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Differential expression and anti-oxidant function of glutathione peroxidase 3 in mouse uterus during decidualization

Xiu Xu [a,b], Jing-Yu Leng [c], Fei Gao [b], Zhen-Ao Zhao [b], Wen-Bo Deng [a], Xiao-Huan Liang [a], Yi-Juan Zhang [c], Zhi-Rong Zhang [c], Ming Li [c], Ai-Guo Sha [d], Zeng-Ming Yang [a,*]

[a] College of Veterinary Medicine, South China Agricultural University, Guangzhou 510642, China
[b] School of Life Science, Northeast Agricultural University, Harbin 150030, China
[c] School of Life Science, Xiamen University, Xiamen 361005, China
[d] Reproductive Medicine Center, Bailu Hospital, Xiamen 361000, China

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ABSTRACT

Glutathione peroxidase 3 (GPX3) is an important member of antioxidant enzymes for reducing reactive oxygen species and maintaining the oxygen balance. *Gpx3* mRNA is strongly expressed in decidual cells from days 5 to 8 of pregnancy. After pregnant mice are treated with GPX inhibitor for 3 days, pregnancy rate is significantly reduced. Progesterone stimulates *Gpx3* expression through PR/HIF1α in mouse endometrial stromal cells. In the decidua, the high level of GPX3 expression is closely associated with the reduction of hydrogen peroxide (H_2O_2). Based on our data, GPX3 may play a major role in reducing H_2O_2 during decidualization.

1. Introduction

Early pregnancy in mammals is vulnerable to stressors [1]. Reactive oxygen species (ROS) is involved in the pathophysiology of infertility and assisted fertility [2,3]. ROS is deleterious by-products of aerobic metabolism, including superoxide ($O_2^{\cdot-}/HO_2^{\cdot}$), hydrogen peroxide ($H_2O_2$), and the hydroxyl radical ($OH^{\cdot}$) [4]. In a healthy body, ROS and antioxidants remain in a balance. Oxidative stress is a disturbance in the pro-oxidant–antioxidant balance [5]. It is estimated that up to 2% of the oxygen consumed by mitochondria is partially reduced to form $O_2^{\cdot-}$, which is subsequently converted to H_2O_2 [6].

In the presence of high H_2O_2 concentration, catalase is most effective for metabolizing H_2O_2. However, the glutathione system plays a critical role in the presence of low concentrations of either H_2O_2 or other peroxides [7]. The key enzyme in the redox cycle responsible for the reduction of H_2O_2 is GPX. The mammalian glutathione peroxidase family consists of 8 classes (i.e., *Gpx1–Gpx8*) [8]. Glutathione peroxidase 3 (GPX3) belongs to the selenocysteine-containing GPX family and is a main antioxidant enzyme in plasma for scavenging ROS derived from normal metabolism or oxidative insult [9]. GPX3 attenuates oxidant stress by reducing H_2O_2 and organic hydroperoxides to their corresponding alcohols [10].

GPX3 is one of the key enzymes in the cellular defense against oxidative stress [11]. *GPX3* expression is down-regulated both in rat and human endometrial adenocarcinoma, regardless of tumor grade or histopathological subtype [11]. *GPX3* is one of the up-regulated genes expressed in receptive phase of human endometrium, which could represent a useful prognostic tool for selecting IVF patients [12]. Compared to natural cycles, *GPX3* expression shows a delay in controlled ovarian stimulation (COS) cycles [13]. Based on our preliminary analysis, *Gpx3* is highly expressed in mouse decidua from days 5 to 8 of pregnancy, suggesting that *Gpx3* may play a role during decidualization. This study was to examine the expression, regulation and function of *Gpx3* during decidualization.

2. Materials and methods

2.1. Animal treatments

Mature mice (CD1 strain) were maintained in a controlled environment (14 h light and 10 h dark cycle). All animal procedures

* Corresponding author.
E-mail address: zmyang@scau.edu.cn (Z.-M. Yang).

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were approved by the Institutional Animal Care and Use Committee of South China Agricultural University. Uteri under pseudopregnancy, delayed implantation and activation, steroid hormonal treatments and deciduoma were collected as described previously [14]. Day 1 is the day of vaginal plug.

2.2. In situ hybridization

Total RNAs from the mouse uterus on day 8 of pregnancy were reverse transcribed and amplified with primers 5′-AGAAAGGAGATGTGAACG and 5′-TAGAATGACTGGGAATGTG (367 bp, NM_001083929). The amplified PCR fragment was recovered from the agarose gel and cloned into pGEM-T plasmid. The cloned fragment of *Gpx3* was further verified by sequencing. The preparation of digoxigenin-labeled cRNA probes and in situ hybridization of *Gpx3* mRNA in mouse uteri was performed as described previously [15].

2.3. Isolation of mouse endometrial stromal cells and in vitro decidualization

Mouse endometrial stromal cells were isolated from day 4 pregnant uteri as previously described [16]. The cell pellets were resuspended in DMEM/F-12 (Sigma) with 2% heat-inactivated FBS (Gibco). Cells were plated onto 35-mm culture dishes at the concentration of 1×10^6 cells/well. After an initial culture for 1 h, the medium was changed to remove free floating cells. In vitro decidualization of endometrial stromal cells was performed as previously described [17].

2.4. Isolation of human endometrial stromal cells and in vitro decidualization

Human endometrial samples were collected from normally cycling women undergoing hysterectomy or endometrial biopsy with written informed consent. All human procedures were approved by the Institutional Committee on the Use of Human Subjects in Medical Research of Bailu Hospital (Xiamen, China). Human endometrial stromal cells were isolated as described previously [18]. In vitro decidualization of human endometrial stromal cells was performed as previously described [19].

2.5. Transfection of Gpx3 siRNA

Transfections of *Gpx3* small interfering RNA (siRNA) were performed according to Lipofectamine 2000 protocol (Invitrogen).

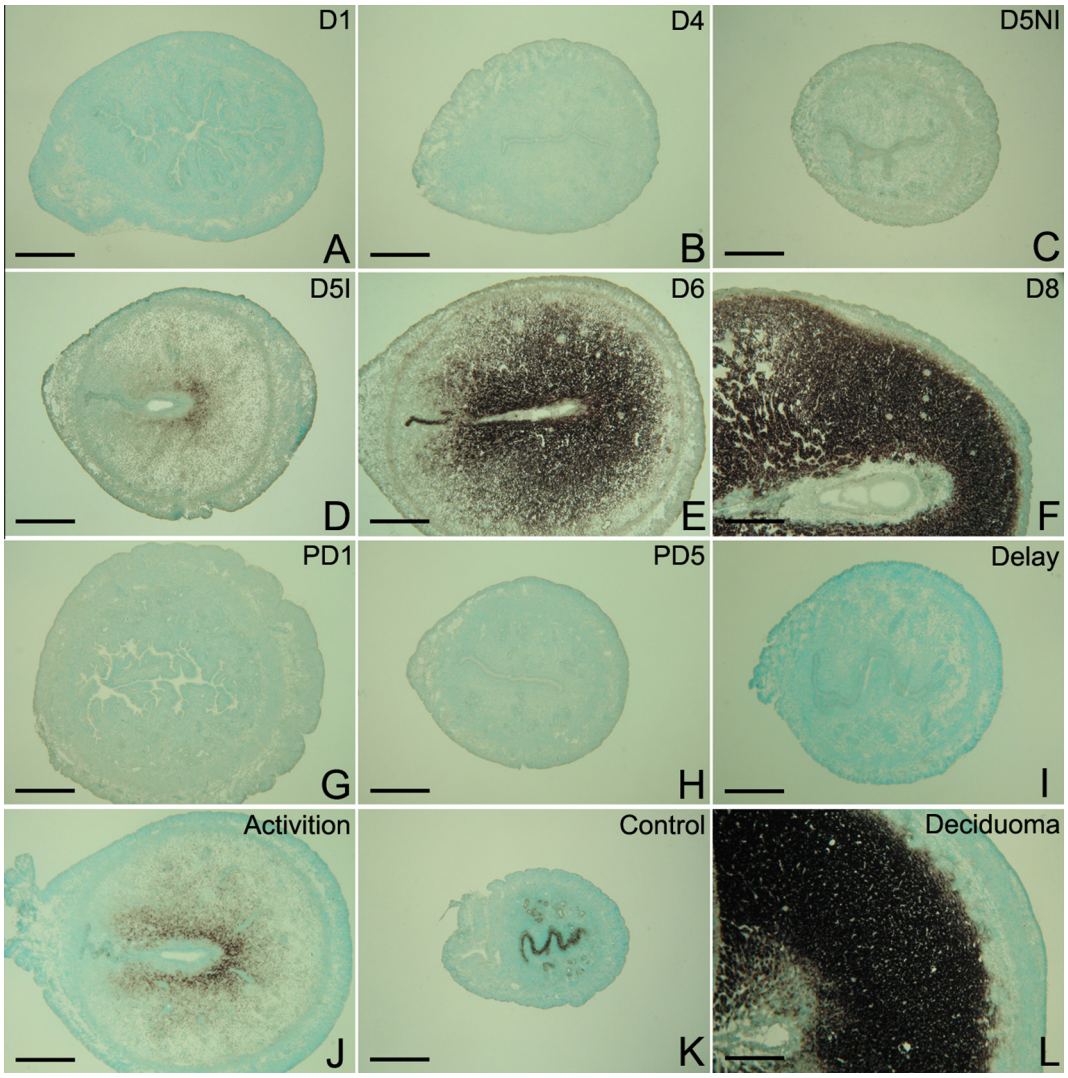

Fig. 1. In situ hybridization of *Gpx3* mRNA expression in mouse uteri. (A) Day 1 of pregnancy; (B) Day 4 of pregnancy; (C) Inter-implantation site on day 5 of pregnancy (D5NI); (D) Implantation site on day 5 of pregnancy (D5I); (E) Day 6 of pregnancy; (F) Day 8 of pregnancy; (G) Day 1 of pseudopregnancy (PD1); (H) Day 5 of pseudopregnancy (PD5); (I) Delayed implantation; (J) Activation of delayed implantation by estrogen; (K) Uninjected uterine horn for control (Control); (L) Uterine horn under artificial decidualization (Deciduoma). Bar = 80 μm.

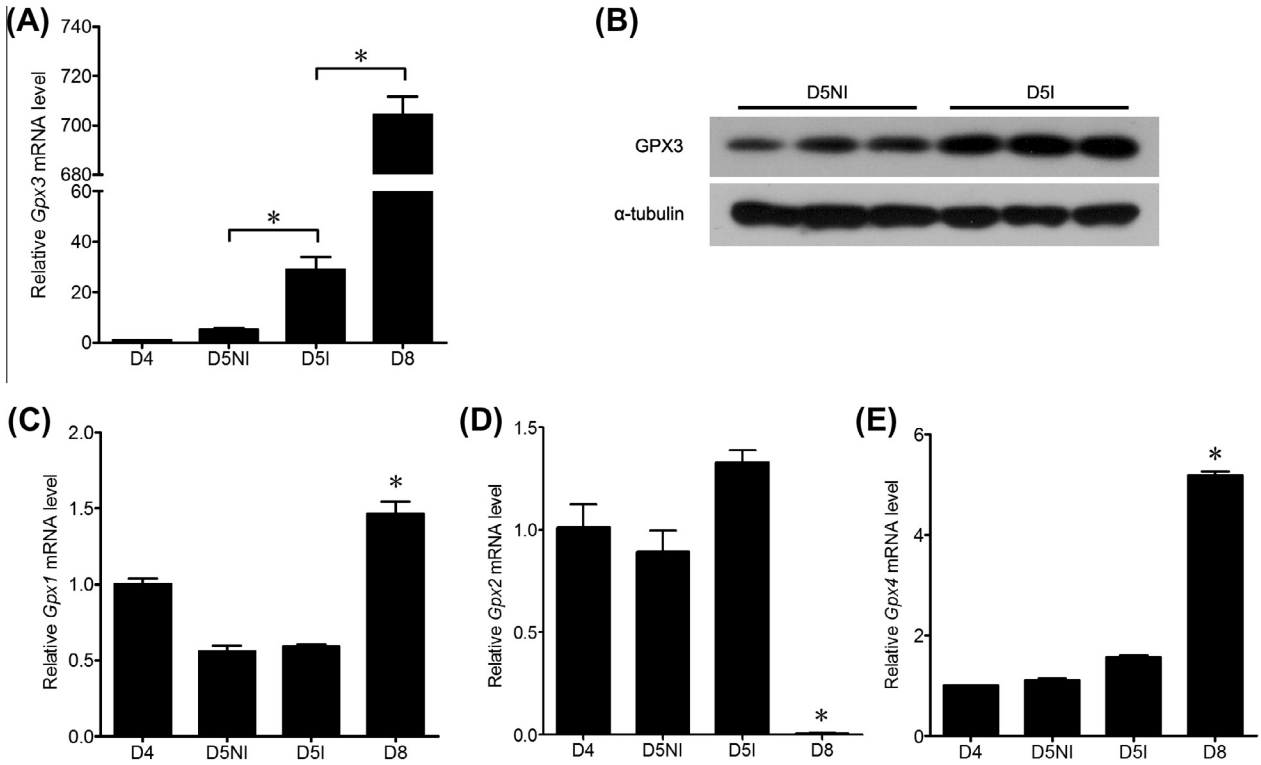

Fig. 2. Expression of GPX family in mouse uteri. (A) *Gpx3* mRNA level; (B) Expression level of GPX3 protein in mouse endometrium at day 5 inter-implantation site (D5NI) and implantation site (D5I); (C) *Gpx1* mRNA level; (D) *Gpx2* mRNA level; (E) *Gpx4* mRNA level (*$P < 0.05$);

siRNAs specific for *Gpx3* and negative control were purchased from GenePharma Co., Ltd. (Shanghai, China).

2.6. Real-time RT-PCR

Real-time RT-PCR was performed as previously described [20].

2.7. Western blot

Western blot was performed as previously described [20].

2.8. Detection of hydrogen peroxide concentration

Detection of hydrogen peroxide concentration in endometrial samples was performed according to the manufacturer's instruction of PeroXOquant™ Quantitative Peroxide Assay Kit (Thermo) with modification.

3. Results

3.1. Localization of Gpx3 expression in mouse uterus during early pregnancy

In situ hybridization was performed to examine the spatial distribution of *Gpx3* mRNA in mouse uterus. There was no visible *Gpx3* mRNA signal in the uteri from days 1 to 4 (Fig. 1, A and B). On day 5 of pregnancy, *Gpx3* mRNA signal was obviously detected in the subluminal stroma immediately surrounding the implanting blastocyst (Fig. 1D), whereas there was no detectable expression of *Gpx3* at the inter-implantation sites (Fig. 1C). From days 6 to 8, *Gpx3* expression was strongly expressed in the decidua and increased during the process of decidualization (Fig. 1, E and F).

3.2. Gpx3 expression during pseudopregnancy, delayed implantation and artificial decidualization

To address whether *Gpx3* expression was induced by embryo, we examined the expression of *Gpx3* during pseudopregnancy. From days 1 to 5 of pseudopregnancy, there was no detectable *Gpx3* mRNA signal in mouse uteri (Fig. 1, G and H). To further check whether the expression of *Gpx3* was induced by the presence of an active blastocyst, a delayed implantation model was used. There was no detectable *Gpx3* expression in mouse uterus under delayed implantation (Fig. 1I). After delayed implantation was activated by estrogen treatment, *Gpx3* expression was highly detected in the subluminal stromal cells surrounding the implanting embryo (Fig. 1J).

Because *Gpx3* was strongly expressed in the decidua during early pregnancy, artificial decidualization was performed to examine whether *Gpx3* was expressed in deciduoma. In the uninjected uterine horn, *Gpx3* mRNA signal was detected in the luminal epithelium and weakly in the glandular epithelium, but not in the stromal cells (Fig. 1K). In the oil-induced uterine horn for artificial decidualization, a strong level of *Gpx3* mRNA expression was observed in the decidualized cells (Fig. 1L).

3.3. Quantification of Gpx3 and other family members in mouse endometrium during early pregnancy

We further quantified the expression levels of *Gpx3* mRNA and its family members by real-time RT-PCR. In order to avoid the influence of myometrium, endometrium was isolated from the whole uterus by squeezing with a needle. *Gpx3* expression was significantly up-regulated in implantation sites on day 5 of pregnancy compared with inter-implantation sites, and increased significantly on day 8 of pregnancy (Fig. 2A). On day 5, the protein level

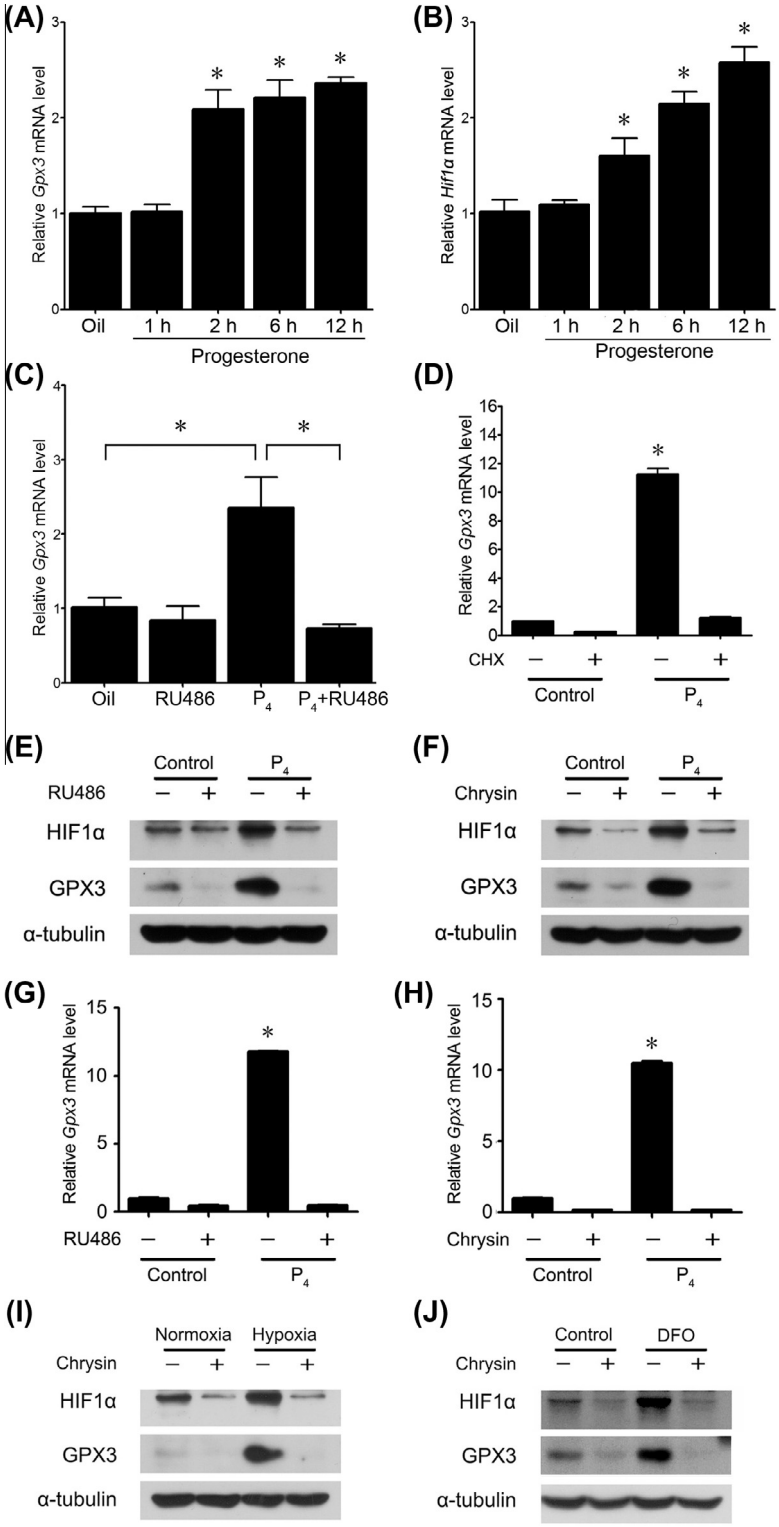

Fig. 3. Progesterone regulates the expression level of GPX3 through PR/HIF1α. (A) *Gpx3* mRNA expression level after progesterone treatment. Ovariectomized mice were treated with an injection of progesterone (1 mg/mouse) for 24 h. Progesterone was dissolved in sesame oil and injected subcutaneously. Controls received the vehicle only (0.1 ml/mouse). (B) *Hif1α* mRNA expression level after progesterone treatment; (C) Expression level of *Gpx3* mRNA 12 h after treatment with progesterone (P_4), RU486 or progesterone plus RU486 (P_4 + RU486). Sesame oil (Oil) was injected as a control. For RU486 (Cayman Chemical, Ann Arbor, MI) treatment, mice were injected with RU486 (0.75 mg/mouse) 1 h before progesterone injection. (D) The up-regulation of *Gpx3* mRNA induced by progesterone was suppressed by translation inhibitor cycloheximide (CHX) at 24 h; (E) The up-regulation of HIF1α and GPX3 protein induced by progesterone was suppressed by RU486 at 24 h; (F) The up-regulation of HIF1α and GPX3 protein induced by progesterone was suppressed by Chrysin, a specific inhibitor for HIF1α at 24 h; (G) The up-regulation of *Gpx3* mRNA induced by progesterone was suppressed by RU486 at 24 h; (H) The up-regulation of *Gpx3* mRNA induced by progesterone was suppressed by Chrysin at 24 h; (I) GPX3 protein expression induced by 2% O_2 (hypoxia) was inhibited by Chrysin at 24 h. Cells were cultured at 20% O_2 (normoxia) as control; (J) GPX3 protein expression induced by deferoxamine mesylate (DFO) was inhibited by Chrysin at 24 h (*$P < 0.05$).

of GPX3 was also up-regulated at implantation site compared with inter-implantation site (Fig. 2B).

Levels of *Gpx1*, *Gpx2* and *Gpx4* expressions did not show obvious changes in the endometrium from days 4 to 5 of pregnancy. On day 8 of pregnancy, both *Gpx1* and *Gpx4* were up-regulated in the endometrium, whereas *Gpx2* was down-regulated (Fig. 2, C–E). Because the expression of *Gpx*5, *Gpx*6 and *Gpx*7 was very weak in the mouse uteri from days 1 to 8 of pregnancy, we got no specific expression signals by real-time RT-PCR.

3.4. Regulation of Gpx3 expression by progesterone in vivo

Because progesterone is essential for mouse embryo implantation [21], ovariectomized mice were used to examine whether *Gpx3* expression is regulated by progesterone in vivo. Compared to control, *Gpx3* expression was significantly induced from 2 to 12 h after progesterone treatment (Fig. 3A).

Because progesterone can up-regulate *Hif1α* through progesterone receptor in vivo [22], we would like to test whether progesterone regulates Gpx3 through Hif1α. Through in silico analysis, we identified a HIF1α binding site in *Gpx3* promoter. Both *Hif1α* and *Gpx3* expressions were significantly induced from 2 to 12 h after progesterone treatment (Fig. 3B), indicating that progesterone may regulate *Gpx3* through *Hif1α*.

We then checked whether *Gpx3* up-regulation by progesterone was mediated through progesterone receptor. *Gpx3* expression was significantly induced by progesterone at 12 h. However, *Gpx3* up-regulation by progesterone was blocked by a pretreatment with RU486 (1 μM), an antagonist for progesterone receptor, suggesting that progesterone regulation on *Gpx3* is mainly mediated through progesterone receptor (Fig. 3C).

3.5. Progesterone stimulates GPX3 expression through PR/HIF1α in vitro

In order to further examine the regulatory mechanism of GPX3 by progesterone, mouse endometrial stromal cells were treated with progesterone and protein synthesis inhibitor cycloheximide (CHX, 1 μg/mL). CHX could significantly inhibit *Gpx3* mRNA induction by progesterone at 24 h (Fig. 3D), showing that other proteins may be involved in the regulation of *Gpx3*. Progesterone should regulate *Gpx3* indirectly.

Stromal cells were then treated with the inhibitor of PR or HIF1α to further verify whether progesterone regulates *Gpx3* through PR and HIF1α. Progesterone-induced up-regulation of both HIF1α and GPX3 expressions was suppressed by RU486 (1 μM) or a *Hif1α* inhibitor (Chrysin, 50 μM) at 24 h (Fig. 3, E-H), indicating that progesterone stimulated GPX3 expression through PR/HIF1α in mouse endometrial stromal cells.

3.6. Effects of hypoxia on GPX3 expression

Since it is reported that hypoxia can induce HIF1α accumulation in all cell types by inhibiting its ubiquitylated degradation pathway [23], stromal cells were treated with either 2% O_2 or deferoxamine mesylate (DFO, 150 μM) for 24 h to determine whether GPX3 expression was stimulated under the hypoxia environment. Both GPX3 and HIF1α were significantly up-regulated by either 2% O_2

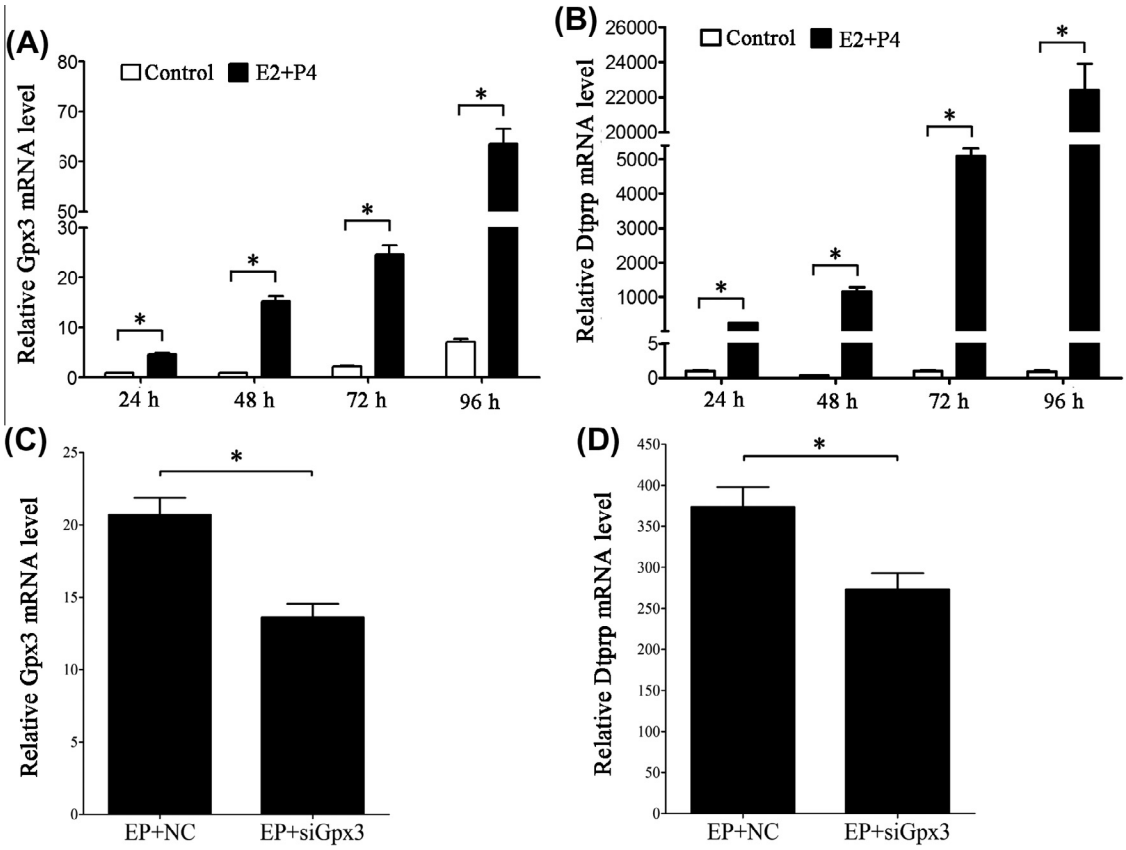

Fig. 4. *Gpx3* action under in vitro decidualization. (A) Expression of *Gpx3* mRNA in mouse endometrial stromal cells during in vitro decidualization (E2 + P4). (B) Expression of *Dtprp* mRNA in mouse endometrial stromal cells during in vitro decidualization (E2 + P4). (C) *Gpx3* expression was significantly down-regulated by *Gpx3* siRNA under in vitro decidualization. EP, E2 + P4; NC, negative control; siGpx3, *Gpx3* siRNA. (D) *Dtprp* expression was significantly inhibited by *Gpx3* siRNA.

or DFO-induced hypoxia. Either 2% hypoxia or DFO-induced GPX3 expression was abrogated by a *Hif1α* inhibitor (Chrysin, 50 μM) (Fig. 3, I and J), indicating that HIF1α is involved in hypoxia-induced GPX3 expression.

3.7. Gpx3 expression during in vitro decidualization

Decidual/trophoblast prolactin-related protein (*Dtprp*) is a reliable marker for decidualization in mice [24,25]. We examined whether *Dtprp* and *Gpx3* was expressed under in vitro decidualization. After mouse stromal cells were induced for in vitro decidualization with both estrogen and progesterone, *Gpx3* expression significantly increased from 24 to 96 h of culture (Fig. 4A). *Dtprp* expression was also significantly induced from 24 to 96 h of culture (Fig. 4B). After GPX3 expression was significantly down-regulated by Gpx3 siRNA under in vitro decidualization (Fig. 4C), Dtprp expression was inhibited (Fig. 4D).

3.8. Post-transcriptional regulation of GPX3 expression by sodium selenite

Uterine stromal cells were treated with different concentrations of sodium selenite to examine its effects on GPX3 protein expression at 24 h. As the concentration of sodium selenite increased, GPX3 protein expression enhanced gradually. But when the concentration of sodium selenite was higher than 0.1 μM, GPX3 protein expression decreased slightly (Fig. 5A). It is possible that a high level of selenium may produce toxic effects since selenium belongs to trace elements. Therefore, culture medium was supplemented with 0.1 μM sodium selenite in rest of the treatments required.

No matter whether sodium selenite was in the medium, both estrogen and progesterone could induce *Gpx3* mRNA expression at 24 h (Fig. 5B). However, GPX3 protein expression was not detected when culture medium didn't contain sodium selenite in the control. Only in the groups containing sodium selenite, both estrogen and progesterone could induce GPX3 protein expression (Fig. 5C), showing that selenium stimulates GPX3 protein expression through post-transcriptional regulation.

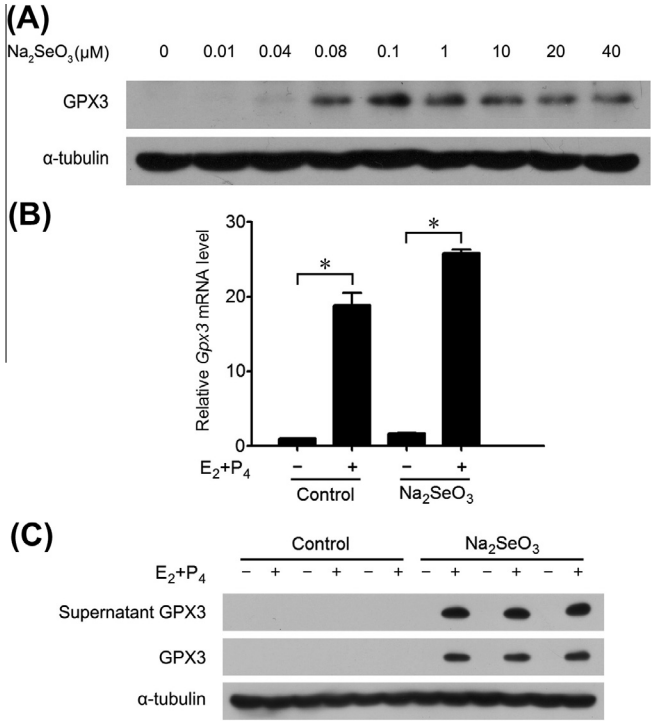

Fig. 5. The post-transcriptional regulation of GPX3 by sodium selenite (Na_2SeO_3) in stromal cells under in vitro decidualization. (A) Effects of different concentrations of sodium selenite (Na_2SeO_3) on the expression level of GPX3 protein at 24 h; (B) Effects of Na_2SeO_3 on the expression level of *Gpx3* mRNA under in vitro decidualization at 24 h; (C) Effects of Na_2SeO_3 on the expression level of GPX3 protein under in vitro decidualization at 24 h (*$P < 0.05$).

3.9. Concentration of hydrogen peroxide in mouse endometrium

The concentration of hydrogen peroxide at implantation sites was significantly lower than that of the inter-implantation sites on day 5 of pregnancy (Fig. 6A). On the contrary, GPX3 was significantly increased in implantation sites on day 5 of pregnancy compared with inter-implantation sites, indicating that GPX3 may play a role in reducing H_2O_2 in mouse endometrium.

3.10. GPX3 reduces H_2O_2 during in vitro decidualization

During in vitro decidualization, the concentration of H_2O_2 in the supernatant of mouse stromal cells was significantly down-regulated, which was blocked by mercaptosuccinic acid, an enzyme activity inhibitor of all GPXs at 96 h (Fig. 6B). Although the reduction of H_2O_2 by GPX3 was inhibited by mercaptosuccinic acid, mercaptosuccinic acid was unable to inhibit GPX3 protein expression induced by both estrogen and progesterone at 96 h (Fig. 6C). The result of real-time RT-PCR showed that *Gpx3* mRNA in decidualized cells was significantly up-regulated (Fig. 6D), *Gpx1* mRNA was significantly down-regulated (Fig. 6E), and *Gpx4* mRNA was invariable in decidualized cells compared with control at 96 h (Fig. 6F). These data indicated that GPX3 also played a major role for reducing H_2O_2 in decidualized cells in vitro.

Gpx3 siRNA could effectively inhibit the expression of GPX3 protein at 48 h (Fig. 6G). *Gpx3* siRNA treatment could block the down-regulation of H_2O_2 concentration in the supernatant of mouse stromal cells undergoing in vitro decidualization at 48 h (Fig. 6H). These data further confirmed that GPX3 could reduce H_2O_2 in decidualized cells in vitro.

3.11. GPX3 reduces H_2O_2 in human stromal cells undergoing in vitro decidualization

Human endometrium stromal cells were used to investigate whether human *GPX3* had similar function as mouse *Gpx3*. The concentration of H_2O_2 in the supernatant of human stromal cells undergoing in vitro decidualization was significantly down-regulated, which was blocked by GPX inhibitor on day 6 (Fig. 7A), indicating that GPX3 had a similar function in human endometrium cells. Mercaptosuccinic acid could inhibit GPX3 enzyme activity, but also could not inhibit GPX3 protein under in vitro decidualization on day 6 (Fig. 7B). *GPX3* mRNA expression was significantly enhanced after in vitro decidualization was induced for 6 days (Fig. 7C). *PRL* and *IGFBP-1* were molecular markers of human decidualization [26,27]. Both *IGFBP1* and *PRL* were significantly up-regulated after stromal cells were induced for in vitro decidualization for 6 days (Fig. 7, D and E).

3.12. Pregnancy rate drops after intraperitoneal injection of mercaptosuccinic acid

In order to investigate the role of GPX during pregnancy, pregnant mice were treated with GPX inhibitor. After intraperitoneal injection of GPX inhibitor for 3 days from day 5 of pregnancy, pregnancy rate was significantly reduced on day 8 of pregnancy (Fig. 8), indicating that GPX may play an important role in the process of implantation and decidualization.

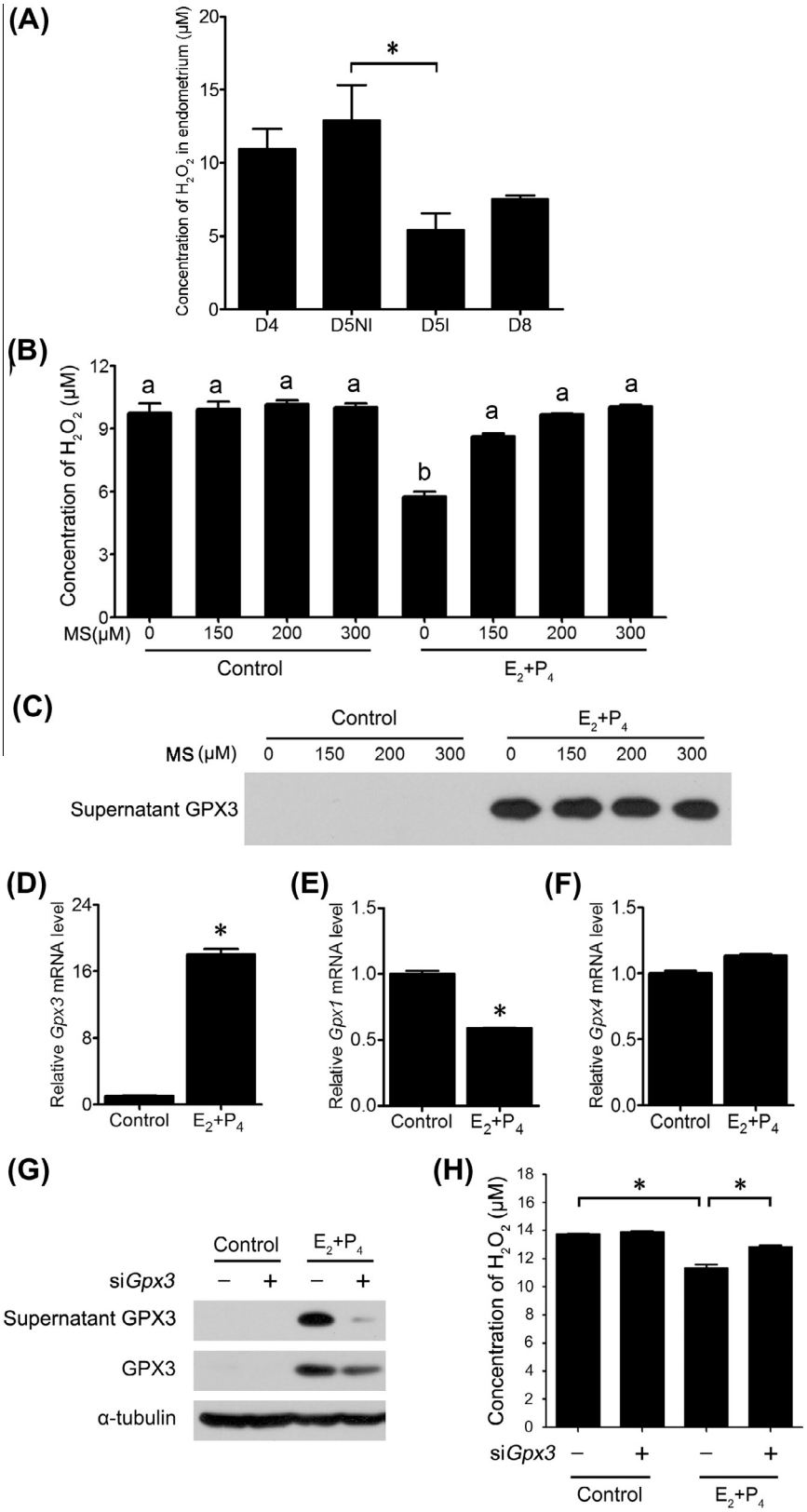

Fig. 6. Effects of GPX3 on hydrogen peroxide (H_2O_2) in mouse decidualized cells. (A) The concentration of H_2O_2 on days 4 (D4), 5 (D5NI and D5I) and 8 (D8) in mouse endometrium. D5NI: inter-implantation site on day 5; D5I: implantation site on day 5; (B) Effects of GPX inhibitor (mercaptosuccinic acid, MS) on H_2O_2 concentration at 96 h; (C) Mercaptosuccinic acid has no effect on GPX3 protein expression at 96 h; (D) *Gpx3* mRNA expression is significantly up-regulated under in vitro decidualization at 96 h; (E) *Gpx1* mRNA level is significantly down-regulated under in vitro decidualization at 96 h; (F) *Gpx4* mRNA expression was not changed under in vitro decidualization at 96 h; (G) Effects of *Gpx3* siRNA on the expression of GPX3 protein at 48 h; (H) Effects of *Gpx3* siRNA on H_2O_2 concentration in the supernatant at 48 h (*$P < 0.05$).

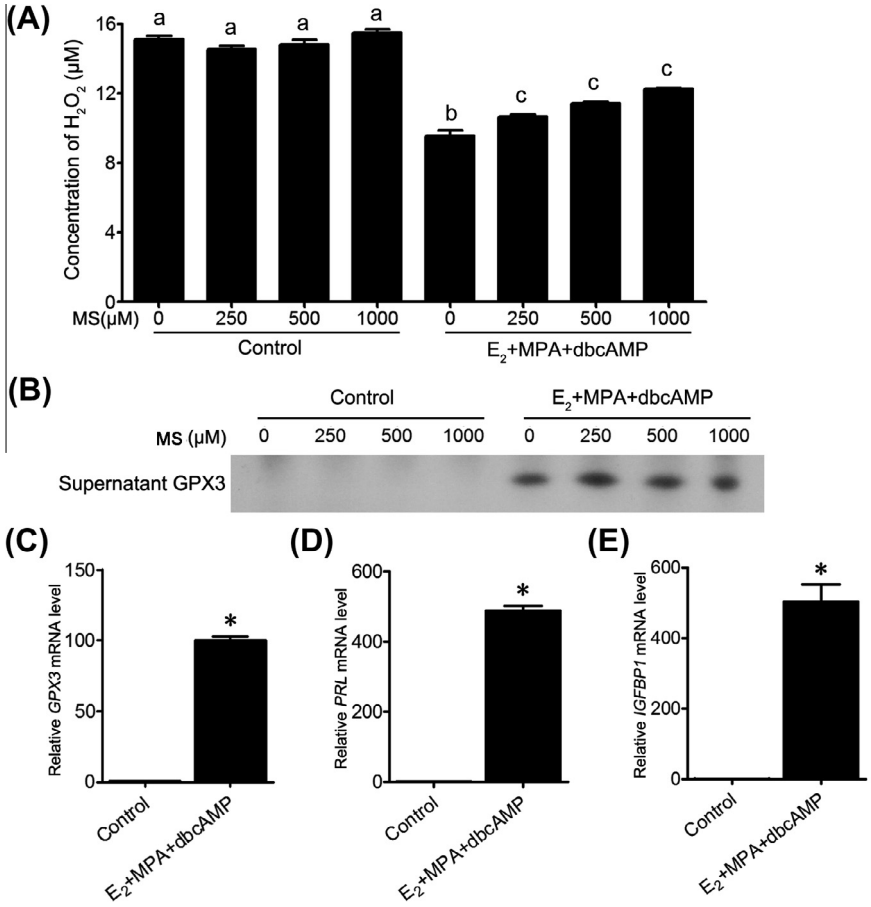

Fig. 7. The concentration of hydrogen peroxide (H_2O_2) in human endometrial stroma cells under in vitro decidualization. (A) Effects of GPX inhibitor (mercaptosuccinic acid, MS) on H_2O_2 concentration on day 6; (B) Mercaptosuccinic acid has no effect on GPX3 protein expression on day 6; In stromal cells under in vitro decidualization, the levels of *GPX3* (C), *PRL* (D) and *IGFBP1* (E) mRNA expression are significantly up-regulated on day 6 ($^*P < 0.05$).

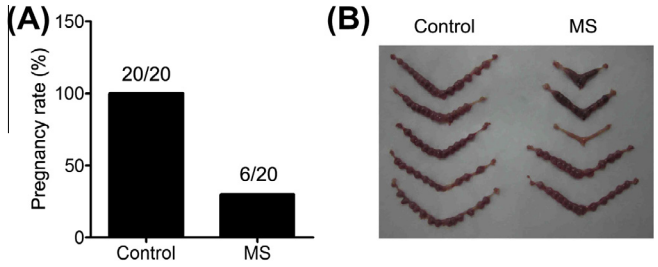

Fig. 8. Effects of mercaptosuccinic acid (MS) on mouse pregnancy. Mercaptosuccinic acid, a specific inhibitor for GPX, was dissolved in saline and injected i.p. (3.5 mg/mouse/day, Sigma) on days 5 (0900 h), 6 (0900 h) and 7 (0900 h) of pregnancy for three times. Controls received saline (0.1 ml/mouse). Mice were sacrificed in the morning of day 8, and ovulation was confirmed by counting the number of corpus luteum. Then uteri were photographed and collected.

4. Discussion

4.1. Specific expression of Gpx3 during decidualization

In this study, we first showed that *Gpx3* is strongly expressed in the decidua from days 5 to 8 of pregnancy. *Gpx3* expression is not detected during pseudopregnancy and under delayed implantation, but is detected after embryo activation and under artificial decidualization. Compared to delayed implantation, *Gpx3* is up-regulated by 12.28-fold after delayed implantation is activated by estrogen [28]. *GPX3* is highly expressed in secretory phase endometrium but only detectable at very low levels in the proliferative phase. Similarly, real-time RT-PCR studies showed an average 50-fold increase in *GPX3* expression in secretory phase versus proliferative phase endometrium [29]. *GPX3* is one of the up-regulated window of implantation genes delayed in controlled ovarian stimulation cycles compared with natural cycles [13].

4.2. Progesterone regulates Gpx3 expression through PR/HIF1α

In this study, *Gpx3* expression is significantly induced by progesterone both in vivo and in vitro. Furthermore, the up-regulation of *Gpx3* by progesterone is significantly blocked by RU486, suggesting that progesterone regulation on *Gpx3* is mainly mediated through progesterone receptor. However, *Gpx3* expression in mouse skeletal muscles is induced both acutely and chronically by estradiol-17β [30].

Blastocyst implantation occurs in a uterine environment low in oxygen (~18 mmHg or 2%), a condition that appreciably changes (~60 mmHg or 8%) [31–33]. Both the luminal epithelium and stroma exhibit *Hif1α* mRNA expression on day 5 of pregnancy. On day 8, *Hif1α* mRNA expression shows a further increase in the decidual bed [22]. The levels of *Hif1α* mRNA shows a gradual increase from 2 h after an injection of P4 and reaches a peak at 24 h [22]. In cultured mouse endometrial stromal cells, the up-regulation of HIF1α and GPX3 protein induced by progesterone is suppressed by RU486, indicating that progesterone stimulates HIF1α and GPX3 expression through PR. Progesterone cannot induce *Hif1α* expression in PR knockout mice, showing that progesterone induces *Hif1α*

though PR [22]. In our study, hypoxia induced by 2% O_2 or DFO also stimulates GPX3 expression. The up-regulation of GPX3 induced by progesterone is suppressed by a *Hif1α* inhibitor, Chrysin. Additionally, *Hif1α* expression in decidua is overlapped with *Gpx3* expression, suggesting that progesterone stimulates GPX3 expression through HIF1α. In silico analysis of *Gpx3* promoter also identifies a HIF1α binding site. Hypoxia is identified as a strong transcriptional regulator of GPX3 expression, in part through the presence of HIF1α binding site [34].

4.3. Gpx3 is involved in decidualization through reducing H_2O_2

We showed that the concentration of H_2O_2 at implantation sites is significantly lower than that of inter-implantation sites on day 5 of pregnancy. The expression of *Gpx3* is significantly increased at implantation sites and other members of GPX family are not significantly up-regulated in implantation sites, suggesting that GPX3 may play a major role in reducing H_2O_2 in mouse endometrium. The concentration of H_2O_2 in the supernatant of stromal cells undergoing in vitro decidualization is significantly down-regulated, which can be blocked by GPX inhibitor or *Gpx3* siRNA. There were similar results from human endometrial stromal cells undergoing in vitro decidualization, indicating that GPX3 has a similar function in human endometrium. It is shown that the endometrium is better equipped to detoxify ROS upon decidualization in vivo [35]. *Gpx3* may play a role in detoxifying ROS since it is strongly expressed in decidua and deciduoma.

Oxidative stress has an important role in the normal functioning of the female reproductive system and in the pathogenesis of female infertility [36,37]. The enzymic activity of placental GPX is also significantly reduced in tissues from preeclamptic women compared to normotensive women [38]. Gpx3 is identified as a potential biomarker for rat endometrial adenocarcinoma as *Gpx3* expression is greatly down-regulated in rat endometrial adenocarcinoma [39].

Although *Gpx3* is strongly expressed in mouse decidua, *Gpx3*(−/−) mice show no overt reproductive phenotypes compared with WT mice [40]. It is still unknown whether other members of GPX family are up-regulated or compensated in *Gpx3*(−/−) mouse uterus. Interestingly, homozygous *Dtprp*-null female mice are viable and fertile, and produce normal litter sizes. However, pregnancy failure is observed when pregnant *Dtprp*-null mice are exposed to hypoxia [41]. Therefore, it is better to check whether *Gpx3*(−/−) mice can reproduce normally under hypoxia or other stress conditions.

In conclusion, GPX3 is strongly expressed in mouse decidua. Both progesterone and hypoxia can stimulate GPX3 expression. GPX3 may be essential for mouse decidualization through reducing H_2O_2.

Conflict of interest

None.

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