in Additional file 2: Table S3. All assays were performed at least thrice, each time in duplicate.

In situ *hybridization*

In situ hybridization with digoxigenin was performed as described [9]. Briefly, frozen Sects. (10 μm) were mounted onto slides coated with poly-L-lysine and fixed in 4% paraformaldehyde (Sigma-Aldrich) solution in phosphate-buffered saline (PBS) at 4 °C. After prehybridization, the sections were hybridized at 45 °C for 4 h in 50% formamide buffer containing digoxigenin-labeled sense or antisense cRNA probes. Subsequently, the sections were incubated with RNase A (20 μg/mL; Takara, Japan) at 37 °C for 20 min, and RNase A-resistant hybrids were incubated with anti-Dig antibody (Roche, Basle, Switzerland) and visualized using NBT/BCIP substrate (Promega, Madison, USA). The nucleus was stained with the Nuclear Fast Red solution (Sigma-Aldrich, N3020). All primers used for in situ hybridization are listed in Additional file 2: Table S4.

Histology and immunohistochemistry

Tissues fixed overnight in 4% paraformaldehyde were dehydrated in a graded ethanol series, cleared in an xylene solution, and embedded in paraffin wax. Paraffin-embedded tissues were then sectioned serially at 5 μm. The sections were dewaxed, hydrated, and stained with hematoxylin/eosin. For immunohistochemistry, the sections were microwaved for antigen retrieval; incubated in methanol/hydrogen peroxide; blocked with bovine serum albumin solution; and incubated overnight with rabbit anti-MAOA (1:200; CST, Danvers, USA), rabbit anti-MAOB (1:200; Proteintech, Chicago, USA), and rabbit anti-FOXO1 (1:200; CST) primary antibodies at 4 °C. After staining, the sections were counterstained with hematoxylin.

Western blotting

Protein extraction and western blotting were performed as described previously. PRL (1:250; Abcam), IGFBP-1 (1:1000; Abcam), MAOA (1:1000; CST), MAOB (1:1000; Proteintech), AKT (1:1000; CST), p-Ser (473) AKT (1:1000; CST), FOXO1 (1:1000; CST), GAPDH (1:2000; CST), and H3 (1:2000; Abmart, Shanghai, China) were used. GAPDH and H3 served as the loading control. The bands were visualized using a chemiluminescent substrate (Super Signal West Pico; Thermo Scientific) according to the manufacturer's instructions. The intensity of the bands was determined by using the Quantity One software, and the quantitative analyses of the gray-scale value of each target protein versus that of individual GAPDH were also performed.

Separation of nucleus and cytoplasm

HESCs were collected using cold PBS, resuspended in lysis buffer (10 mM NaCl, 3 mM $MgCl_2$, 0.4% Nonidet P-40, and 10 mM Tris–HCl, pH 7.5) in the presence of protease inhibitor, and the nuclei were pelleted, which allowed the separation of the cytoplasm and nucleus for further validation.

Enzyme-linked immunosorbent assay (ELISA) kits for Epi and NE

On day 4 after treatment with Epi, NE, and MAOs inhibitor ($n=3$ per group), the cell culture medium was collected and stored at − 80 °C for preparation. The cells were washed with PBS three times and then centrifuged, collected, and stored at − 80 °C for preparation. The concentrations of supernatant and cell protein monoamines

were measured. ELISA kits for Epi (E-EL-0045c, elabscience, China) and NE (E-EL-0047, elabscience) were used to determine the monoamine levels. These assays were performed according to the instructions provided by the manufacturer. Each sample was detected at least twice and analyzed using the Microsoft Excel 2010 software.

Statistical analysis

Statistical analysis was performed with the IBM SPSS Statistics 21 program (SPSS Inc., Chicago, USA). Data are presented as the means ± SD or SEM. Comparison of the means between the two groups was accomplished by Student's *t*-test. For multiple comparisons, one-way analysis of variance (ANOVA) was applied to assess differences in the mean values among the groups. $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$, and $^{****}P<0.0001$, versus the control group were analyzed by ANOVA.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12915-024-01966-2.

Additional file 1: Fig. S1 Dynamic protein levels of MAOs in utero during implantation in mice. (A) The protein pattern of MAOA in the uteri on D1, D4, D5, D6, and D8. (B) The protein pattern of MAOB in the uteri on D1, D4, D5, D6, and D8. Fig. S2 Oil induced decidualization for 72 h. Fig. S3 Embryo implantation before MAOs activity intervention

Additional file 2: Table S1 Basal patients information for human endometrial biopsy. Table S2 Basal information for control and EPF. Table S3 Quantitative real-time PCR primer sequences. Table S4 In situ hybridization primers for mouse and human

Additional file 3. Raw data for Figs. 1, 2, 4, 5, and 6

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Authors' contributions

JW and ZY performed assisted reproductive technology; HW and SK designed the study; PH analyzed the data and wrote the paper. LQ and XH prepared the original draft. XL and SH collected clinical samples; FM prepared Figs. 1 and 2. LW and QS prepared tables and participate in revising manuscript. All authors have read and reviewed the final manuscript.

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Availability of data and materials

The data that support the study are available upon reasonable request to the corresponding author. RNA sequencing data used in this study are accessible with the following link: https:// www.ncbi.nlm.nih.gov/sra/PRJNA838733. All source data underlying the graphs and charts presented in the main figures are presented as Supplementary data. Supplementary data have been uploaded to Figshare and can be accessed through the following link: https://doi.org/https://doi.org/10.6084/m9.figshare.20102084. All data generated or analyzed during this study are included in this article and its supplementary information files. Supporting data values for Figs. 1, 2, 4, 5, and 6 are provided as Additional file 3: Raw data.

Declarations

Ethics approval and consent to participate

The study was conducted in accordance with the principles laid out in the Declaration of Helsinki. Approval for this study was obtained from the Ethics Committee of the Second Affiliated Hospital of Fujian Medical University (Research Ethics Committee No. 20235). All samples were obtained from the Department of Reproductive Medicine of the Second Affiliated Hospital of Fujian Medical University, and all the patients signed the informed consent form before any study-specific procedures were performed.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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