Decidual expression and regulation of fatty acid desaturase 3 during mouse decidualization

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Short title: Fads3 during mouse decidualization

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Decidualization is required for the successful establishment of pregnancy in rodents and primates. Fatty acid desaturase 3 (Fads3) belongs to the fatty acid desaturase family, which is a crucial enzymes for highly unsaturated fatty acid biosynthesis. However, the expression, regulation and function of Fads3 during early pregnancy in mice are still unknown. In this study, we examined Fads3 expression, regulation and function during mouse decidualization. The expression of Fads3 is detected in the subluminal stromal cells at implantation site on day 5 of pregnancy, but not at inter-implantation site and in day 5 pseudopregnant uteri. Compared to delayed implantation, Fads3 is strongly expressed after delayed implantation is activated by estrogen treatment. From days 6 to 8, Fads3 mRNA signals are significantly detected in the decidua. In ovariectomized mice, estrogen significantly stimulates Fads3 expression. However, estrogen has no effect on Fads3 expression in ovariectomized ERα deficient mice, suggesting that estrogen regulation on Fads3 expression is ERα-dependent. When ovariectomized mice were treated with progesterone, Fads3 expression is significantly increased by progesterone. Progesterone stimulation on Fads3 expression is also detected in cultured stromal cells, which is abrogated by RU486 treatment. These data indicate that progesterone upregulation on Fads3 expression is progesterone receptor-dependent. Fads3 knockdown by siRNA reduces in vitro decidualization of mouse stromal cells. Taken together, Fads3 may play an important role during mouse decidualization.

Keywords: Fads3; uterus; progesterone; implantation; decidualization
Embryo implantation and decidualization are essential for the establishment of pregnancy in rodents and primates. After embryo implantation occurs, the stromal cells surrounding the implanting blastocyst begin to proliferate and differentiate into large, round decidual cells. This process is known as decidualization. Impaired decidualization can lead to adverse outcomes such as implantation failure and miscarriage (Carson et al. 2000). In mice, decidualization is stimulated by the blastocyst, and can also be induced by scratching the endometrium or intraluminal injection of oil (Gellersen & Brosens 2014). The establishment of pregnancy is orchestrated by ovarian estrogen and progesterone, which are crucial for implantation and decidualization in mammals (Dey et al. 2004). The physiological effects of estrogen and progesterone are mediated by binding to their cognate receptors, estrogen receptors (ER) and progesterone receptor (PR). ERα knockout mice show ovarian dysfunction and estrogen insensitivity of the uterus (Schomberg et al. 1999). PR knockout mice are infertile due to the defects in embryo implantation and decidualization (Mulac-Jericevic et al. 2000). However, the molecular mechanism during decidualization is still unclear.

Highly unsaturated fatty acids (HUFA), especially arachidonic acid (20:4n-6) and docosahexaenoic acid (DHA, 22:6n-3), are metabolically required for many developmental processes. The dysfunction in the metabolism of highly unsaturated fatty acids will result in cardiovascular disease, cancers, and diabetes (Lee et al. 2016). HUFA, a long chain subgroup of poly unsaturated fatty acids, can be biosynthesized from linoleic acid (LA, 18:2n-6) and α-linolenic acid (ALA, 18:3n-3) (Zhang et al. 2017).

Fatty acid desaturases are essential enzymes that mediate the introduction of double bonds at specific positions in a long-chain fatty acid (Nakamura & Nara 2004). Fatty acid desaturase 1 and 2 (FADS1 and FADS2) catalyze the biosynthesis of HUFA. FADS1 introduces a double bond at the Δ5 position of 20-carbon fatty acids (Kothapalli et al. 2016). FADS2 catalyzes the introduction of a double bond at the Δ4, Δ6 or Δ8 position, which is a rate limiting step for the biosynthesis of eicosapentaenoic acid.
(DHA) (Park et al. 2016). Arachidonic acid is the precursor for the biosynthesis of prostaglandins, prostacyclins, and leukotrienes (Lee et al. 2016). Arachidonic acid can be synthesized from linoleic acid (LA) utilizing three enzymatic steps. Two of these steps are catalyzed by FADS1 and FADS2 (Hester et al. 2014). FADS3 is the third member of the FADS gene cluster and evolutionarily results from a gene duplication event with Fads1 and Fads2 (Zhang et al. 2017). However, FADS3 function remains elusive. Based on the 62% and 70% sequence homology with Fads1 and Fads2, Fads3 may be a new fatty acid desaturase (Blanchard et al. 2011). Recent data indicate that Fads3 plays a role during early development as an enhancer of HUFA biosynthesis and/or regulation (Zhang et al. 2017).

Based on our SAGE analysis, Fads3 expression is significantly higher at the implantation site than inter-implantation in mouse uterus (Ma et al. 2006), suggesting a putative involvement of Fads3 in early pregnancy. However, the expression, regulation, and function of Fads3 in mouse uterus during early pregnancy are still unknown. In this study, we showed that Fads3 is strongly expressed in mouse decidua and up-regulated by progesterone.

**MATERIALS AND METHODS**

**Animal treatments**

Mature CD-1 mice were caged in a temperature- and light-controlled environment (12 h of light and 12 h of dark) with free access to regular food and water. All animal procedures were approved by the Institutional Animal Care and Use Committee of South China Agricultural University.

Adult female mice were mated with fertile or vasectomized males to induce pregnancy or pseudopregnancy (day 1 is the day of vaginal plug). On days 1-4, pregnancy was confirmed by flushing embryos from oviducts or uterus. On day 5, the implantation sites were visualized through intravenous injection of 0.1 ml of 1% Chicago blue dye (Sigma Aldrich, St. Louis, MO, USA) in saline.
Delayed implantation was induced by ovariectomizing pregnant mice at 08:30-09:00 on day 4 of pregnancy. Progesterone was subcutaneously injected (1 mg/mouse, Sigma Aldrich) from days 5 to 7. Estradiol-17 (25 ng/mouse, Sigma Aldrich) was given to progesterone-primed delayed mice to activate blastocyst implantation on day 7 of pregnancy. On day 8 of pregnancy, the mice were sacrificed to collect uteri after estrogen treatment. The activation of implantation sites were identified through intravenous injection of 0.1 ml of 1% Chicago blue dye. Delayed implantation was confirmed by flushing blastocysts from one horn of the uterus on day 8 of pregnancy and uteri were collected for further analysis.

To induce the artificial decidualization, pseudopregnancy mice were infused 10 µl of sesame oil (S3547; Sigma Aldrich) into one uterine horn on day 4, whereas another uninjected horn served as a control. The uteri were collected on day 8 of pseudopregnancy.

To determine the effects of steroid hormones on Fads3 expression, the ovariectomized mice rested for 2 weeks to eliminate circulating ovarian steroids. Mice were injected subcutaneously with estradiol-17β (100 ng/mouse) or progesterone (1 mg/mouse). The control mice were injected subcutaneously with sesame oil (100 µl/mouse). Mice were sacrificed and the uteri were collected after the hormone injections for 24h.

To estimate whether Fads3 expression was ERα-dependent, wild-type (WT) or ERα knock-out (ERα KO) mice were ovariectomized and rested for 2 weeks and then injected subcutaneously with estradiol-17β(100 ng/mouse). The control mice were treated with sesame oil as vehicle. The uteri were collected after the estradiol-17β injections for 24h.

In Situ Hybridization

Total RNAs from mouse uteri on day 5 of pregnancy were reverse transcribed and amplified with the specific primers for mouse Fads3. The amplified fragment of Fads3 was cloned into pGEM-T plasmid,
and verified by sequencing. The primers of T7 and SP6 were used to amplify Fads3 fragment from the pGEM-Fads3 plasmids. Digoxigenin-labeled antisense or sense cRNA probes were transcribed in vitro using a digoxigenin RNA labeling kit (Roche Applied Science, Indianapolis, IN, USA).

As previously described (Ding et al. 2018), frozen sections (10 µm) were mounted on 3-aminopropyltriethoxysilane (A3648; Sigma Aldrich)-treated slides and fixed in 4% paraformaldehyde solution in PBS. Hybridization was performed at 55 °C for 16 h. Digoxigenin-labeled Fads3 sense probe was used as negative control. Following hybridization and post-hybridization washes, sections were incubated with sheep anti-digoxigenin antibody conjugated to alkaline phosphatase (1:5,000; Roche Applied Science). Endogenous alkaline phosphatase activity was inhibited with 2 mM levamisole. All of the sections were counterstained with 1% methyl green. The positive signal was visualized as a dark brown color.

**Isolation and culture of uterine stromal cells**

Mouse uterine stromal cells were isolated as described previously (Liang et al. 2014). Briefly, uterine horns from day 4 pregnant mice were split longitudinally, washed with Hanks’ balanced salt solution (HBSS; Sigma), and digested with 1% (w/v) trypsin (Amresco, Cleveland, USA) and 6 mg/ml dispase (Roche Applied Science) in HBSS for 1 h at 4 °C followed by 1 h at room temperature and 10 min at 37 °C. After rinsing 3 times with HBSS, the remaining tissues were incubated in 6 ml of HBSS containing 0.15 mg/ml collagenase I (Invitrogen, Waltham, MA, USA; 17100-017) at 37 °C for 30 min. The digested tissues were shaken and filtered through a 70 µm wire gauze filter and centrifuged to collect the stromal cells. The isolated cells were grown in DMEM/F-12 medium containing 2% heat-inactivated fetal bovine serum (FBS, Biological Industries, Cromwell, USA). After an initial culture for 1 h, the medium was changed to remove unattached epithelial cells. The stromal cells were cultured in DMEM/F-12 (D2906; Sigma-Aldrich) containing 10% (v/v) charcoal-stripped FBS (cFBS, Biological Industries). To induce in vitro decidualization, cells were treated with 10 nM estrogen and 1 µM progesterone in DMEM/F12 containing 2% cFBS for different time points.
Stromal cells were also treated with 1 µM of progesterone for 24 h. For RU486 treatment, cells were pretreated with 1 µM of RU486 (Cayman, Ann Arbor, USA) or JNK kinase inhibitor (Cayman) 1 h and then treated with progesterone.

**siRNA transfection**

The siRNA for mouse Fads3 and nonspecific siRNA were designed and synthesized by Ribobio Co., Ltd. (Guangzhou, China). The stromal cells were transfected with siRNA using Lipofectamine 2000 (Invitrogen) according to the manufacturer’s protocol. After cells were transfected for 6 h, the stromal cells were induced for *in vitro* decidualization for 48 h.

**Real-time PCR**

Total RNAs were extracted from the whole uteri or cultured cells by using TRIZOL (TaKaRa, Dalian, China) and reverse transcribed into cDNA by following the PrimeScript reverse transcriptase reagent kit (TaKaRa). Then real-time PCR was performed with SYBR Premix Ex Taq kit (RR820A TaKaRa) on the CFX96 TOUCHTM (BIO-RAD, Hercules, USA). The specific Primer sequences of each gene used for real-time were provided in Table 1. The $2^{\Delta\Delta C_{T}}$ method was used to analyze the data from real-time PCR.

**Western blot analysis**

Western blot was performed as described previously (Liang *et al.* 2014). Briefly, proteins for Western blot were extracted from cultured stromal cells by homogenization lysis buffer including 50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 1% Triton X-100, and 0.25% sodium deoxycholate. The concentration of proteins was measured by BCA kit (Applygen, Beijing, China). The samples (10 µg per lane) were separated by 10% SDS-PAGE gel and transferred onto PVDF membranes (Millipore, German) followed by blocking with 5% non-flat milk (Sangon, Shanghai, China). PVDF membranes were probed with the corresponding antibodies for FADS3 (ABclonal, Wuhan, China) and β-Actin (Cell Signaling, Danvers, USA) overnight at 4°C. Membranes were then incubated with the matched
secondary antibodies conjugated with HRP (1:5000). Signals were detected with the ECL kit (Pierce Biotechnology, Rockford, USA).

**Statistical analysis**

All of the experiments were independently repeated at least 3 times. The difference between two groups was compared by Student’s t test. The multiple comparisons were performed with one way ANOVA. Data were presented as the mean ± standard error. In all cases, p<0.05 was considered significantly different.

**RESULTS**

**Fads1, Fads2 and Fads3 mRNA expression during early pregnancy**

In situ hybridization was used to examine the spatial distribution of Fads1, Fads2 and Fads3 mRNA in mouse uteri during early pregnancy. There were no visible Fads1 and Fads2 mRNA signals in the uteri from days 1 to 4 of pregnancy. On day 5 of pregnancy, Fads1 and Fads2 mRNA signals were weakly detected in the subluminal stromal cells surrounding the implanting blastocyst at implantation sites. On days 6 and 8, Fads1 and Fads2 mRNA signals were faintly detected in the decidua (Fig. 1).

From days 1 to 4 of pregnancy, there was no visible Fads3 mRNA signal in the uteri. On day 5 of pregnancy, Fads3 mRNA was detected specifically in the subluminal stromal cells surrounding the implanting blastocyst at implantation sites (Fig. 1). On days 6 and 8, Fads3 mRNA signals were highly detected in the decidua (Fig. 1).

Because the mRNA signals for Fads1 and Fads2 were weakly detected in mouse uteri from days 1 to 8 of pregnancy, no further analysis was performed on Fads1 and Fads2.

**Fads3 mRNA expression during pseudopregnancy, delayed and activated implantation**
Because Fads3 was strongly detected at implantation sites, we also checked Fads3 expression at inter-implantation sites. However, there was no detectable signal at inter-implantation sites (Fig. 2A). Data from real-time RT-PCR also showed that Fads3 level at implantation site was significantly higher than that at inter-implantation sites on day 5 of pregnancy (Fig. 2B).

To further address whether the expression of Fads3 is dependent on the embryo, we examined the expression of Fads3 during pseudopregnancy. There was no detectable Fads3 signal in mouse uteri on day 5 of pseudopregnancy (Fig. 2A).

Delayed implantation model was applied to assess whether Fads3 expression is regulated by active blastocyst. When embryo implantation was delayed by ovariectomy, there was no detectable Fads3 mRNA signal in the uteri. After delayed implantation was terminated by estrogen, Fads3 mRNA was detected in the subluminal stromal surrounding the blastocyst of implantation sites (Fig. 2A).

Fads3 mRNA expression during artificial decidualization

Because Fads3 expression was strongly detected in the decidua on day 8 of pregnancy, real-time RT-PCR was used to verify Fads3 expression. Compared to inter-implantation site, Fads3 expression level was significantly higher at implantation sites on day 8 of pregnancy (Fig. 3A). Then artificial decidualization model was used to determine whether Fads3 is induced by oil injection. There was no detectable Fads3 mRNA signal in the control uterine horn, while a high level of Fads3 mRNA expression was detected in the deciduoma of oil-injected uterine horn (Fig. 3B). Real-time PCR results also showed that Fads3 mRNA signals were strongly up-regulated in deciduoma compared with control (Fig. 3C).

Steroid hormonal regulation on Fads3

Because estrogen and progesterone are crucial for mouse decidualization (Dey et al. 2004), ovariectomized mice were used to determine whether Fads3 expression was regulated by estrogen and progesterone. After ovariectomized mice were treated with estrogen, Fads3 expression was weakly
detected in the luminal epithelium and stromal cells compared with control (Fig. 4A). Real-time PCR analysis showed that Fads3 mRNA level was remarkably enhanced compared with control uteri. However, estrogen had no stimulating effect on Fads3 expression in ovariectomized ERα knockout mice (Fig. 4B), suggesting that estrogen regulation of Fads3 expression is ERα-dependent.

When ovariectomized mice were treated with progesterone, Fads3 mRNA signals were weakly seen in uterine luminal epithelium and stromal cells (Fig. 4A). Data from real-time PCR indicated that Fads3 expression was significantly increased compared with control (Fig. 4C). Furthermore, Fads3 expression was also obviously increased when cultured stromal cells were treated with progesterone, which was abrogated by RU486 treatment (Fig. 4D), suggesting that progesterone regulation of Fads3 expression is progesterone receptor-dependent. In the cultured stromal cells, the expression of Fads3 was inhibited by JNK kinase inhibitor, and progesterone induction on Fads3 expression was slightly inhibited by the inhibitor for JNK kinase (Fig. 4E).

**Fads3 mRNA expression during *in vitro* decidualization**

Because Fads3 is strongly expressed in the decidual cells, in vitro decidualization model was used to examine whether Fads3 is involved in mouse decidualization. After stromal cells were induced for *in vitro* decidualization, there was a significant induction of Dtprp, Prl3c1 and Bmp8a (Fig. 5), reliable markers for mouse *in vitro* decidualization (Bany & Cross 2006; Kelleher *et al.* 2017; Kimura *et al.* 2001; Li *et al.* 2017). Meanwhile, Fads3 expression was also significantly induced under in vitro decidualization for 12, 24, 48 and 72 h, respectively (Fig. 5B).

To investigate the function of Fads3 during decidualization, the expression of Fads3 was silenced by the specific siRNA. After Fads3 expression was knockdowned by Fads3 siRNA (Fig. 5C-D), there was a remarkable reduction for the expression levels of Dtprp, Prl3c1 and Bmp8a (Fig. 5E-G). Meanwhile, based on *in situ* hybridization, Fads3 shares a co-localization with Abp1, which is essential to mouse embryo implantation and decidualization (Liang *et al.* 2010). In this study, Abp1
expression was remarkably reduced following Fads3 siRNA treatment (Fig. 5H).

Among prostaglandins synthesized from arachidonic acid, PGE2 has been proved to be essential to decidualization (Kennedy et al. 2007). When stromal cells were treated with PGE2 under in vitro decidualization, PGE2 had no obvious effects on Fads3 expression (Fig. 6A). Although PGE2 had no stimulating effects on both Dtprp and Prl3c1 levels, PGE2 was able to rescue the inhibitory effect of Fads3 siRNA on both Dtprp and Prl3c1 levels (Fig. 6B-C).

**DISCUSSION**

In this study, we showed that Fads3 is strongly expressed in the decidual cells at implantation sites. Furthermore, Fads3 expression is not detected at inter-implantation sites, in day 5 pseudopregnant uterus and under delayed uterus, suggesting that Fads3 expression is embryo-dependent during peri-implantation period.

Under in vitro decidualization, knockdown of Fads3 expression by siRNA leads to a significantly reduction of Dtprp, Bmp8a and Prl3c1, which are reliable markers for mouse in vitro decidualization (Bany & Cross 2006; Kelleher et al. 2017; Kimura et al. 2001; Li et al. 2017). These data suggest that Fads3 may play a role during mouse decidualization. Based on the high homology with FADS1 and FADS2, Fads3 should be important for arachidonic acid (Pedrono et al. 2010). Our previous study showed that arachidonic acid can activate the cytosolic phospholipase A2a (cPLA2a)/cyclooxygenase-2 (COX-2) pathway, which is essential during embryo implantation and decidualization in mouse uterus (Zhao et al. 2012). cPLA2a catalyzes arachidonic acid release from membrane phospholipids (Murakami et al. 2000). cPLA2a is crucial for successful pregnancy because of cPLA2a KO mice show small litters, abnormal uterine spacing of embryos and often exhibit pregnancy failures (Bonventre et al. 1997, Song et al. 2002). Conditional deletion of mouse uterine Trp53 (p53(d/d)), leads to premature uterine senescence and preterm birth, and also causes an increase in arachidonic and docosahexaenoic acid (Lanekoff et al. 2016). These data suggest that a balance in
arachidonic acid should be essential for decidual development. It is possible that Fads3 is involved in mouse decidualization through arachidonic acid production.

Furthermore, arachidonic acid can be converted to prostaglandin H2 (PGH2) by COX2, a crucial rate-limiting enzyme. COX2 null mice resulted infertility with multiple defects in ovulation, embryo implantation and decidualization in female reproduction. These defects can be restored by exogenous prostaglandin administration in both of cPLA2α and COX2 null mice (Lim et al. 1997, Song et al. 2002). Indomethacin, an inhibitor of COX2, can suppress the generation of PGE2 and result in embryo implantation failure (Hamilton & Kennedy 1994). PGE2 is essential to decidualization (Kennedy et al. 2007). In this study, PGE2 is able to rescue the inhibitory effect of Fads3 siRNA on both Dtprp and Prl3c1 levels although PGE2 has no stimulating effect on Fads3 expression, suggesting that Fads3 may be involved in PGE2 synthesis. Additionally, our previous study reported that Afp1 mRNA is highly expressed in the decidual cells in mouse uterus and plays a role during mouse decidualization (Liang et al. 2010). Based on data from in situ hybridization, Fads3 is co-localized with Afp1 in the primary decidual zone in mouse uterus, suggesting that Fads3 may have a connection with Afp1 during mouse decidualization. In this study, knockdown of Fads3 expression by siRNA leads in a significant reduction on Afp1 expression during in vitro decidualization, suggesting that Fads3 may regulate mouse decidualization through Afp1.

In our study, the levels of Dtprp, Prl3c1 and Bmp8 are partially inhibited by Fads3 siRNA. The failure of complete inhibition of decidualization may be caused by the partial inhibition of Fads3 siRNA on Fads3 expression and the possible involvement of both Fads1 and Fads2. Both Fads1 and Fads2 are also weakly expressed in the primary decidual zone on day 5 of pregnancy. It is known that FADS1 and FADS2 participate in the biosynthesis of arachidonic acid from linoleic acid (Hester et al. 2014). However, the molecular mechanism of Fads3 action needs to be further explored.

Estrogen is crucial for blastocyst implantation in rodents (Zhang et al. 2013). Conditional knock-out ERα in mouse uteri results in implantation failure (Mulac-Jericevic et al. 2000). Our study showed...
that Fads3 expression level was significantly higher after ovariectomized mice were treated with estrogen. However, estrogen has no effects on Fads3 expression in ERα-deficient ovariectomized mice, suggesting that estrogen regulates Fads3 expression via ERα in mouse uteri.

In this study, progesterone stimulated Fads3 expression in ovariectomized mouse uterus and cultured uterine stromal cells, which was abrogated by progesterone receptor antagonist RU486. These results indicate that progesterone stimulates Fads3 expression though PR. Recent studies showed that Fads3 is a target gene for MYCN, NK-kB or p63 transcription factors, which could be involved in cell proliferation, differentiation and apoptosis (Yan et al. 2007, Gu et al. 2008). Decidualization is characterized by stromal cells proliferation, differentiation and apoptosis. Furthermore, within the mouse mammary gland, NF-kB is up-regulated by progesterone. NF-kB is also a regulator of COX2 expression in many cell types (Schmedtje et al. 1997). It is possible that progesterone may regulate Fads3 through NF-kB. We also showed that JNK-1 may be involved in progesterone regulation on Fads3 because progesterone induction of Fads3 is decreased by JNK-1 inhibitor.

In conclusion, Fads3 is strongly expressed in the decidual cells and regulated by estrogen or progesterone. Knockdown of Fads3 can reduce decidualization.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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**Figure legends**

**Figure 1.** In situ hybridization of Fads1, Fads2 and Fads3 mRNA in mouse uteri during early pregnancy, showing the localization of Fads1, Fads2 and Fads3 mRNA in mouse uteri on days 1 (D1), days 4 (D4), days 5 (D5), days 6 (D6) and 8 (D8). On each day of pregnancy, 3-5 mice were used. At least 3 implantation sites per mouse were used for *in situ* hybridization and a representative section was shown. Asterisk (*), embryo; Bar=300 µm.

**Figure 2.** Expression of Fads3 mRNA during pseudopregnancy and under delayed implantation. (A) *In situ* hybridization of Fads3 expression at inter-implantation site on day 5 of pregnancy (D5 NI), on day 5 of pseudopregnancy, and under delayed implantation and activated uteri. D5 NI, inter-implantation site on day 5; PD5, day 5 of pseudopregnancy; Asterisk (*), embryo. Bar=300 µm. Three mice were used in each group. At least 3-5 sections from each mouse were used for *in situ* hybridization. A representative section was shown. (B) Real-time PCR analysis of Fads3 expression between inter-implantation and implantation on day 5. D5 IS, implantation site on day 5. Uteri from 3 mice were used for real time PCR. Three independent repeats were performed.

**Figure 3.** Expression of Fads3 mRNA during artificial decidualization. (A) Real-time PCR analysis of Fads3 expression between inter-implantation and implantation on day 8. D8-NI, inter-implantation site on day 8; D8-IS, implantation site on day 8. (B) *In situ* hybridization of Fads3 expression under artificial decidualization. Bar=300 µm. (C) Real-time PCR analysis of Fads3 expression under artificial decidualization. Uteri from 3 mice were used for real time PCR. Three independent repeats were performed. At least 3-5 sections from each mouse were used for *in situ* hybridization. A representative section was shown.

**Figure 4.** Steroid hormones regulation on Fads3 mRNA expression. (A) *In situ* hybridization of Fads3 expression after ovariectomized mice were treated with sesame oil, estrogen, and progesterone, respectively. (B) Real-time PCR of Fads3 mRNA expression after WT or ER KO ovariectomized mice
were injected with estrogen. (C) Real-time PCR of Fads3 mRNA expression after WT ovariectomized mice were injected with progesterone. (D) Real-time PCR of Fads3 mRNA expression after stromal cells were treated with progesterone, or progesterone plus RU486. Con, control; E, estrogen; P, progesterone; group P+RU486, Co-treatment with progesterone plus RU486. (E) Effects of JNK inhibitor on progesterone-induced Fads3 expression. Stromal cells were pretreated with JNK inhibitor for 1 h and then treated with progesterone. Three mice were used in each group. At least 3-5 sections from each mouse were used for in situ hybridization. A representative section was shown. All experiments were repeated at least 3 times.

**Figure 5.** Expression and function of Fads3 under *in vitro* decidualization. (A) Real-time PCR of Dtprp mRNA expression under *in vitro* decidualization from 12 h to 72 h. (B) Real-time PCR of Fads3 mRNA expression during *in vitro* decidualization. Fads3 expression was obviously up-regulated compared to the control from 12 h to 72 h. (C) Fads3 mRNA expression after stromal cells were transfected with Fads3 siRNA and the nonspecific siRNA (NC) under *in vitro* decidualization for 48 h. (D) Western blot analysis showed that Fads3 protein level was significantly reduced by Fads3 siRNA. (E) Effects of Fads3 siRNA on Dtprp expression. (F) Effects of Fas3 siRNA on Prl3c1 expression. (G) Effects of Fads3 siRNA on Bmp8a expression. (H) Effects of Fads3 siRNA on Abp1 expression. Con, control; group E+P, Co-treatment with estrogen plus progesterone. Stromal cells from 3-6 mice were isolated, pooled and used for cell culture and treatments in triplicates. All experiments were repeated at least 3 times.

**Figure 6.** Effects of PGE2 on Fasd3 action during in vitro decidualization. (A) PGE2 has no effects on Fads3 expression. (B) PGE2 has no stimulating effects on Dtprp, but can rescue the inhibitory effect of Fads3 siRNA on Dtprp level. (C) PGE2 has no stimulating effects on Dtprp, but can rescue the inhibitory effect of Fads3 siRNA on Prl3c1. Stromal cells from 3-6 mice were isolated, pooled and used for cell culture and treatments in triplicates. All experiments were repeated at least 3 times.
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Figure 1

209x260mm (300 x 300 DPI)
Figure 2

209x297mm (300 x 300 DPI)
Figure 3

209x297mm (300 x 300 DPI)
Figure 4

209x297mm (300 x 300 DPI)
Figure 5

209x297mm (300 x 300 DPI)
Figure 6

The mRNA relative expression of Fads3

The mRNA relative expression of Dppp

The mRNA relative expression of Pck1

209x297mm (300 x 300 DPI)