The high concentration of progesterone is harmful for endometrial receptivity and decidualization

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Progesterone is required for the establishment and maintenance of mammalian pregnancy and widely used for conservative treatment of luteal phase deficiency in clinics. However, there are limited solid evidences available for the optimal timing and dose of progesterone therapy, especially for the possible adverse effects on implantation and decidualization when progesterone is administrated empirically. In our study, mouse models were used to examine effects of excess progesterone on embryo implantation and decidualization. Our data indicate that excess progesterone is not only harmful for mouse implantation, but also impairs mouse decidualization. In excess progesterone-treated mice, the impaired LIF/STAT3 pathway and dysregulated endoplasmic reticulum stress may lead to the inhibition of embryo implantation and decidualization. It is possible that the decrease in birth weight of excess progesterone-treated mice is due to a compromised embryo implantation and decidualization. Furthermore, excess progesterone compromises in vitro decidualization of human endometrial stromal cells.
unstimulated cycles\textsuperscript{13}. More studies should be done to determine the optimal dosage and possible adverse effects on implantation and decidualization when P is used empirically in clinical trial for luteal phase support\textsuperscript{18}.

On the other hand, ovarian stimulation program is routinely used to induce multiple ovulation in human in vitro fertilization (IVF), which inevitably leads to ultra-physiological level of P on the day of hCG administration, defined as ‘premature luteinization’. Premature luteinization may be caused by multiple follicles, the overdose of gonadotropins and poor ovarian response. The frequency of elevated serum P level varies between 5% and 38% due to the discrepancy on stimulation regimen, method of P assessment and P cut-off level\textsuperscript{19}. It is still controversial whether the high P serum level at the end of follicular phase has any adverse impacts on ongoing pregnancy outcome\textsuperscript{20}. Several studies show that there is no significant difference on IVF pregnancy outcome between normal and high P serum level (\(\geq 0.9\ \text{ng/ml}\)) on hCG day\textsuperscript{21,22}. However, premature P elevation (\(\geq 1.5\ \text{ng/ml}\)) in stimulated IVF cycles seems to have a detrimental influence on the pregnancy outcome\textsuperscript{23–25}. Therefore, further evidences are badly needed to clarify these controversial issues.

In this study, the effects of P at different concentrations on embryo implantation and decidualization were evaluated in mouse models. Effects of excess P on human in vitro decidualization were also examined. Our data suggested that endometrial receptivity and decidualization are compromised by a high level of P in mice, and human in vitro decidualization is also impaired by supplementation of excess P.

### Results

#### Effects of excess P on mouse endometrium receptivity.
Leukemia inhibitory factor (LIF) is strongly expressed in the glandular epithelium and required for mouse implantation\textsuperscript{26}. The phosphorylation of Stat3, as a receptivity marker on day 4 of pregnancy in mice, is at the downstream of LIF\textsuperscript{27}. Therefore, pregnant mice were treated with 1.4 and 8 mg P/mouse on day 3 9:00, compared to control, the level of LIF mRNA expression on day 4 9:00 was inhibited by 4 or 8 mg/mouse P (Fig. 1A). Accordingly, the level of phosphorylated Stat3 in the luminal epithelium was sharply decreased on days 4 of pregnancy after day 3 pregnant mice were treated with 4 mg P/mouse, (Fig. 1B and C).

When pregnant mice are treated with 4 mg P/mouse on days 3 and 4, the number of implantation sites are significantly reduced compared to control at midnight on day 4 of pregnancy (Fig. 1D and E). To evaluate whether excess P has any negative effect on embryo development, then we examined the blastocyst development at the 14:00 of day 4. The morphology of blastocysts from different dose of P treated mice is normal and similar to vehicle control (Fig. 1F).

#### Effects of excess P on the expression of PR, estrogen receptor (ER) and P target genes.
Because P executes its function through PR, effects of excess P on PR expression were examined. The levels of total PRB and PRAB expression were reduced by 4 or 8 mg/mouse P, not by 1 mg/mouse P (Fig. 2A and B). Compared to control, the level of PR immunostaining was also decreased by 4 mg P/mouse (Fig. 2C).

In mouse uterus, P inhibits its estrogen-induced cell proliferation\textsuperscript{28}. Therefore, effects of excess P on ER were also examined. Compared to control, ER immunostaining was slightly inhibited in 4 mg P-treated mouse uterus (Fig. 2C).

Ihh and Areg are P target genes and essential for mouse embryo implantation\textsuperscript{29–31}. When day 3 pregnant mice were treated with different concentrations of P, the levels of both Ihh and Areg were obviously downregulated by 4 or 8 mg/mouse P. Ihh expression was also reduced by 1 mg/mouse P (Fig. 2D and E).

#### Effects of excess P on mouse decidualization and birth weight.
In order to analyze effects of excess P on mouse decidualization, day 3 pregnant mice were treated with different doses of P daily (from days 3 to 7). Compared to control, the weight of implantation sites on day 8 was significantly declined by 1, 4 and 8 mg/mouse P (Fig. 3A and B). In order to exclude effects of excess P on embryonic development, pseudo-pregnant mice under artificial decidualization were treated with 4 mg/mouse P. Treatment of 4 mg/mouse P caused a significant decrease on the weight of decidualoma (Fig. 3C and D). To further verify effects of excess P on mouse decidualization, mouse stromal cells under in vitro decidualization were treated with P. Under in vitro decidualization, Dttprp, a marker for mouse decidualization\textsuperscript{32}, was significantly induced, while Dttpr expression was significantly suppressed by 4 and 20 \(\mu M\), but not by 0.8 \(\mu M\) P (Fig. 3E).

Because treatment of excess P during early pregnancy had significant effects on embryo implantation and decidualization, we would like to explore whether these effects during early pregnancy affect the whole pregnant outcome. After day 3 pregnant mice were treated with 1, 4 and 8 mg/mouse P daily for 5 days from days 3 to 7, respectively, the birth weight of P treated mice was significantly reduced by 1 and 8 mg/mouse P, not by 4 mg/mouse P (Fig. 3F).

#### Effects of excess P on the expression of P and estrogen target genes in ovarioctomized mice.
After ovarioctomized mice were treated with different concentrations of P daily for 3 days, real time PCR was performed to analyze gene expression. The expression levels of total PRAB (Fig. 4A) and PRB (Fig. 4B) were significantly inhibited by different concentrations of P. Both of Ihh (Fig. 4C) and Areg (Fig. 4D) were up-regulated by 2, 4 and 8 mg/mouse P. However, estrogen target gene LTF was significantly suppressed by different concentrations of P (Fig. 4E).

#### Effects of excess P on endoplasmic reticulum stress.
ER stress is shown to be required for mouse decidualization\textsuperscript{33}. Ovarioctomized mice were treated with different concentrations of P to examine its effects on ER stress. In P-treated uterus, endoplasmic reticulum stress was activated, especially for GRP78/p-eIF2α/ATF4 pathway (Fig. 5A). P treatment had little effects on IRE1α/XBP1 pathway. Spliced XBP1 (sXBP1) mRNA level didn’t show obvious changes following P treatments (Fig. 5B). Previous investigation indicated that GRP78/IRE1α/XBP1 pathway is physiologically activated in mouse decidualization\textsuperscript{34}. Then in order to analyze if excess P has any effect on ER stress of day 8 uteri, day 3 pregnant mice were treated with 4 mg/mouse P daily from days 3 to 7 for 5 days, we found that GRP78/eIF2α/ATF4 pathway was aberrantly upregulated by excess P in decidua of day 8 (Fig. 5C).

Similarly, excess P didn’t show any obvious effects on IRE1α/XBP1 pathway because spliced Xbp1 remained unchanged following P treatments (Fig. 5D).
Effects of excess P on human in vitro decidualization. In mice, we showed that decidualization was impaired by excess P treatments. Then we would like to examine effects of excess P on human in vitro decidualization. Under human in vitro decidualization, there was a significant increase for the expression levels of IGFBP-134.
FOXO1 and PLZF, the well-known markers for human in vitro decidualization. At the same time, the expression levels of IGFBP-1, FOXO1 and PLZF were significantly suppressed by excess P in a dosage-dependent manner (Fig. 6A–C).
Discussion

The endometrium is a highly hormone responsive tissue. Under the influence of steroid sex hormones, the endometrium undergoes dynamic changes prepared for embryo implantation and decidualization. Estrogen and P are the major mediators for embryo implantation and decidualization. Estrogen is critical for determining the endometrium's responsiveness to P, which is essential for successful pregnancy. The balance of estrogen and P in the endometrium is finely tuned to support embryo implantation and decidualization, ensuring the proper environment for the developing embryo. Understanding these hormonal interactions is crucial for reproductive health and the success of assisted reproductive technologies.
the duration of implantation window. A high level of estrogen will lead to the close of implantation window\(^9\). Estrogen administration during early gestation can disrupt implantation\(^40\). The increased estrogenic responses caused from uterine deletion of gp130 or Stat3 also result in implantation failure\(^41\). Although effects of excess estrogen on receptivity and decidualization have been extensively explored, whether excess P affects receptivity and decidualization remains to be clarified. P is widely used to treat women with threatened abortion for maintaining pregnancy. However, up to now, there is no real consensus on the timing, dose and routes of P administration\(^42,43\).

LPD ubiquitously exists in IVF with controlled ovarian hyper-stimulation and other pathological conditions. The aetiology and diagnosis criterion of LPD are still not well established in current clinics\(^44\). It is empirical for

Figure 4. Effects of excess P in ovariectomized mice. Ovariectomized mice were treated with oil or different concentrations of P daily for 3 days and the relative mRNA expression of these genes to GAPDH were detected by real-time PCR. (A) PRAB. (B) PRB. (C) Ihh. (D) Areg. (E) Ltf. The real-time values are normalized to the Rpl7 expression level and indicated as the mean ± SEM. n = 3. *P < 0.05.
clinicians to treat all the possible LPD with P for that they may ignore the adverse effects of excessive supplementation of P on pregnancy. Almost all of current evidence suggests that luteal phase support using P can play positive effects on main pregnancy outcome. While studies conducted by Kyrou et al. don't show any improvement of pregnancy rates in women received routine P supplementation. In spite of the potential risks for excess P supplementation, little attention has been paid to the possible detrimental impacts of excess P supplementation on pregnancy outcome. Our data showed that excessive P dramatically destroys decidualization process in mice and humans in a dosage-dependent manner, which may give a reference on the clinical use dosage of P. The side-effects of excessive administration of P on the reproduction outcomes should be carefully taken into consideration.

There are accumulating evidences indicating that premature P over 1.5 ng/ml in stimulated IVF cycles seems to have detrimental influences on the pregnancy outcome. High P level (1.7 ng/ml) before oocyte retrieval is associated with an obvious reduction of endometrial receptivity. The gene expression profile of the endometrium is indeed affected when P level is above 1.5 ng/ml at the end of the follicular phase. Elevated P levels on the day of hCG during the initial fresh cycle are correlated with poor pregnancy in the fresh transfer cycles but not in subsequent frozen-thawed embryo transfer cycles. A previous retrospective study including 4,106 IVF/ICSI cycles reported that patients with P ≥ 2 ng/mL exhibit more high-quality embryos than patients with P < 1 ng/mL, while our data show that excess P has no detrimental effect on embryo development, which suggests that it is endometrium, not the oocyte, that is compromised by P elevation. In a recent study, women in GnRH down-regulation cycles were treated with different dose of P (2.5, 5, 10, 40 mg/day) and compared to control group female with normal ovulation. The high dose of P (≥5 mg/day) is harmful for endometrium receptivity due to aberrant gene expression in spite of a normal histology. Our data also showed a harmful impact on mouse receptivity and decidualization in excess P group, in addition to impaired decidualization of human endometrial stromal cells in vitro.

In our study, excess P-treated mice exhibit a lower birth weights than control, which is in line with previous studies that neonatal birth weights are lower in fresh blastocyst transfer cycles after controlled ovarian stimulation than in frozen-thawed embryo transfer cycles without ovarian stimulation. Another latest retrospective analysis indicates that in fresh embryo transfers cycle, patients with elevated P levels (>2.0 ng/mL) suffer from lower birth weight compared to P levels ≤ 2.0 ng/mL counterparts. The cumulating evidences indicate that it is not advisable to perform embryo transfer for patients with high levels of P in fresh cycle.

Successful implantation requires a synchronous cross-talk between a competent blastocyst and a receptive endometrium. The primary masters that coordinate the endometrium receptivity are estrogen and P. In natural mouse reproduction cycle, uterine epithelial cell proliferation is stimulated by a pre-ovulatory estrogen. P secreted from newly formed corpus luteum enhances uterine stromal cell proliferation. The combined actions of

**Figure 5.** Effects of excess P on endoplasmic reticulum stress. (A) The protein expressions of GRP78, p-IRE1α, p-eIF2α and ATF4 of ovariectomized mice treated with oil or different concentrations of P daily for 3 days were detected by Western blot. (B) The mRNA expression of XBP1 of implantation sites in mice treated with oil or 4 mg/mouse P daily for 3 days were detected by agarose gel electrophoresis. (C) GRP78, p-IRE1α, p-eIF2α and ATF4 levels of implantation sites in mice treated with oil or 4 mg/mouse P daily from days 3 to 7 were detected by Western blot. (D) The mRNA expression of spliced and un-spliced XBP1 of implantation sites in mice treated with oil or 4 mg/mouse P daily from days 3 to 7 were detected by agarose gel electrophoresis.
P4 and estrogen is required to establish receptivity for implantation. In our study, Lif expression and p-Stat3 immunostaining were inhibited by excess P. LIF expression is also downregulated following ablation of epithelial PR or PRA overexpression in whole uterus or uterine epithelium. However, ER immunostaining was reduced by excess P. These evidences suggest that the downregulation of PR and ER may contribute to the decrease of Lif expression.

As an estrogen-responsive gene, LIF is crucial for uterine receptivity and implantation, for that its deletion leads to implantation failure in mice. There is a decline of LIF in endometrial glandular epithelium of women with recurrent implantation failure after IVF. As a direct downstream target of LIF, signal transducer and activator of transcription 3 (STAT3) is phosphorylated during the establishment of uterine receptivity. Implantation is impaired when STAT3 phosphorylation is inhibited or uterine conditional deletion of STAT3 is performed. It is reported that endometrial p-STAT3 is reduced in some women with unexplained infertility. In our study, a significant reduced expression of LIF and p-STAT3, resulted from excess P treatment, may underline the deficiency in embryo implantation.

Indian hedgehog (Ihh) has been identified as a target gene of P and is expressed in epithelium, mediating epithelial-mesenchymal interactions in the mouse uterus. Conditional knockout of Ihh in the murine uterus results in infertility for defective embryo implantation and decidualization. Another P regulated gene, amphiregulin (Areg), has also been identified as a receptivity marker for implantation. In our study, Ihh and Areg are conspicuously down-regulated by excess P in mouse uterus on day 4 pregnancy, indicating a compromised endometrium receptivity.

Perturbation of endoplasmic reticulum (ER) protein homeostasis leads to the accumulation of misfolded proteins in its lumen and subsequently causes a stress that is called ER stress, consisting of three pathways: glucose regulated protein 78 (GRP78)/inositol requiring enzyme 1 alpha (IRE1a)/X-box protein 1 (XBPI) signaling pathway, activating transcription factor 6 (ATF6) pathway and pancreatic ER kinase (PERK)/eukaryotic translation initiation factor 2a (eIF2a)/activating transcription factor 4 (ATF4) pathway. GRP78/IRE1a/
XBP1 pathway is activated and essential during mouse decidualization. Excessive or chronic ER stress is harmful to mouse decidualization. Sustained endoplasmic reticulum stress-induced apoptosis in decidualization may play an ignominious role in early pregnancy loss. The markers of ER stress, GRP78, IRE1α, and spliced XBP1 (sXBP1), are significantly increased in fetal membranes and myometrium after term and preterm labor. Excessive potentiation of uterine ER stress fails to maintain uterine caspase-3 and 7 levels, leading to preterm birth. Our results showed that excess P can promote ER stress by predominantly up-regulating GRP78/eIF2α/ATF4 pathway in ovariectomized mice. Similarly, GRP78/eIF2α/ATF4 pathway is activated by excess P4 in day 8 pregnant mouse uterus, resulting in a repression of decidualization. In humans, developmentally impaired embryos elicit an anomalous endoplasmic stress response in human decidua cells. PERK/eIF2α and ATF6 signaling pathway is activated in fetal growth restriction. Actually, in present study, mice received excess P deliver a lower birth weight, with an aberrant elevation of GRP78/eIF2α/ATF4 signaling at implantation sites on day 8 pregnancy.

In conclusion, our study indicates that excess P has a detrimental effect on endometrium receptivity and decidualization. Excess P treatment may cause fetal growth restriction through compromising embryo implantation and decidualization in mouse models.

Materials and Methods

Animal Treatments. All animal experiments were approved by Animal Care and Use Committee of South China Agricultural University. All of the experiments were carried out in accordance with the approved guidelines by South China Agricultural University. Adult CD1 mice were housed in a temperature- and light-controlled environment with 14 h light: 10 h dark cycle. Pregnant or pseudo-pregnant female mice (8-10 weeks) were obtained by mating with fertile or vasectomized males of the same strain (day 1 is the day of vaginal plug), respectively. From days 1–4, pregnancy was verified by flushing the embryos from the fallopian tube and uterus, respectively. The implantation sites on day 5 were confirmed by tail intravenous injection of Chicago blue dye (Sigma). Artificial decidualization was performed as previously described.

In order to examine the effects of P on the expression of endometrial receptivity-related genes, pregnant mice were subcutaneously injected with 1, 4, and 8 mg/mouse P (Sigma, dissolved in 100 μl sesame oil) at 9:00 on day 3 of pregnancy. The control mice received 100 μl of sesame oil. Mice were sacrificed at 9:00 on day 4 of pregnancy to collect uteri for further analysis.

For examining the effects of excess P on implantation sites, pregnant mice were subcutaneously injected with 4 mg/mouse P (Sigma, dissolved in 100 μl sesame oil) twice (9:00 on day 3 and 9:00 on day 4). The control mice received 100 μl sesame oil/mouse. Mice were sacrificed at midnight on day 4 to collect uteri for counting implantation sites. Blastocysts were flushed from uterine horns of mice at 14:00 on day 4 of those mice.

To analyze the effects of excess P on the weight of mouse implantation sites on day 8, pregnant mice were subcutaneously injected with 1, 4, and 8 mg P/mouse (Sigma, dissolved in 100 μl sesame oil) daily from 9:00 on day 3 to 9:00 on day 7 for 5 days. The control mice received 100 μl sesame oil/mouse. Mice were sacrificed at 9:00 on day 8 to collect uteri for weighing implantation sites.

To exclude the effects of P on embryonic development, pseudo-pregnant mice induced for artificial decidualization were treated with a daily injection of 4 mg/mouse P (dissolved in 100 μl sesame oil) on days 5, 6, and 7 of pseudo-pregnancy. Uteri were collected and weighed on day 8 of pseudo-pregnancy.

Ovariectomized mice were subcutaneously injected with 1, 4, and 8 mg/mouse P (Sigma, dissolved in 100 μl sesame oil) daily for 3 days. The control mice received 100 μl sesame oil. Mice were sacrificed 24 h after last injections to collect uteri for further analysis.

Immunohistochemistry. Immunohistochemistry was performed as described previously. Briefly, paraffin-embedded uterine sections were deparaffinized in xylene, rehydrated through a graded series of ethanol, and washed in water. Antigen retrieval was performed in 0.01 M sodium citrate buffer (pH 6.0) by microwaving for 10 min. Endogenous horseradish peroxidase (HRP) activity was inhibited with 3% H2O2 for 15 min. After blocked with 10% horse serum at 37°C for 1 h, sections were incubated with rabbit anti-PR (1:1200, #MA5-14505, Thermo Fisher Scientific, MA, USA), rabbit anti-ER (1:2000, #sc-7207, Santa Cruz Biotechnology, TX USA), rabbit anti-p-Stat3 (1:400, #9145, Cell Signaling Technology, MA, USA), rabbit anti-p-Stat1 (1:400, #9145, Cell Signaling Technology, MA, USA), rabbit anti-p-Stat6 (1:400, #9145, Cell Signaling Technology, MA, USA), and rabbit anti-PR (1:400, #9145, Cell Signaling Technology, MA, USA), diluted in 10% horse serum at 4°C overnight, respectively. Followed by washing and incubating with biotin-labeled goat anti-rabbit IgG antibodies (Zhongshan Golden Bridge, Beijing, China) for 30 min, then sections were incubated with streptavidin-HRP complex (Zhongshan Golden Bridge, Beijing, China) for 30 min. The positive signals were visualized using DAB Horseradish Peroxidase Color Development Kit according to the manufacturer’s protocol (Zhongshan Golden Bridge, Beijing, China). The sections were counterstained with hematoxylin.

Isolation and treatment of mouse endometrial stromal cells. Primary endometrial stromal cells were enzymatically isolated from day 4 pregnant mice and cultured as described previously. Briefly, mouse uteri were digested with Hanks’ balanced salt solution (Sigma) containing 1% trypsin (AMRESCO) and 6 mg/ml dispase (Roche). Luminal epithelial cells were removed after HBSS washing. After the remaining uteri were treated with 0.15 mg/ml collagenase I (Invitrogen), endometrial stromal cells were collected and cultured in DMEM/F12 (Sigma) containing 10% charcoal-treated FBS (Biological Industries, Israel). For inducing in vitro decidualization, primary endometrial stromal cells were treated with 10 nM estradiol-17β and 1μM P. Under in vitro decidualization, cultured stromal cells were treated with 0.8, 4 and 20 μM P (Sigma), respectively. The highest treatment dose of P has no toxic effect on cell viability.
### Table 1. Primers used in this study.

| Gene   | Primer sequences (5′-3′) | Accession number | Size (bp) | Application       |
|--------|--------------------------|------------------|-----------|-------------------|
| Ihh    | GCTGAAGGGGACTCTAACCACAGAGAAGAGACAC | NM_010544.2 | 118 | Real-time PCR     |
| LIF    | AGCCCAAAATTGCTCCTTCTCCTCAGATGCTGTTTTC | NM_008522 | 119 | Real-time PCR     |
| LIF    | AAAAGCTGATGCCCCGCTCCGACTCTCTGATTAA | NM_008501 | 98  | Real-time PCR     |
| Areg   | CTCGCAAGGGGACTAGCAGCTTGTGGCTTATTACGCGTTC | NM_007904.3 | 105 | Real-time PCR     |
| Hand2  | GCTATACGCCTACTGAGCGCTTCTGTCTTCTTCTTCT | NM_010402.4 | 114 | Real-time PCR     |
| PRB    | GCTTTGTGATATTATACGCTATTGCTGGGCTGAGC | NM_008829.2 | 153 | Real-time PCR     |
| PRAB   | TATACCAGCTTCTGACCCGCTTATGAGCTGCTTACC | NM_008829.2 | 137 | Real-time PCR     |
| RPL7   | GCAGATGTCGACCTGAGATCTACCTTTGGCTTACTCCATGGTAA | M29016 | 129 | Real-time PCR     |
| Dnpp   | AGCCGAAGATCAGCTGCCACTTGATGTCCTGACACECATGAA | NM_010088 | 119  | Real-time PCR     |
| Xbp1   | GAGGAGGAGTTGTTTATTTAACGGGCTGAGGGAAGGAGGAGAA | NM_0013842.3 | 447/421 | Real-time PCR     |
| IFGBP1 | CCAACTGCAAGAAGATGAGTGAACAGCACCACGAGAC | NM_000596.2 | 87  | Real-time PCR     |
| FOXO1  | CGAGGTCGAAGAAGGAAAATTCGAGGGCGAAGAATGTAC | NM_002015 | 105 | Real-time PCR     |
| PLZF   | TCACATAGCCGCGACCACTGAGGCTGTAACATGTC | NM_006064.0 | 144  | Real-time PCR     |
| GAPDH  | GAAGGTGAAGGTCGGAGTTGATGGCAACAATATCCACTT | BC023632 | 94  | Real-time PCR     |
| RPL7   | CTGGTCTGCAGAACCACCTCCTTTCCTCTCCCAT | NM_000971 | 194  | Real-time PCR     |

### Culture and in vitro decidualization of human endometrial stromal cells. Immortalized human endometrial stromal cells (hESC) were purchased from the American Type Culture Collection (ATCC CRL-4003TM) and cultured according to the manufacturer’s instructions. Briefly, stromal cells were cultured in DMEM/F12 (Sigma) supplemented with 10% charcoal-stripped FBS (CS-FBS, Biological Industries) at 37°C in a humidified chamber with 5% CO2. To induce decidualization in vitro, stromal cells were treated with 1 μM Medroxyprogesterone 17-acetate (MPA, Sigma) and 0.5 mM dibutyryl cAMP (db-cAMP, Sigma) in DMEM/F12 with 2% CS-FBS for 6 days. The medium was changed every 48 h. Under in vitro decidualization, stromal cells were treated with 0.032, 0.16, 0.8, 4, and 20 μM P (Sigma) for further analysis, respectively. The highest treatment dose of P has no significant toxic effect on cell viability.

### Real-time PCR and detection of spliced XBPI. Real-time PCR was performed as previously described. Briefly, total RNAs from each sample were isolated using TRizol reagent kit (Invitrogen), digested with RQ1 deoxyribonuclease I (Promega, Fitchburg, WI) and reverse-transcribed into cDNA with PrimeScript reverse transcriptase reagent kit (TaKaRa). For real time PCR, cDNA was amplified using a SYBR Premix Ex Taq kit (TaKaRa) on the CFX96 Touch™ Real-Time System (Bio-Rad). Data from real-time PCR were analyzed using the 2^ΔΔCt method and normalized to Rpl7 or GAPDH expression.

### Western blot analysis. Western blot was performed as previously described. Briefly, protein lysates were separated by SDS polyacrylamide gel electrophoresis and transferred onto a PVDF membrane. Membranes were incubated overnight at 4°C with each primary antibody, including anti-GRP78 (sc-1050, Santa Cruz Biotechnology, TX USA) and anti-Tubulin (#2144, Cell Signaling Technology, MA USA). Then the membrane was incubated in 5% non-fat milk containing HRP-conjugated secondary antibody (1:5000) for 1 h. Signals were detected by ECL Chemiluminescent kit (Millipore).

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Author Contributions
Y.X.L. designed and performed experiments including major experiments, analyzed the data and wrote the manuscript; L.L., Z.Y.J. performed mouse treatments and real-time RT-PCR; X.H.L. and X.W.G. contributed to data interpretation and analysis. Z.M.Y. designed, supervised the study, and wrote the manuscript. All authors commented on the manuscript.

Additional Information
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