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An exaggerated epinephrine-adrenergic receptor signaling impairs uterine decidualization in mice

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ABSTRACT

Our understanding of the relationship between stress-derived epinephrine and early pregnancy failure remains incomplete. Here, we explored the effect of epinephrine exposure on early pregnancy and pseudopregnancy in mice. Increased expression of adrenergic receptors *Adra1b, Adra2b and Adrb2* was observed during decidualization and post-implantation embryogenesis was delayed or survival impaired. Epinephrine treatment also impaired decidualization in both the gravid and pseudopregnant uterus, suggesting the effect on decidualization was independent of the conceptus. This included a suppression of endometrial stroma cell proliferation and of key decidualization regulators, including COX2, BMP2 and WNT4. Collectively, these data demonstrate that maternal epinephrine exposure during early pregnancy impairs uterine decidualization and embryo development, underlying early pregnancy failure.

1. Introduction

Early pregnancy loss is a common event in women of childbearing age with both personal and societal implications in public health. Previous studies have shown that nearly 40% miscarriages occur during early pregnancy, and this proportion is higher following *in vitro* fertilization–embryo transfer [1,2]. Critical changes in the uterus are required to establish and maintain pregnancy, including decidualization of endometrial stromal cells [3]. Some of the essential signals that regulate decidualization include cyclooxygenase 2 (COX2), bone morphogenetic protein 2 (BMP2) and wingless-related MMTV integration site 4 (WNT4) [4,5].

Recurrent pregnancy loss is generally believed to be a multifactorial problem involving the interaction of diverse genetic and environmental factors [6]. Maternal stress may be an important consideration underlying pregnancy loss. For example, several studies have reported that psychological support of women during early pregnancy decreased the rate of spontaneous miscarriage [7,8]. Epidemiological data also show a strong association between maternal stress during early pregnancy and other pregnancy complications {Gold, 2007 #1109;Neugebauer, 1996 #1110} [9]. Anxiety is associated with epinipherine release from the adrenal medulla, elevating plasma levels of this stress hormone [10]. Studies in women with unexplained recurrent pregnancy loss have also found elevated blood flow resistance in the uterine arteries [11] and increased plasma adrenomedullin {Nakatsuka, 2003 #1107;Nakatsuka, 2003 #1107} [12]. However, our knowledge of epinephrine's role during pregnancy loss remains incomplete.

The uterus has an extensive sympathetic innervation [13], and disrupting β2-adrenoceptor (Adrb2) signaling results in aberrant oviductal embryo transport [14]. In addition, transient activation of Adrb2 during preimplantation stages in the mouse alters embryo spacing, leading to significant increase in postimplantation losses [15]. However, it is not clear how stress-induced sympathetic activation might affect the process. In the present study, we showed that maternal epinephrine exposure during early post-implantation development in mice severely uterine decidualization and embryonic development, resulting in early pregnancy loss.

2. Materials and methods

2.1. Animals

Adult CD1 mice (7–8 weeks old) were purchased from Vital River Laboratories Co. Ltd. All animal experiments were approved by the

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animal ethic committee and the Institutional Animal Care. All mice were housed on a 12 h light/12 h dark cycle (light on 07:00 AM-19:00 PM) with free water and food supply at a temperature of 23 °C. Nulliparous females were mated with males overnight and the morning (08:00 AM) finding of the vaginal plug was designated as day 0 of gestation (GD 0). Dams received a daily intravenous injection of epinephrine (MedChem Express, Monmouth Junction, USA) in saline at 0830 h from GD 4 to GD 6 and dams were euthanized on GD 7 (0900 h). Maternal blood samples were collected for analysis of serum progesterone (P4) levels were measured by radioimmunoassay (RIA). The dosage of epinephrine was referenced or modified as described previously [16–20]. Mice receiving sterile saline alone served as the control.

2.2. Artificial decidualization treatment schedules

Pseudopregnancy was produced by mating females with vasectomized males of the same strain (Day 0 = vaginal plug). To induce decidual cell reaction (Fig. 3A), sesame oil (25 μl) was infused in one uterine horn at Day 3 of pseudopregnancy; the non-infused contralateral horn served as a control. Mice were injected with epinephrine (100 μg/mouse) intraperitoneally 15 min after intraluminal infusion of oil. The control animals received only the vehicle. Uterine weights of the infused and non-infused horns were recorded 96 h later and the fold increase in uterine weight was taken as an index of decidualization [21].

2.3. Uterine stromal cell culture

Uterine stromal cells were isolated, cultured and induced differentiation as previously described with some modifications [22].Three to four pseudopregnant Day 3 mouse uterine horns were cut into small pieces (2–3 mm). Tissue pieces were first digested in 3 ml fresh medium (HBSS antibiotic; Gibco, Grand Island, USA) containing 6 mg/ml dispase (Gibco) and 25 mg/ml pancreatin (Sigma-Aldrich, USA), and then incubated in fresh medium (3 ml) containing 0.5 mg/ml collagenase (Sigma-Aldrich) at 37 °C for 30 min. The digested cells were passed through a 70-μm filter to obtain the stromal cells. Cells were plated at 60-mm dishes and 24-wells plates, containing phenol red-free Dulbecco modified Eagle medium (DMEM) and Ham F-12 nutrient mixture (1:1) (Gibco) with 10% charcoal-stripped fetal bovine serum (CS-FBS) and antibiotic. Two hours later, the medium was replaced with fresh medium (DMEM/F-12, 1:1) with 10% CS-FBS. The next morning, the medium was replaced with DMEM/F12 containing 1% C-FBS, E2 (10 nM; Sigma-Aldrich), P4 (1 μM; Sigma-Aldrich) and antibiotic to induce decidualization. The media was changed every 48 h, and cells were collected at 0 h and 96 h after decidualization induction.

2.4. Tissue collection

After euthanasia, the uteri were harvested and washed thrice in physiological saline. Implantation sites were frozen or stored in liquid nitrogen for protein and RNA analyses. Some tissue samples were fixed in 4% paraformaldehyde (PFA) in 10 mM PBS (pH = 7.4) for histologic examination.

2.5. Polymerase chain reaction PCR

A total of 1–3 μg RNA was used to synthesize cDNA. The expression levels of different genes were validated by real-time reverse transcription polymerase chain reaction (RT-PCR) TaqMan analysis using the ABI 7500 sequence detector system according to manufacturer's instructions (Applied Biosystems, Waltham Massachusetts, USA). All the primers for PCR are listed in Table 1. All the PCR experiments were repeated at least three times.

Table 1
Quantitative real-time PCR primer sequences.

Genes	Primer sequence5'–3'		GenBank accession no.
Adra1a	Forward	TTCTGCAACATCTGGGCGG	NM_013461.4
	Reverse	GCTGCCTCCAGCCGAACA	
Adra1b	Forward	GACCTGCTGTTGAGCTTCA	NM_007416.4
	Reverse	AGAGGCCCGATGGAGATGA	
Adra1d	Forward	GTCACCAACTATTTCATCG	NM_013460.5
	Reverse	GAGATGGTGCAGAGGCT	
Adra2a	Forward	GAGGAAGAGGAGGACCCA	NM_007417.5
	Reverse	TCGGTCCCGTTCCAGCT	
Adra2c	Forward	CAGAACCTCTTCCTGGTGT	NM_007418.3
	Reverse	TGCGTCACCGACCAGTAGC	
Adra2b	Forward	GTGAGCCGAGCATTGGAGTA	NM_009633.4
	Reverse	GAGAAGCTGGCAGTGTTTGG	
Adrb1	Forward	GTGCTGGTGATCGTGGCCAT	NM_007419.2
	Reverse	GCAGAAGAAGGAGCCGTACTC	
Adrb2	Forward	ATCGTCCTGGCCATCGTG	NM_007420.3
	Reverse	ATCACGCACAGGGTCTC	
Adrb3	Forward	ACCAACGTGTTCGTGACTTC	NM_013462.3
	Reverse	CACCACTGGCTCATGATGG	
Gapdh	Forward	GACAACTCACTCAAGATTGT	NM_001289726.1
	Reverse	CAGCTCTGGGATGACCTTGC	

2.6. In situ hybridization

In situ hybridization with digoxygenin was performed as described [23]. Briefly, frozen sections (10 μm) were mounted onto poly-L-lysine-coated slides and fixed in 4% paraformaldehyde (Sigma-Aldrich) solution in PBS at 4 °C. After prehybridization, sections were hybridized at 45 °C for 4 h in 50% formamide buffer containing digoxygenin-labeled sense or antisense cRNA probes. After hybridization, 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 then visualized by NBT/BCIP substrate (Promega, Madison, USA). The nucleus was stained with the Nuclear Fast Red solution (Sigma-Aldrich, N3020). All the primers for in situ hybridization are listed in Table 2.

2.7. Histology and immunohistochemistry

Tissues fixed overnight in 4% PFA were dehydrated in a graded ethanol series, cleared in a xylene solution, and then embedded in paraffin wax. To paraffin-embedded tissues were sectioned serially at 5 μm. The sections were dewaxed, hydrated, and stained with H/E. For immunohistochemistry, the sections were microwaved for antigen retreival, incubated in methanol/hydrogen peroxide, blocked with bovine serum albumin solution, and incubated overnight with rabbit anti-KI67 (1:200; Epitomics, California, USA), rabbit anti-PRL8A2 (1:200; Abcam, Cambridge, USA), rabbit anti-FOXO1 (1:200; CST, Danvers, USA) and mouse anti-MEIS1 (1:200, Abcam) primary antibodies at 4°. After

Table 2
In situ hybridization probe primer sequences.

Genes	Primer sequence5'–3'		GenBank accession no.
Adra1b	Forward	GACCTGCTGTTGAGCTTCA	NM_007416.4
	Reverse	AGAGGCCCGATGGAGATGA	
Adra2b	Forward	GTGAGCCGAGCATTGGAGTA	NM_009633.4
	Reverse	GAGAAGCTGGCAGTGTTTGG	
Adrb2	Forward	ATCGTCCTGGCCATCGTG	NM_007420.3
	Reverse	ATCACGCACAGGGTCTC	
Bmp2	Forward	GGTTTGGCAACCCGAGACGC	NM_007553.3
	Reverse	CTCCAGCCAAGTGCTGACAC	
Ptgs2	Forward	GATCATAAGCGAGGACCTG	NM_011198.4
	Reverse	CTCTCCTATGAGTATGAGTC	
Wnt4	Forward	GTGGCGTGCTAGGCACGAG	NM_009523.2
	Reverse	CTTTGGTCAGAGGCAGCCAC	

staining, sections were counterstained with hematoxylin.

2.8. Western blot analysis

Protein extraction and Western Blot analysis were performed as described previously [24]. Tissues were lysed in 150 mM NaCl, 1% Triton X-100, 1% sodium deoxycholate, 0.1% SDS, 50 mM Tris, pH 8.0, and a protease inhibitor cocktail (Roche). The samples were kept on ice for 30 min and then centrifuged at 13 000 rpm for 30 min at 4 °C. The concentration of protein was detected with a bicinchocinic acid protein assay kit according to the manufacturer's instructions (Beyotime Institute of Biotechnology, Shanghai, China). Proteins were subjected to 12% polyacrylamide gel electrophoresis and then transferred onto a polyvinylidene difluoride membrane (Millipore). After the transfer, membranes were blocked with 5% skim milk in Tris-buffered saline with 0.1% Tween 20 (TBST) for 2 h at RT and incubated overnight with primary antibodies in TBST. Antibodies to COX2 (1:1000; eBioscience, California, USA), WNT4 (1:1000; Abcam), BMP2 (1:1000; Abcam), CEBPB (1:250; Santa Cruz, California, USA), FOXO1 (1:1000; CST), MEIS1 (1:1000; Abcam), PRL8A2 (1:1000; Abcam), and β-Actin (1:2000; Sigma, St. Louis, USA) were used. After incubation with the primary antibodies, the membranes were washed in TBST three times and then incubated with their specific secondary antibodies (1:3000; Zhongshan Golden Bridge Biotechnology Co., Beijing, China) in 5% silk milk for 2 h at RT. Bands were visualized using Thermo Super signal West Pico chemiluminescent substrate according to the manufacturer's instructions. The relative band intensity was acquired by using Quantity One software.

2.9. Statistical analysis

Statistical analysis was performed with the IBM SPSS Statistics 21 program (SPSS Inc., Chicago, USA). Comparison of means was performed using the independent-samples Student t-test. The data are shown as means ± SEM. Differences were considered statistically significant at $P < 0.05$.

3. Results

3.1. The expression of adrenergic receptors in uterine stroma is increased during early pregnancy

To examine the potential roles of adrenergic signaling during early pregnancy, we screened the expression profile of different adrenergic receptors in the pregnancy mouse uterus. *Adra1b, Adra2b and Adrb2* mRNA were less expressed on GD 3 when uteri enter the receptive status and GD 4 with embryo implantation completing, while increased on GD 5 with the onset of decidualization and were abundant on GD 7 with the development of decidualization (Fig. 1A). Meanwhile, using in situ hybridization analyses, we observed that *Adra1b, Adra2b and Adrb2* were specifically expressed in the uterus stromal cells surrounding the developing embryo, but not in the epithelial cells on GD 4. Strong hybridization signal of *Adra1b, Adra2b and Adrb2* appeared within the primary decidua by GD 5. As gestation proceeds, the positive signaling of *Adra1b, Adra2b and Adrb2* was attenuated in the primary decidua, but enhanced in the secondary decidua by GD 7 (Fig. 1B–D). This dynamic uterine expression pattern of adrenergic receptors, *Adra1b, Adra2b and Adrb2*, motivated us to study the potential roles of adrenergic signaling in the early pregnancy events.

3.2. Epinephrine exposure during early pregnancy resulted in aberrant embryo development

To assess the impacts of adrenergic signaling on early pregnancy, we get post-implantation mice injected with different dosages of epinephrine as shown in Fig. 2A. Low-dosage of epinephrine (25 μg/mouse) groups had no adverse effort on delivery date, while high-dosage of epinephrine (100 μg/mouse) treatment resulted in decreased delivery rate, in which only one out of five mice successfully delivered (Fig. 2B). However, high dosage of epinephrine administered during early pregnancy had no effect on the number of litter size and neonatal weight among delivered mice (Fig. 2C). Meanwhile, we found that the serum level of progesterone was not affected by the high dosage of epinephrine on GD 7 (Fig. 2D). Early resorption sites were observed following the high dosage of epinephrine, but not in the controls or mice with low dosage of epinephrine treatment (Fig. 2E). Embryos at the trilaminar disc stage were observed in the control litters as expected; however, embryos were delayed in dams treated with the high dosage of epinephrine on GD 6 (Fig. 2F). The average weight of implantation sites (30.04 ± 1.83 vs 22.69 ± 1.99; $P < 0.01$), vertical axis length of embryo (Fold change: 1.0 ± 0.03 vs 0.8 ± 0.07, $P < 0.01$) and embryonic area (Fold change: 1.0 ± 0.12 vs 0.31 ± 0.23, $P < 0.01$) on GD 7 were decreased in mice injected with epinephrine compared with control mice (Fig. 2F–I). These results indicated that maternal dosing with epinephrine delayed or disrupted early post-implantation development.

3.3. Epinephrine exposure during early pregnancy resulted in decidualization defect

Since stromal cell decidualization is essential for early pregnancy [3], we next detected the effect of epinephrine on decidualization. In vehicle groups, strong Alkaline phosphatase (ALP) activity was detected in most stromal cells on GD 5, while only in decidual cells of the antimesometrial pole on GD 7. However, the expression pattern of ALP activity in mice treated with epinephrine was delayed, indicating the impaired decidual function (Fig. 3A). We further detected the expression of prolactin family 8 subfamily a member 2 (PRL8A2), COX2 and WNT4, the well-known markers for uterine stromal differentiation during decidualization. Epinephrine treated mice showed decreased expression of COX2, WNT4 and PRL8A2 on GD 7 compared with the vehicle groups (Fig. 3B). Immunofluorescence assay confirmed that there was no obvious difference of the localization and expression levels of PRL8A2 between two groups on GD 5, while the number of PRL8A2-positive decidual cells was obviously reduced in mice treated with epinephrine on GD 7 (Fig. 3C). In addition, several critical transcriptional factors during decidualization, including Foxhead box O1 (FOXO1), Meis Homeobox 1 (MEIS1) and CCAAT Enhancer Binding Protein Beta (CEBPB) were decreased in mice treated with epinephrine compared with the vehicle groups on GD 7 (Fig. 3D). Immunohistochemistry assay further showed that the numbers of FOXO1 and MEIS1 positive decidual cells were obviously reduced in the uteri of mice treated with exaggerated epinephrine on GD 7 (Fig. 3E and F). These results suggested that epinephrine exposure during early pregnancy resulted in impaired decidualization.

3.4. Epinephrine exposure impaired uterine decidualization independent of embryo development

To further examine the important role of epinephrine on uterine decidualization, we utilized an artificial decidualization model to exclude the interference of embryo development defect (Fig. 4A). Firstly, we analyzed the expression pattern of *Adra1b, Adra2b and Adrb2* in the artificial decidualization mouse uterus by in situ hybridization. The first elevation of *Adra1b, Adra2b and Adrb2* transcripts was apparent in decidual cells suibjacent to the epithelium at 24 h after oil infusion and widly expressed throughout the deciduum by 48 h (Fig. 4B). At 96 h of oil stimulation, we observed impaired decidualization and decreased decidual tissue weight (22.10 ± 2.47 vs 11.33 ± 4.06, $P < 0.05$) following epinephrine exposure (Fig. 4C and D). To determine if decidual cells were a direct target of epinephrine, we evaluated induced decidual cell cultures for the response to 10 μM epinephrine based on

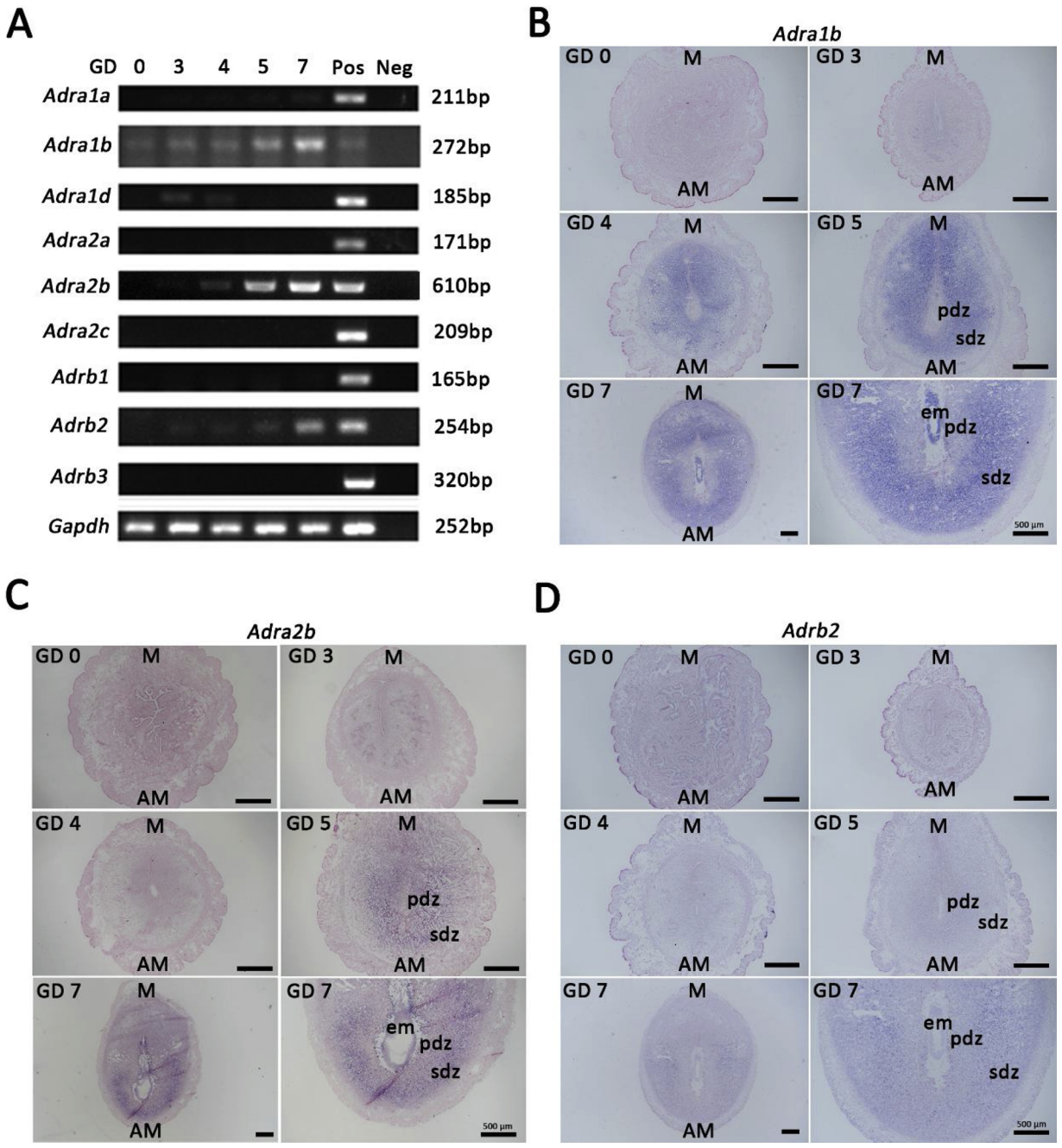

Fig. 1. Dominant expression of *Adra1b, Adra2b and Adrb2* mRNA in decidua during pregnancy. (A) The expression profile of different adrenergic receptors in mouse uterus on GD 0, 3, 4, 5, 7. *Pos*, positive control; *Neg*, negative control. (B–D) In situ hybridization revealed the expression pattern of *Adra1b, Adra2b and Adrb2* in the uteri on GD 0, 3, 4, 5, 7 (bar = 500 μm). em, embryo; pdz, primary decidual zone; sdz, secondary decidual zone; M, mesometrial pole; AM, antimesometrial pole.

the differentiation marker PRL8A2 (Fig. 4E and F). Taken together, these results confirmed that epinephrine directly impaired decidualization independent of maternal or conceptual influences.

3.5. Epinephrine exposure led to abnormal uterine stromal cell proliferation and differentiation

To reveal the underlying causes accounting for decidualization defect in dams treated with epinephrine, we profiled endometrial cell proliferation with Ki67 immunostaining. Both control and epinephrine-treated dams showed robust Ki67-immunoreactive protein in the deciduum at 12 h and 24 h post oil stimulation; however, dams treated with epinephrine showed markedly reduced numbers of Ki67-positive cells in the antimesometrium after 48 h of oil stimulation (Fig. 5A). We analyzed the expression of several key genes regulating decidualization, including *Ptgs2* (encoding COX2), *Wnt4* and *Bmp2*. While *Ptgs2, Wnt4* and *Bmp2* were normally expressed in stromal component of the vehicle group after oil stimulation, these expressions were almost completely abolished in the dams treated with epinephrine (Fig. 5B–D). Western blot assay confirmed an epinephrine-induced decrease in COX2, WNT4 and BMP2 protein bands at 48 h after oil infusion (Fig. 5E). These results indicated that epinephrine inhibited stromal proliferation and differentiation under conditions normally conducive to decidualization.

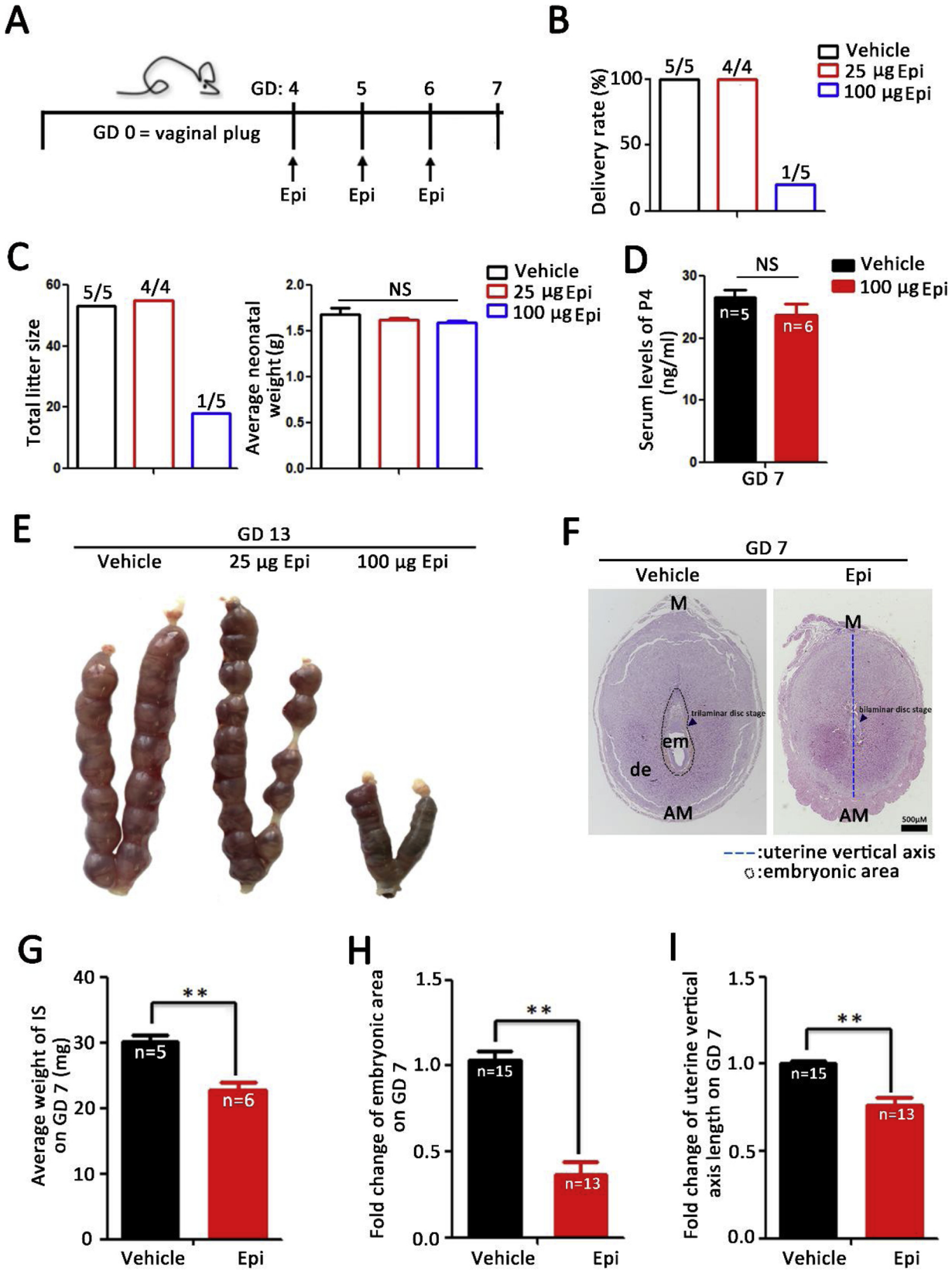

Fig. 2. Epinephrine exposure during early pregnancy caused aberrant embryo development. (A) Epinephrine administration schedules. (B) Delivery rate of mice treated with different dosage of epinephrine. (C) The total litter size and average neonatal weight in different dosage of epinephrine. (D) Serum levels of P4 on GD 7 between vehicle and high dosage of epinephrine groups. (E) The dose of 100 μg/mouse epinephrine caused embryonic early resorption in early post-implantation stage. (F) Gross morphology of GD 7 implantation sites. An embryo of trilaminar disc stage in the control (left panel) and an embryo of bilaminar disc stage in the mice treated with 100 μg/mouse epinephrine (right panel) (bar = 500 μm). (G) Average wet weight of implantation sites (IS) on GD 7. Numbers within the bars indicate the number of pregnant mice examined. (H) Fold change of embryonic area on GD 7. (I) Fold change of IS uterine axis length on GD 7. **$P < 0.01$, data are shown as means ± SEM. Epi, epinephrine; M, mesometrial pole; AM, antimesometrial pole; em, embryo; de, decidua.

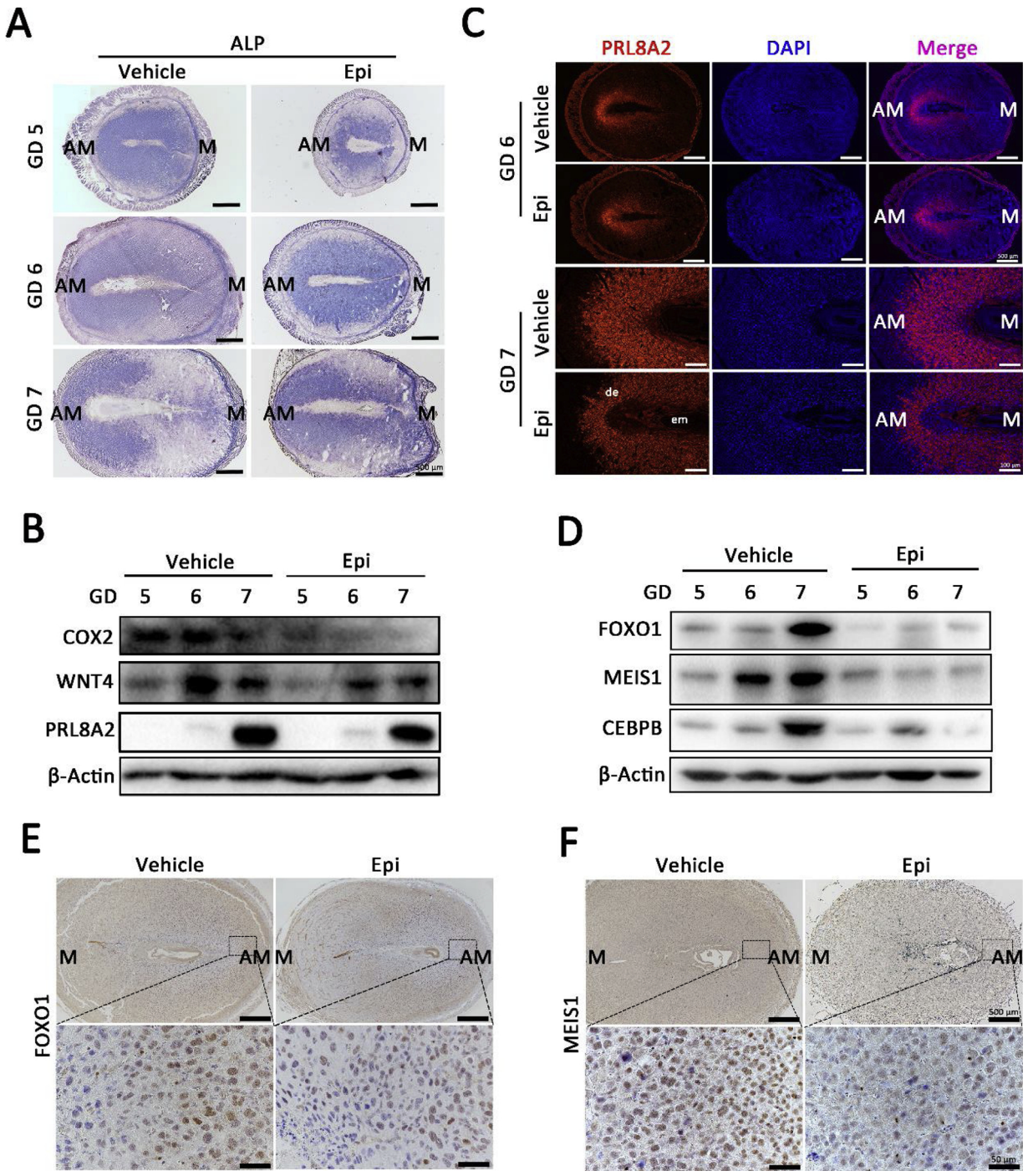

Fig. 3. Exogenous epinephrine induced abnormal decidualization at post-implantation uteri. (A) Alkaline phosphatase staining of GD 5–7 implantation sites in vehicle and 100 μg/mouse epinephrine administered mice (bar = 500 μm). (B) The protein levels of COX2, WNT4 and PRL8A2 were detected by Western blot from GD 5 to GD 7 of pregnancy uteri tissues. (C) Immunofluorescence staining of PRL8A2 in decidual tissue of mice treated with 100 μg/mouse epinephrine and control mice (bar = 500 μm). (D) The protein levels of decidualization associated TFs were observed by western blot from GD 5 to GD 7 of pregnancy uteri tissues. The protein levels of these factors were reduced by time-dependent assay with epinephrine administration. (E–F) Immunohistochemistry was used to detect the difference in expression of FOXO1 and MEIS1 in both groups on GD 7. Embryos at the trilaminar disc stage were found in the vehicle but delayed in litters exposed to 100 μg/mouse epinephrine (Scale bars were showed in pictures). Epi, epinephrine; M, mesometrial pole; AM, antimesometrial pole.

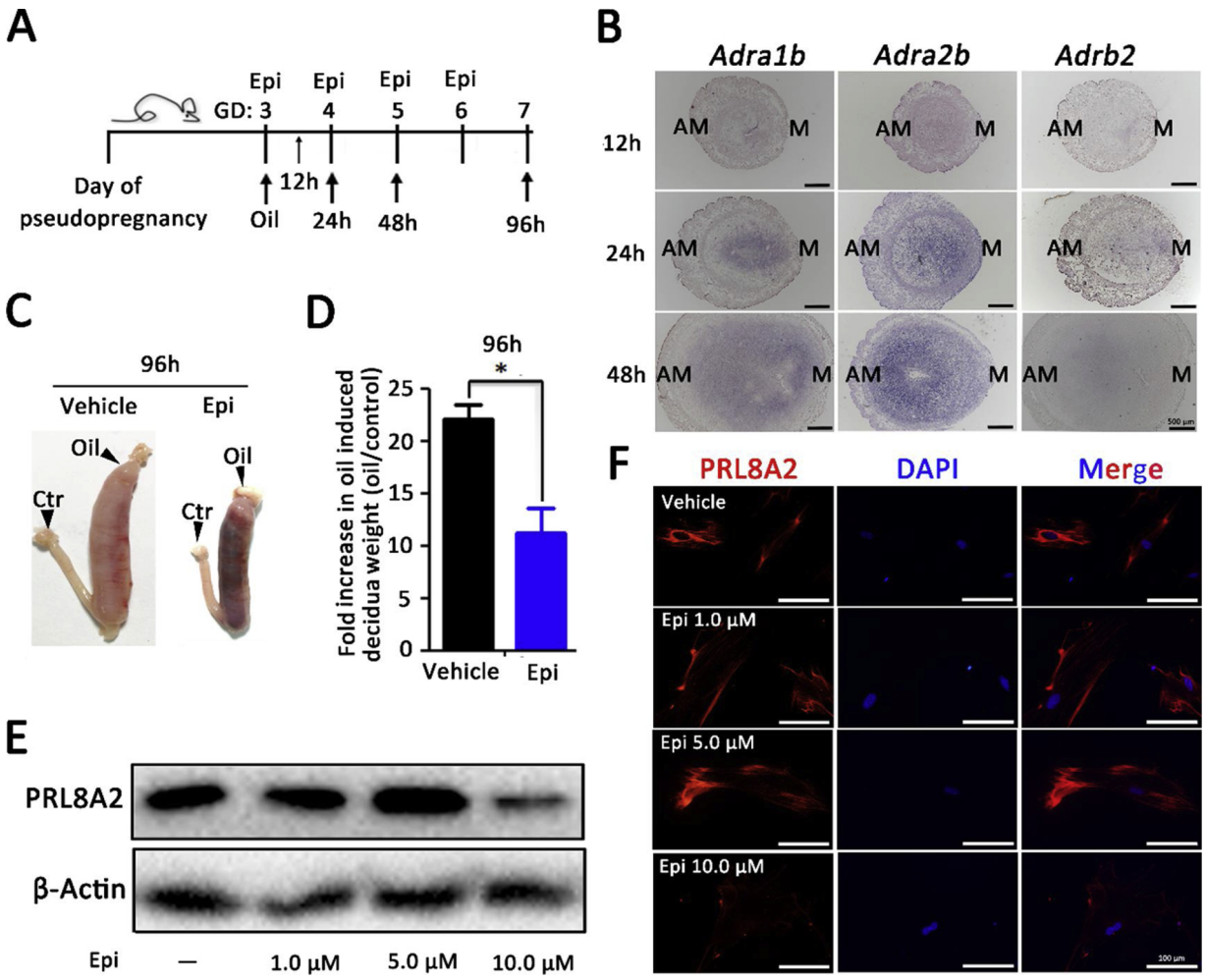

Fig. 4. Epinephrine exposure impaired uterine decidualization independent of embryo development. (A) Artificial decidualization treatment schedules. (B) In situ hybridization analysis of *Adra1b, Adra2b and Adrb2* in the uteri after oil induced 12 h, 24 h and 48 h (bar = 500 μm). (C) Morphological differences of pseudopregnant uterus after 96 h of oil induction between vehicle and 100 μg/mouse epinephrine treatment. Ctr, Non-decidualization; Oil, artificial decidualization; (D) The fold increase after oil induced decidual weight (*P < 0.05). (E) Western blotting analysis of PRL8A2 in differentiated primary uterine stromal cells treated with different dosage of epinephrine for 72 h. (F) Immunofluorescence analysis of PRL8A2 in differentiated primary uterine stromal cells treated with different dosage of epinephrine for 72 h (bar = 100 μm). Epi, epinephrine; de, decidua; M, mesometrial pole; AM, antimesometrial pole.

4. Discussion

Embryo implantation in rodents and primates consists of tightly controlled events, including the location of blastocyst, the attachment of uterine lumen, and the differentiation of the uterine stromal cells. Decidualization is critical to the establishment of maternal-fetal communication and early post-implantation development by providing a microphysiological environment for nourishment, placentation, immune tolerance, and modeling of the implantation chamber. Impaired endometrial stroma proliferation and differentiation is associated with recurrent miscarriage, pre-eclampsia, intrauterine growth restriction and unexplained infertility in the clinical setting [25–27]. Results from the present study demonstrate a role for peri-implantation adrenergic signaling in successful establishment and maintenance of the early mouse embryo. These results, with exogenous epinephrine, imply a direct relationship between the maternal stress hormone and decidualization of the uterine stroma, with adverse consequences to early postimplantation development and embryo survival.

Elevated epinephrine is associated with stress-induced chronic disorders of cardiovascular, immune, cancer, and behavioral systems. The major physiologic triggers of adrenaline release are diverse and may include physical threat, excitement, noise, bright lights, and high or low ambient temperature [28]. These stimuli are processed in the central nervous system and invoke fear, anxiety, nervous and depression [29] in the mother. Previous investigations on pathogenesis of recurrent spontaneous abortion have focused on psychosocial factors in a 'psychoneuroendocrine network' that can contribute to pregnancy miscarriage [30,31]. The serum concentration of epinephrine in mice is 561.2 ± 157.4 pg/ml under physiological conditions but increases to 1169.3 ± 340.2 pg/ml after minor stressors such as acute running. Low or high doses of exogenous epinephrine (0.5 mg/kg and 2 mg/kg, respectively) has been applied to mimic the acute running response [32], and 200 μg/mouse exaggerates epinephrine-liganded adrenergic receptor signaling during early pregnancy in mice [15]. In the present study, treatment with 100 μg/mouse epinephrine led to impaired decidualization in both natural pregnancy and pseudopregnancy, affecting both stromal cell proliferation and differentiation. We believe these changes with exogenous epinepherine invoke a physiologically-relevant response. Although the majority of exposed mice in the high-dose group did not carry pregnancy to term, the successful litters were comparable in size to the vehicle and low dose epinephrine groups. These data suggested individual variations in the susceptibility of the outbred CD1 mice evaluated here and may have additional stressors on top of the controlled exposure conditions.

Adrenaline is a potent stimulator of cyclic AMP production in microvascular endothelial cells [33] and uterine smooth muscle [34], but

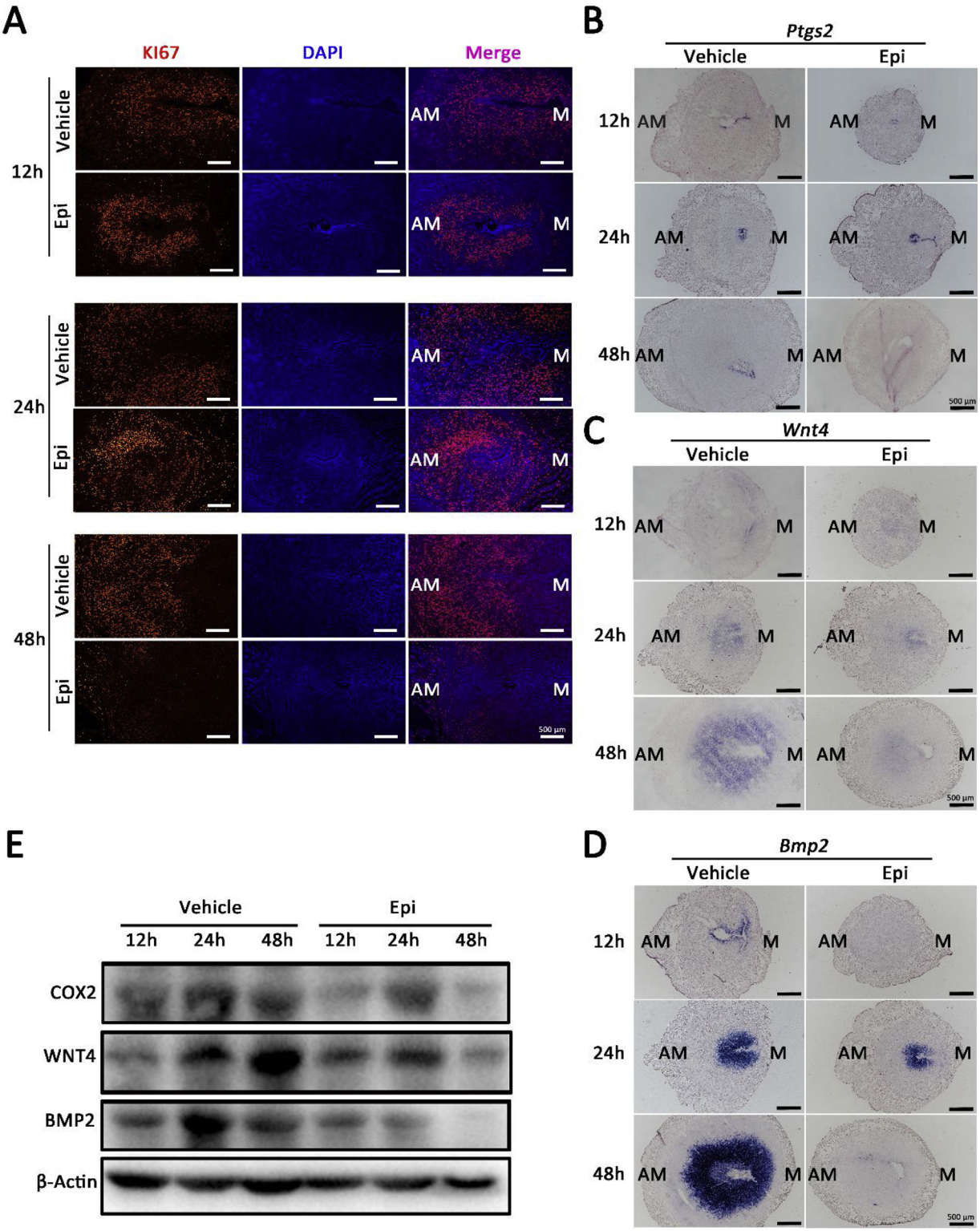

Fig. 5. Exogenous epinephrine hampered uterine stromal cells proliferation and differentiation during decidualization. (A) Detection of cell proliferation by ki67 immunofluorescence in the uteri after oil induced 12 h,24 h and 48 h (bar = 500 μm). (B–D) In situ hybridization revealed the expression pattern of *Ptgs2, Wnt4* and *Bmp2* in the uteri after oil induced 12 h, 24 h and 48 h between vehicle and epinephrine administration (bar = 500 μm). (E) The protein levels of COX2, WNT4 and BMP2 were observed by western blot. The protein levels of these factors were reduced by time-dependent assay with epinephrine administration. Epi, epinephrine; M, mesometrial pole; AM, antimesometrial pole.

the potential roles during decidual morphogenesis is not clear. Previous investigations revealed a peri-implantation adenosine surge in the murine antimesometrium that coincided with expression of ecto-5'-nucleotidase in the primary decidua followed by a precipitous drop in local adenosine between GD 6 and GD 7 coincident with marked expression of adenosine deaminase in the secondary decidua [35]. These changes were accompanied by localized expression of the A2b adenosine receptor within the primary decidua by GD 5, suggesting a role for adenosine signaling during remodeling of the implantation chamber in mice [36]. Our results found strong hybridization of *Adra1b, Adra2b and Adrb2* transcripts within the primary decidua by GD 5, and secondary decidua by GD 7. Cross-talk between these G-protein coupled receptor systems infers complex regulation of second messengers systems such as cyclic AMP in the early postimplantation uterus.

Disruption in Adrb2 signaling affect oviductal embryo transport [11] and spacing at implantation [12]. Our results demonstrate differential expression of adrenergic receptor subtypes in decidual cells after embryo implantation and impairment with exogenous epinephrine. Exaggerated epinephrine-adrenergic receptor signaling has adverse consequences to early post-implantation development, leading to resorption in the pregnant mouse. Variations in individual susceptibility, as observed here, may reflect what is observed in human populations. These findings suggest exaggerated epinephrine-adrenergic receptor signaling is a risk factor for early pregnancy failure and miscarriage, which provided a basic experimental clue in explaining how maternal stress at early stages could adversely affect the early post-implantation uterus. However, the physiological significance of adrenergic receptor function and pathophysiology of pregnancy and embryogenesis warrants further study.

Author contributions

Jinxiang Wu, Ruiwei Jiang, Haibin Wang and Jinhua Lu designed research; Jinxiang Wu, Shuangbo Kong and Chuanhui Guo performed the research; Jinxiang Wu, Jianqi Wang and Ruiwei Jiang analyzed the data; Jinxiang Wu, Shuangbo Kong and Ruiwei Jiang wrote the paper.

Declaration of Competing Interest

The authors declare no conflict of interest.

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