

Differential expression and regulation of *Ido2* in the mouse uterus during peri-implantation period

Dang-Dang Li · Xin-Yuan Liu · Chuan-Hui Guo · Liang Yue · Zhan-Qing Yang · Hang Cao · Bin Guo · Zhan-Peng Yue

Received: 22 July 2014 / Accepted: 1 October 2014 / Published online: 19 November 2014 / Editor: T. Okamoto
© The Society for In Vitro Biology 2014

Abstract *Ido2* is involved in tryptophan catabolism and immunity, but its physiological functions remain poorly understood. This study was undertaken to examine the expression and regulation of *Ido2* gene in mouse uterus during the peri-implantation period. The results showed that *Ido2* mRNA was highly expressed on day 4 of pregnancy and in the delayed implantation uterus. On days 5–8 of pregnancy, a low level of *Ido2* expression was observed in the uteri. Simultaneously, *Ido2* mRNA was also lowly expressed in the decidualized uterus. In the uterine stromal cells, 8-Br-cAMP could inhibit the expression of *Ido2* mRNA. Moreover, *Ido2* mRNA expression was gradually decreased after the stromal cells were treated with estrogen and progesterone and reached a nadir at 96 h. Further study found that overexpression of *Ido2* could downregulate the expression of decidualization marker genes PRL, IGFBP1, and *Dtprp* under in vitro decidualization, while inhibition of *Ido2* with devo-1-methyl-tryptophan (D-1-MT) could upregulate the expression of these marker genes. Under in vitro decidualization, overexpression of *Ido2* could suppress the proliferation of uterine stromal cells and elevate the expression of *Bax* and *MMP2* genes. On the contrary, *Ido2* inhibitor D-1-MT could enhance the proliferation of stromal cells and expression of *Bcl2* gene but decline the *Bax/Bcl2* ratio. In the uterine stromal cells, estrogen and progesterone could induce the expression of *Ido2* mRNA. These data

indicate that *Ido2* may be important for mouse embryo implantation and decidualization.

Keywords *Ido2* · Implantation · Decidualization · Uterus · Mouse

Introduction

Tryptophan is an essential amino acid that is required for the biosynthesis of proteins and an immediate precursor of serotonin which has been identified within the female reproductive tract in ovaries, uterus, oviducts, placenta, and ovarian follicular fluid (Doherty *et al.* 2011; Li *et al.* 2013). High tryptophan level in culture medium could inhibit embryo development in vitro up to the blastocyst stage (McKiernan *et al.* 1995). Moreover, serotonin whose biosynthesis was dependent on tryptophan availability could disrupt implantation and impair decidualization (Mitchell *et al.* 1983; Mitchell and Hammer 1983). Thus, the redundant tryptophan must be metabolized during early pregnancy. In mammals, the kynurenine pathway was the major route for the oxidative degradation of tryptophan which was performed independently by indoleamine 2,3-dioxygenase-1 (*Ido1*), tryptophan 2,3-dioxygenase (*Tdo2*), and the recently discovered indoleamine 2,3-dioxygenase-2 (*Ido2*) (Austin *et al.* 2010; Fatokun *et al.* 2013). *Ido1* and *Tdo2* were the rate-limiting enzymes in the metabolism of tryptophan and could catalyze the oxidative cleavage of L-tryptophan pyrrole ring to form *N*-formylkynurenine (Thackray *et al.* 2008). Targeted disruption of *Ido1* or *Tdo2* could decrease the catabolism of tryptophan and increase the level of whole body serotonin (Stone and Darlington 2002; Kanai *et al.* 2009). Previous studies have demonstrated that *Ido1* and *Tdo2* were expressed in the placenta and uterus, and closely related to the process of decidualization (Sedlmayr *et al.* 2002; Kudo *et al.* 2004;

Dang-Dang Li and Xin-Yuan Liu contributed equally to this work.

D.-D. Li · X.-Y. Liu · C.-H. Guo · Z.-Q. Yang · H. Cao · B. Guo (✉) · Z.-P. Yue
College of Veterinary Medicine, Jilin University, Changchun, People's Republic of China
e-mail: guobin79@jlu.edu.cn

L. Yue
College of Clinical Medicine, Jilin University, Changchun 130062, People's Republic of China

Mei *et al.* 2012; Li *et al.* 2013). Tdo2 could induce the decidualization of uterine stromal cells, while Ido1 could inhibit the process of decidualization (Kudo *et al.* 2004; Mei *et al.* 2012; Li *et al.* 2013). In Ido1-deficient mice, there was a strong relative upregulation of Ido2 expression in the epididymis and macrophages, indicating that Ido2 could compensate for Ido1 loss (Fukunaga *et al.* 2012; Metz *et al.* 2014).

Ido2 was located immediately downstream of the *Ido1* gene on chromosome 8 and structurally similar to the *Ido1* gene (Ball *et al.* 2007; Fatokun *et al.* 2013). In human and mouse, Ido1 and Ido2 proteins shared 43% homology at the amino acid level but very little homology with the Tdo2 protein (Ball *et al.* 2007; Fatokun *et al.* 2013). Although Ido2 was involved in tryptophan catabolism, its physiological role was still yet to be defined. Only several studies found that Ido2 was expressed in the dendritic cells and essential for Ido1-dependent induction of regulatory T (Treg) cells (Metz *et al.* 2014; Trabanelli *et al.* 2014), suggesting that Ido2 has immunomodulatory effects. Simultaneously, Ido2 was also observed in the uterus, ovary, and placenta (Ball *et al.* 2009). However, the expression and regulation of Ido2 in mouse uterus during the peri-implantation were still not defined so far. Therefore, this study was undertaken to examine the expression and regulation of *Ido2* gene in mouse uterus during the peri-implantation period in order to provide insight into the physiological function of Ido2 during embryo implantation and decidualization.

Materials and Methods

Animal treatments. Matured mice (Kunming White strain) were caged in a controlled environment with a cycle of 14L:10D. All animal procedures were approved by the Institutional Animal Care and Use Committee of Jilin University. To confirm reproducibility of results, at least three mice per group were used in each stage or treatment in this study.

Pregnancy and pseudopregnancy. Adult female mice were mated with fertile or vasectomized males of the same strain to induce pregnancy or pseudopregnancy by cocaging, respectively (day 1 = day of vaginal plug). On days 1–4, pregnancy was confirmed by recovering embryos from the oviducts or uterus. The implantation sites on day 5 were identified by intravenous injection of 0.1 ml of 1% Chicago blue (Sigma, St. Louis, MO) in 0.85% sodium chloride.

Delayed implantation and activation. To induce delayed implantation, pregnant mice were ovariectomized under ether anesthesia at 08:30–09:00 AM on day 4 of pregnancy. Progesterone (P4) (1 mg/mouse; Sigma) was injected subcutaneously to maintain delayed implantation from days 5 to 7.

Estrogen (E2) (25 ng/mouse, Sigma) was given to progesterone-primed delayed-implantation mice to activate blastocyst implantation. The mice were sacrificed to collect uteri at 24 h after estrogen treatment. The implantation sites were identified by intravenous injection of Chicago blue solution. Delayed implantation was confirmed by flushing the blastocysts from the uterus.

Artificial induced decidualization. Artificial decidualization was induced by intraluminally infusing 25 μ l of sesame oil into one uterine horn on day 4 of pseudopregnancy, while the contralateral uninjected horn served as a control. The uteri were collected on day 8 of pseudopregnancy. Decidualization was confirmed by weighing the uterine horn and histological examination of uterine sections.

Isolation of uterine stromal cells and in vitro decidualization. Uterine stromal cells from day 4 of pregnancy were isolated and cultured as previously described (Li *et al.* 2013). Uterine stromal cells were induced for in vitro decidualization with fresh medium supplemented with progesterone (1 μ M) and estrogen (10 nM) in Dulbecco's modified Eagle's medium (DMEM)-F12 with 2% charcoal-treated fetal bovine serum (FBS) (Biological Industries Ltd., Kibbutz Beit Hemeek, Israel). In addition, uterine stromal cells were also treated with cyclic adenosine monophosphate (cAMP) analogue 8-bromoadenosine-cAMP (8-Br-cAMP, 500 μ M).

Steroid hormonal treatments in vitro. Cultured stromal cells were treated with 100 nM of progesterone or 0.1 nM of estrogen, respectively. Then cells were collected at 0, 1, 3, 6, 12, and 24 h for further quantitative analysis by real-time polymerase chain reaction (PCR). All steroids were dissolved in ethanol. Controls received the vehicle only.

Plasmid construction and transfection. Mouse Ido2 cDNA fragment was amplified by PCR from mouse uterus using the following primers: 5'-AAGCTTATGGAGCCTCAAAGTCAGAGC-3' and 5'-CTCGAGCTAAGCAC CAGGACACAGG-3' in which HindIII and XhoI sites were underlined. The amplified product was purified and cloned into pGEM-T vector. Both pGEM-T-Ido2 and pcDNA3.1 vectors were cut by HindIII/XhoI (TaKaRa, Dalian, China) at 37°C for 1 h, and then the fragment was ligated into pcDNA3.1 with T4 ligase (Promega, Madison, WI) at 4°C overnight to construct pcDNA-Ido2 (pc-Ido2). An empty pcDNA3.1 expression vector was served as control.

Transfection of uterine stromal cells was performed according to the manufacturer's protocol for lipofectamine 2000 (Invitrogen, Carlsbad, CA). After transfection with control plasmid (empty pcDNA3.1 vector) or pc-Ido2 plasmid,

stromal cells from day 4 pregnancy mice were collected or induced for in vitro decidualization for 48 h.

Real-time PCR. Total RNAs from mouse uteri or cultured cells were isolated using TRIPURE reagent according to the manufacturer's instructions (Roche, Indianapolis, IN) and reverse-transcribed into cDNA using M-MLV reverse-transcriptase (Promega). Reverse transcriptase was performed at 42°C for 60 min with 2 µg total RNA in 25 µl volume. For real-time PCR, cDNA was amplified using FS Universal SYBR Green Real Master (Roche) on BIO-RAD CFX96™ Real-Time Detection System. The conditions used for real-time PCR were as follows—95°C for 3 min, followed by 40 cycles of 95°C for 15 s and 60°C for 1 min. All reactions were run in triplicate. The results were analyzed using CFX Manager Software. After analysis using the $2^{-\Delta\Delta C_t}$ method, data were normalized to Gapdh expression. Primer sequences for real-time PCR were listed in Table 1.

Cell proliferation. Proliferation assays were performed using MTS reagent (Promega) according to the manufacturer's directions. Uterine stromal cells were seeded at a density of 1×10^5 /well in 96-well plates and cultured in the DMEM/F12 medium containing 2% heat-inactivated FBS. Cells were treated with estrogen and progesterone with/without 100 µM Ido2 inhibitor devo-1-methyl-tryptophan (D-1-MT), or transfected pc-Ido2 plasmid. Finally, 20 µl of MTS reagent was added to each well and incubated for 4 h. Absorbance was measured at 490 nm using a 96-well plate reader. Every experiment was performed in triplicate.

Statistics. All the experiments were independently repeated at least three times. The significance of difference was analyzed by one-way ANOVA or independent-samples *T* test using the SPSS software program (SPSS Inc., Chicago, IL). The differences were considered significant at $P < 0.05$.

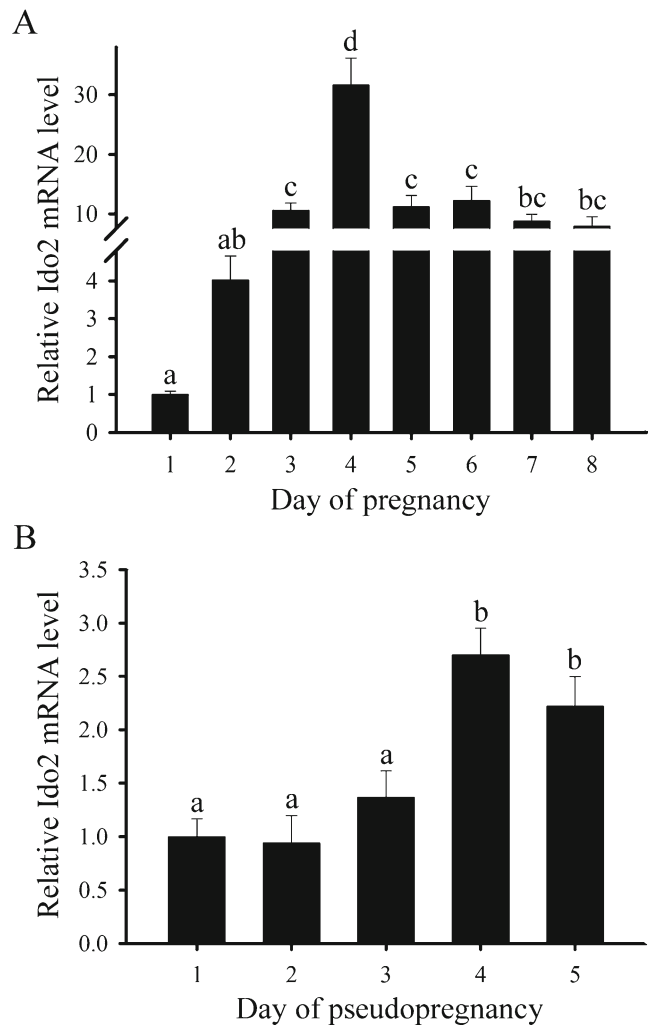


Figure 1. Ido2 mRNA expression during early pregnancy and pseudopregnancy. (A) Real-time PCR analysis of Ido2 expression in mouse uterus on days 1–8 of pregnancy. (B) Real-time PCR analysis of Ido2 expression in mouse uterus on days 1–5 of pseudopregnancy. Data are shown mean \pm SEM. Bars with different letters at the top differ significantly.

Table 1. Primers for real-time PCR

Gene	Primer sequence	Length of amplified fragment (bp)
<i>Ido2</i>	ACATGCCGCCTTCCCATAAG GGCATGGGGTGAGGGATTAG	227 bp
<i>PRL</i>	GGGACACTCCTCCTGTTGC CACACGGTCAAACAGCTCTC	117 bp
<i>IGFBP1</i>	TCGCCGACCTCAAGAAATGG GACACACCAGCAGAGTCCAG	197 bp
<i>Dtprp</i>	AGCCAGAAATCACTGCCACT TGATCCATGCACCCATAAAA	119 bp
<i>Bax</i>	GACTCTCCCCGAGAGGTCTT GTCCAATGTCCAGCCCATGA	171 bp
<i>Bcl2</i>	GGGCGCATCGTGGCCTTCTT GCGTCCCAGCCTCCGTTGTC	158 bp
<i>MMP2</i>	GGATACCCCAAGCCACTGAC ACGACGGCATCCAGGTTATC	212 bp
<i>MMP9</i>	GCACCTCCCCTATGTGTCC CAAGGATTGTCTGCCGGACT	209 bp
<i>Gapdh</i>	GCCTTCCGTGTCTCTACCC TGCCTGCTTCACCACCTTC	102 bp

Results

Ido2 mRNA expression in mouse uterus during early pregnancy. To quantify Ido2 mRNA expression in mouse uterus on days 1–8 of pregnancy, real-time PCR was performed. The result showed that Ido2 mRNA expression was gradually increased from days 1 to 4 of pregnancy and reached a peak on day 4, then declined and reached a nadir on days 7 and 8 of pregnancy (Fig. 1A).

Ido2 mRNA expression during pseudopregnancy and under delayed implantation. To address whether Ido2 expression was dependent on the embryo or active blastocyst, Ido2 expression during pseudopregnancy and under delayed implantation was examined. On days 1–5 of pseudopregnancy, Ido2 mRNA was highly expressed on day 4 by real-time PCR, although Ido2 expression was detected through days 1–5 (Fig. 1B). In the delayed-implantation uterus, a significantly higher level of Ido2 mRNA expression was detected compared with the activated implantation uterus (Fig. 2A).

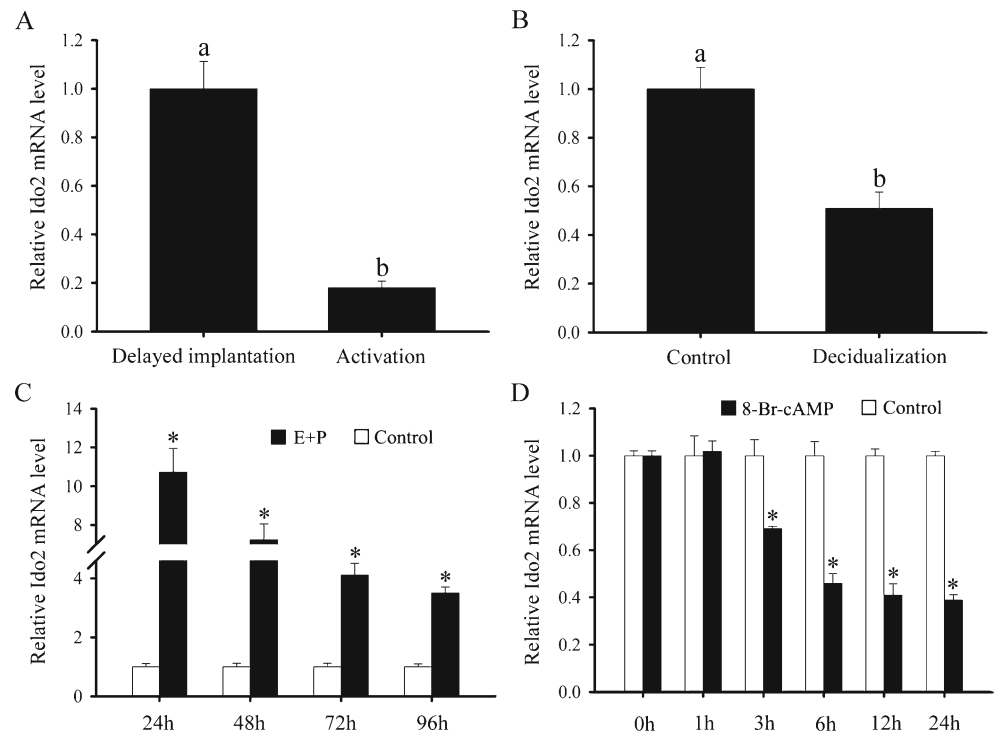
Ido2 mRNA expression during decidualization. To confirm whether Ido2 expression was correlated to the process of decidualization, we examined the expression of Ido2 under artificial decidualization and in vitro decidualization. Under artificial decidualization, a relatively low level of Ido2 expression was observed in the decidualized uterus compared with the uninjected control uterine horn (Fig. 2B). Previous study found that progesterone, estrogen, and cAMP could together

induce the decidualization of uterine stromal cells (Logan *et al.* 2013). Thus, we isolated stromal cells and treated them with estrogen and progesterone, or cAMP analogues 8-Br-cAMP to induce in vitro decidualization. The results found that Ido2 expression was gradually decreased along with the development of decidua and reached a nadir at 96 h, although Ido2 expression was higher in the stromal cells treated with estrogen and progesterone compared with that in the control cells (Fig. 2C). In the in vitro cultured stromal cells, treatment of 8-Br-cAMP resulted in a decline of Ido2 mRNA level which reached the minimum at 24 h (Fig. 2D).

The effects of Ido2 on decidualization. To examine whether Ido2 played a role in the process of decidualization, we observed the effects of Ido2 on the expression of prolactin (PRL), insulin-like growth factor binding protein 1 (IGFBP1), and decidual/trophoblast PRL-related protein (Dtprp) which were the established molecular markers for decidualization. The results showed that inhibition of Ido2 with D-1-MT could enhance the expression of PRL, IGFBP1, and Dtprp under in vitro decidualization (Fig. 3E–G), while overexpression of Ido2 could inhibit the expression of PRL, IGFBP1, and Dtprp (Fig. 3B–D), and significantly elevate the expression of Ido2 (Fig. 3A).

The effects of Ido2 on stromal cells proliferation. Because decidualization involves in extensive proliferation of uterine stromal cells, we treated the stromal cells with Ido2 inhibitor D-1-MT or transfected uterine stromal cells with the pc-Ido2

Figure 2. Real-time PCR analysis of Ido2 expression in mouse uteri and uterine stromal cells. (A) Real-time PCR analysis of Ido2 expression in mouse uterus during delayed implantation and activation. (B) Real-time PCR analysis of Ido2 expression in mouse uterus under artificial decidualization. (C) Real-time PCR analysis of Ido2 expression in in vitro decidualization of uterine stromal cells. Asterisks denote significance ($P < 0.05$) from the control group. (D) Real-time PCR analysis of Ido2 expression in the uterine stromal cells after 8-Br-cAMP treatment.



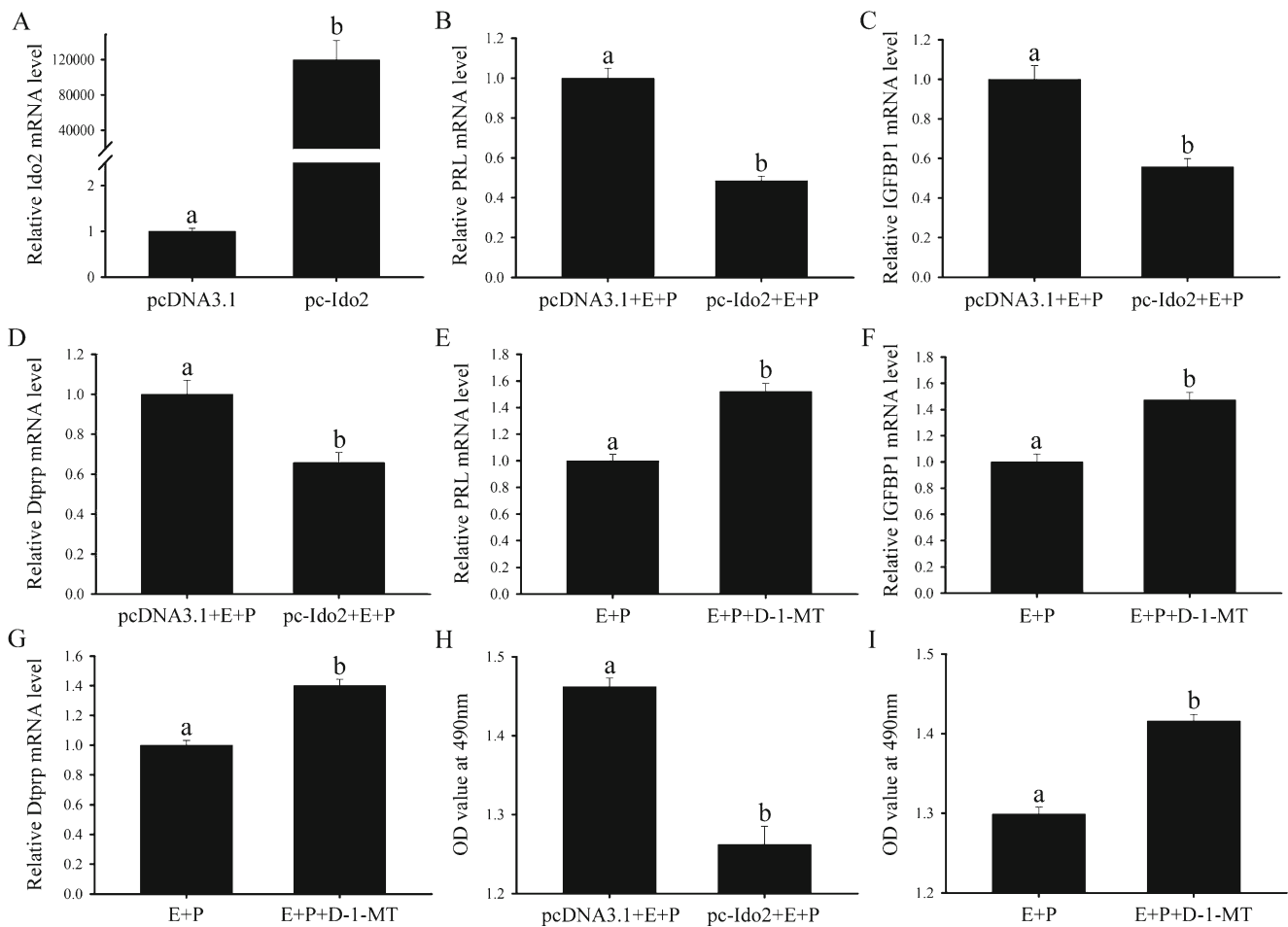


Figure 3. Effects of Ido2 on decidualization. (A) Ido2 mRNA expression following Ido2 overexpression. (B–D) Effects of Ido2 overexpression on the expression of *PRL*, *IGFBP1*, and *Dtprp* genes. After transfection with control plasmid (empty pcDNA3.1 vector) or Ido2 overexpression plasmid (pc-Ido2) for 6 h, the stromal cells were induced for in vitro decidualization with estrogen and progesterone. (E–G) Effects of D-1-

MT on the expression of *PRL*, *IGFBP1*, and *Dtprp* genes. Stromal cells were treated with estrogen and progesterone with/without Ido2 inhibitor D-1-MT. (H) Uterine stromal cells were treated with estrogen and progesterone for 48 h after transfection pc-Ido2 plasmid and analyzed by MTS assay. (I) Uterine stromal cells treated with estrogen and progesterone with/without D-1-MT were determined by MTS assay.

expression plasmid under in vitro decidualization and examined the effects of Ido2 on the proliferation of uterine stromal cells. The results showed that D-1-MT could obviously stimulate the proliferation activity of uterine stromal cells under in vitro decidualization (Fig. 3I). In contrast, proliferation activity of uterine stromal cells displayed a significant decrease after transfection with pc-Ido2 plasmid (Fig. 3H).

The effects of Ido2 on the expression of Bax, Bcl2, MMP2, and MMP9. Under in vitro decidualization, the expression of Bcl2 was suppressed by overexpression of Ido2 and enhanced by Ido2 inhibitor D-1-MT (Fig. 4C and D). In contrast, overexpression of Ido2 could elevate the expression of *Bax* and *MMP2* genes while Ido2 inhibitor D-1-MT could inhibit the expression of *MMP2* (Figs. 4A and 5A, B). Interestingly, D-1-MT could not restrain the expression of *Bax* gene (Fig. 4B). The *Bax/Bcl2* ratio was raised after transfection of pc-Ido2 plasmid but declined after the stromal cells were treated with

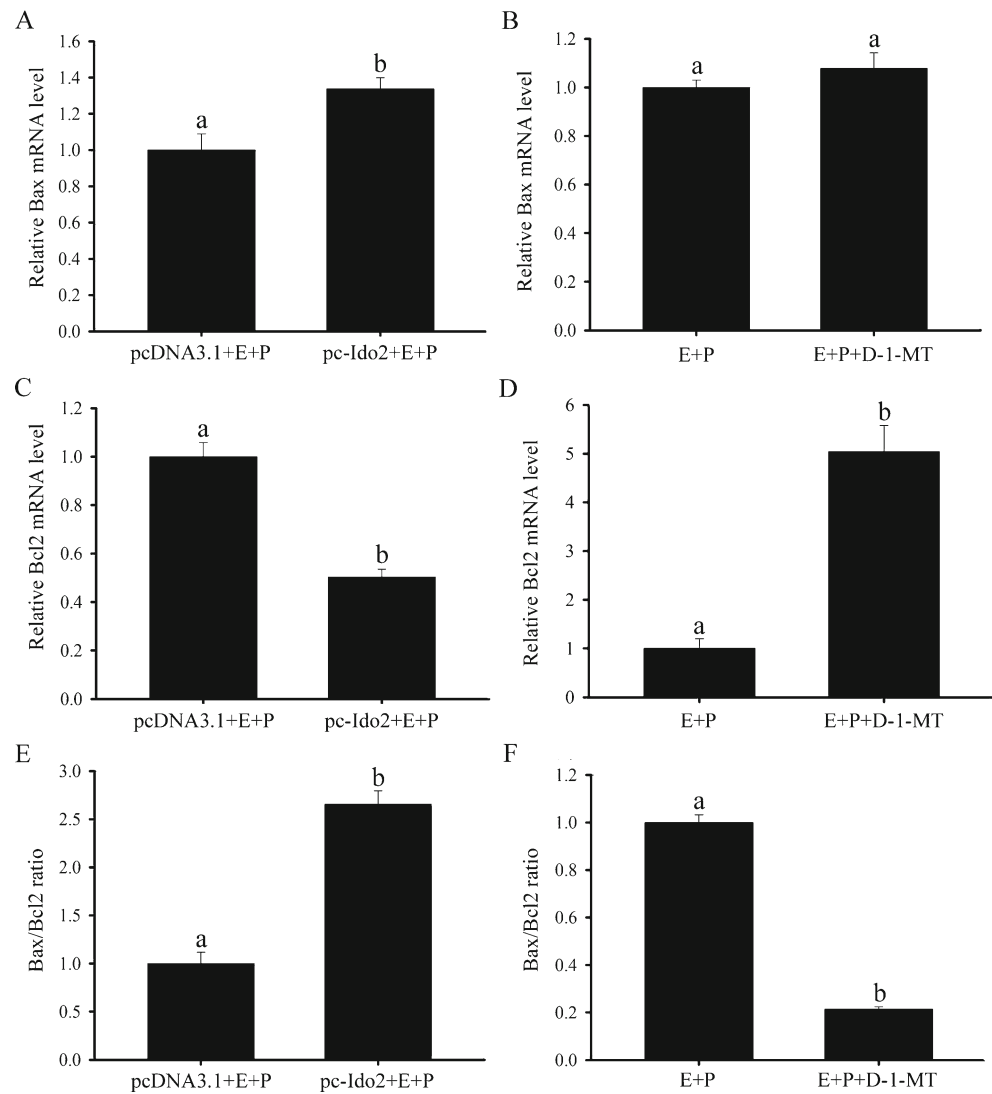
D-1-MT (Fig. 4E and F). However, Ido2 overexpression and D-1-MT treatment had no obvious effects on the expression of *MMP9* gene (Fig. 5C and D).

Steroid hormonal regulation on Ido2 expression. In the in vitro cultured stromal cells, progesterone treatment resulted in an increase of Ido2 mRNA level which reached the highest level at 12 and 24 h (Fig. 6B). After the uterine stromal cells were treated by estrogen, Ido2 mRNA expression was gradually elevated and reached a peak at 3 h, then declined and reached the lowest level at 24 h (Fig. 6A).

Discussion

Ido2 is involved in tryptophan catabolism and immunity, but its biological functions remain poorly understood. The present

Figure 4. Effects of Ido2 on the expression of Bax and Bcl2 under in vitro decidualization. (A) Effects of Ido2 overexpression on the expression of *Bax* gene. (B) Effects of D-1-MT on the expression of *Bax* gene. (C) Effects of Ido2 overexpression on the expression of *Bcl2* gene. (D) Effects of D-1-MT on the expression of *Bcl2* gene. (E) Effects of Ido2 overexpression on the Bax/Bcl2 ratio. (F) Effects of D-1-MT on the Bax/Bcl2 ratio.

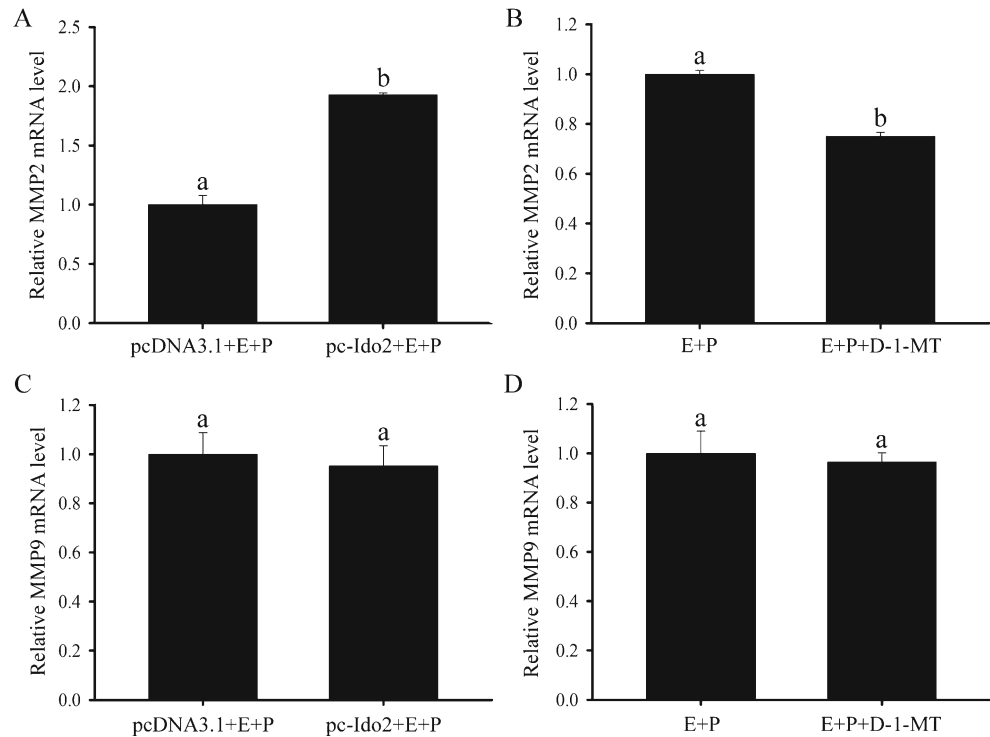


study was undertaken to examine the expression and regulation of Ido2 in mouse uterus during the peri-implantation period by real-time PCR. Our results showed that Ido2 was highly expressed in the mouse uterus on day 4 of pregnancy and in the delayed implantation uterus, implying that uterine tryptophan metabolism may enhance in this period. Further studies found that depletion of tryptophan could result in the inhibition of T cell activation and expansion of Treg cells, and further alter the T cell-mediated immune responses (Frumento *et al.* 2002; Curti *et al.* 2007). Indeed, Ido2 was required for the generation of Treg cells (Metz *et al.* 2014). Inhibition or deficiency of Ido2 could reduce the number of Treg cells which could play very important role in the induction of maternal tolerance to the semi-allogeneic fetus and the expression of Th2-type cytokines IL-4 and IL-6 which could promote allograft tolerance (Saito *et al.* 2007; Merlo *et al.* 2014; Metz *et al.* 2014; Trabanelli *et al.* 2014). These

results suggest that Ido2 may be necessary to prevent immunological rejection of fetal allografts and prepare for embryo implantation.

Uterine stromal cell decidualization is crucial for successful embryo implantation and maintenance of pregnancy (Zhang *et al.* 2013). On days 6–8 of pregnancy, Ido2 was lowly expressed in mouse uteri. The similar expression pattern was also noticed with Ido1. However, tryptophan-degrading activity was still observed on days 5.5–9.5 of post-coitus and not inhibited by the Ido1 inhibitor 1-methyl-L-tryptophan (Suzuki *et al.* 2001; Minatogawa *et al.* 2003). These results demonstrated that tryptophan metabolism on days 6–8 of pregnancy was not attributable to Ido1 and Ido2, but attributable to Tdo2. Indeed, a high level of Tdo2 mRNA signal was also detected in the decidua on days 6–8 of pregnancy (Li *et al.* 2013). Moreover, Tdo2 might induce the decidualization of uterine stromal cells (Li *et al.* 2013). In the artificial decidualized uterus, the expression of Ido2 mRNA was lower compared

Figure 5. Regulation of Ido2 on the expression of MMP2 and MMP9 under in vitro decidualization. (A) Effects of Ido2 overexpression on the expression of MMP2. (B) Effects of D-1-MT on the expression of MMP2. (C) Effects of Ido2 overexpression on the expression of MMP9. (D) Effects of D-1-MT on the expression of MMP9.



with that in control uterus. Previous studies have evidenced that injection of sesame oil resulted in an increase of uterine cAMP level which could induce the decidualization of uterine stromal cells in the normal cell culture and three-dimensional collagen gel culture (Rankin *et al.* 1977; Gellersen and Brosens 2003; Tsuno *et al.* 2009; Schutte and Taylor 2012; Tome *et al.* 2014; Yano *et al.* 2014). The present results illustrated that cAMP analogue 8-Br-cAMP inhibited the expression of Ido2 in the uterine stromal cells. However, Ido2 expression was higher in the stromal cells treated with estrogen and progesterone to induce in vitro decidualization compared with that in the control cells. The discrepancy indicated that the process of estrogen/progesterone-induced in vitro decidualization might consist of several pathways, including the intracellular cAMP signaling cascade. Indeed, previous study also found that inhibition of the cAMP signaling cascade did not suppress decidualization of uterine stromal cells (Wang *et al.* 2007). Taken together, these results suggest that Ido2 can be involved in the decidualization of mouse uterine stromal cells.

Decidualization is a process where uterine stromal cells undergo extensive proliferation, apoptosis, and differentiation into polyploid decidual cells (McConaha *et al.* 2011; Zhang *et al.* 2013). Under in vitro decidualization, inhibition of Ido2 with D-1-MT could induce the proliferation of stromal cells, while overexpression of Ido2 could suppress the proliferation of stromal cells. Simultaneously, Ido2 could also enhance the expression of Bax and Bax/Bcl2 ratio which promoted the apoptosis of stromal cells (Dai *et al.* 2000). Further study

found that Ido2 inhibitor D-1-MT could stimulate mammalian target of rapamycin (mTOR) which was a central coordinator in cell proliferation, differentiation, and apoptosis (Chen *et al.* 2009; Metz *et al.* 2012). The decreasing mTOR activity might affect the decidualization of stromal cells and further induce their apoptosis (Chen *et al.* 2009). These results indicate that Ido2 may prevent stromal cell proliferation and promote its apoptosis by affecting the mTOR activity under in vitro decidualization, and further inhibit the process of decidualization. Decidual cells were characterized by the increased expression of *PRL*, *IGFBP1*, and *Dtprp* genes which were the established molecular markers for decidualization and their pavement morphology (Tawadros *et al.* 2007). The present results revealed that inhibition of Ido2 with D-1-MT could enhance the expression of *PRL*, *IGFBP1*, and *Dtprp* under in vitro decidualization, while overexpression of Ido2 could inhibit the expression of these marker genes, which further verified the inhibition effect of Ido2 on decidualization. Additionally, decidualization was also involved in the uterine extracellular matrix remodeling which could be mediated by matrix metalloproteinases (MMPs) (Bany *et al.* 2000; Dey *et al.* 2004; McConaha *et al.* 2011). MMP2 and MMP9 were able to degrade the numerous components of the extracellular matrix (Bany *et al.* 2000). In this study, Ido2 could promote the expression of MMP2, but not MMP9 under in vitro decidualization. Moreover, MMP2 was also lowly expressed in the uterus undergoing oil-induced decidualization compared with the uninjected

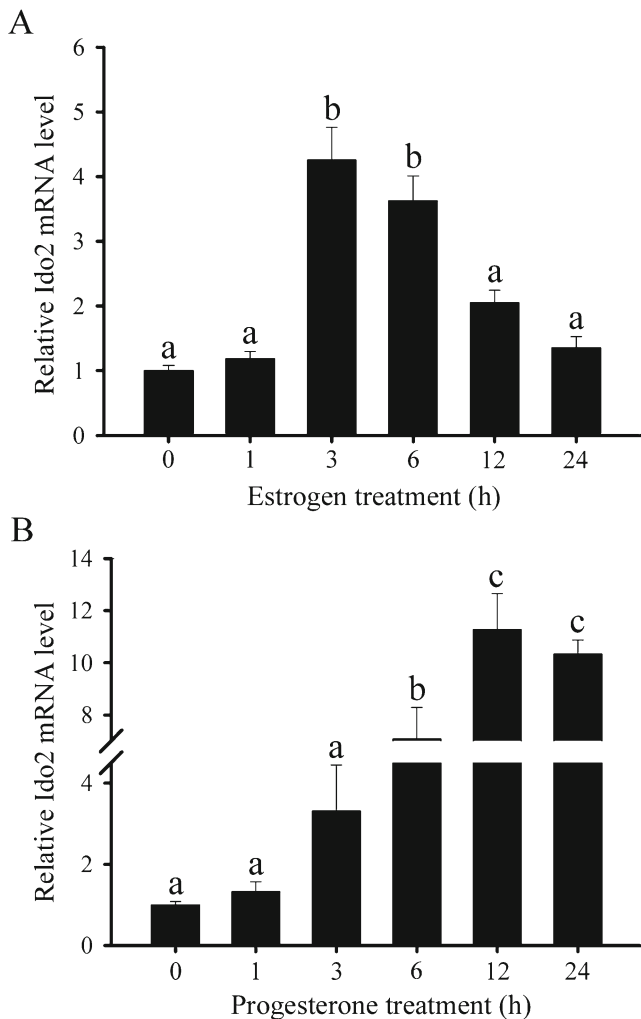


Figure 6. Hormonal regulation of Ido2 expression. (A) Real-time PCR analysis of Ido2 expression in the uterine stromal cells after estrogen treatment for 0, 1, 3, 6, 12, and 24 h. (B) Ido2 expression after stromal cells were treated with progesterone.

control uterus (Bany *et al.* 2000). These results state that Ido2 may influence the extracellular matrix remodeling of uterine stromal cells through MMP2 under in vitro decidualization.

It has been well established that ovarian estrogen and progesterone were required for embryo implantation and decidualization (Dey *et al.* 2004; Zhang *et al.* 2013). In the present study, Ido2 was highly expressed on day 4 of pregnancy when the level of progesterone was gradually increased accompanied by a surge of estrogen. The similar expression was observed in the pseudopregnant mouse uterus. These results indicate that the expression of Ido2 may be under the control of maternal hormones and not related to the presence of embryo. Indeed, estrogen and progesterone induced the expression of Ido2 mRNA in the uterine stromal cells.

In summary, Ido2 may be important for mouse embryo implantation and decidualization and regulated by estrogen and progesterone in the uterine stromal cells.

Acknowledgment This work was financially supported by Special Funds for Scientific Research on Public Causes (201303119), National Natural Science Foundation of China (31472158 and 31372390), and College Student Innovation Experimental Program of Jilin University (2013B85402).

References

- Austin CJ, Mailu BM, Maghzal GJ, Sanchez-Perez A, Rahlfs S, Zocher K, Yuasa HJ, Arthur JW, Becker K, Stocker R, Hunt NH, Ball HJ (2010) Biochemical characteristics and inhibitor selectivity of mouse indoleamine 2,3-dioxygenase-2. *Amino Acids* 39:565–578
- Ball HJ, Sanchez-Perez A, Weiser S, Austin CJ, Astelbauer F, Miu J, McQuillan JA, Stocker R, Jermin LS, Hunt NH (2007) Characterization of an indoleamine 2,3-dioxygenase-like protein found in humans and mice. *Gene* 396:203–213
- Ball HJ, Yuasa HJ, Austin CJ, Weiser S, Hunt NH (2009) Indoleamine 2,3-dioxygenase-2; a new enzyme in the kynurenine pathway. *Int J Biochem Cell Biol* 41:467–471
- Bany BM, Harvey MB, Schultz GA (2000) Expression of matrix metalloproteinases 2 and 9 in the mouse uterus during implantation and oil-induced decidualization. *J Reprod Fertil* 120:125–134
- Chen X, He J, Ding Y, Zeng L, Gao R, Cheng S, Liu X, Wang Y (2009) The role of MTOR in mouse uterus during embryo implantation. *Reproduction* 138:351–356
- Curti A, Pandolfi S, Valzasina B, Aluigi M, Isidori A, Ferri E, Salvestrini V, Bonanno G, Rutella S, Durelli I, Horenstein AL, Fiore F, Massaia M, Colombo MP, Baccarani M, Lemoli RM (2007) Modulation of tryptophan catabolism by human leukemic cells results in the conversion of CD25+ into CD25+ T regulatory cells. *Blood* 109:2871–2877
- Dai D, Moulton BC, Ogle TF (2000) Regression of the decidualized mesometrium and decidual cell apoptosis are associated with a shift in expression of Bcl2 family members. *Biol Reprod* 63:188–195
- Dey SK, Lim H, Das SK, Reese J, Paria BC, Daikoku T, Wang H (2004) Molecular cues to implantation. *Endocr Rev* 25:341–373
- Doherty LF, Kwon HE, Taylor HS (2011) Regulation of tryptophan 2,3-dioxygenase by HOXA10 enhances embryo viability through serotonin signaling. *Am J Physiol Endocrinol Metab* 300:E86–93
- Fatokun AA, Hunt NH, Ball HJ (2013) Indoleamine 2,3-dioxygenase 2 (IDO2) and the kynurenine pathway: characteristics and potential roles in health and disease. *Amino Acids* 45:1319–1329
- Frumento G, Rotondo R, Tonetti M, Damonte G, Benatti U, Ferrara GB (2002) Tryptophan-derived catabolites are responsible for inhibition of T and natural killer cell proliferation induced by indoleamine 2,3-dioxygenase. *J Exp Med* 196:459–468
- Fukunaga M, Yamamoto Y, Kawasoe M, Arioka Y, Murakami Y, Hoshi M, Saito K (2012) Studies on tissue and cellular distribution of indoleamine 2,3-dioxygenase 2: the absence of IDO1 upregulates IDO2 expression in the epididymis. *J Histochem Cytochem* 60:854–860
- Gellersen B, Brosens J (2003) Cyclic AMP and progesterone receptor cross-talk in human endometrium: a decidualizing affair. *J Endocrinol* 178:357–372
- Kanai M, Funakoshi H, Takahashi H, Hayakawa T, Mizuno S, Matsumoto K, Nakamura T (2009) Tryptophan 2,3-dioxygenase is a key modulator of physiological neurogenesis and anxiety-related behavior in mice. *Mol Brain* 2:8
- Kudo Y, Hara T, Katsuki T, Toyofuku A, Katsura Y, Takikawa O, Fujii T, Ohama K (2004) Mechanisms regulating the expression of indoleamine 2,3-dioxygenase during decidualization of human endometrium. *Hum Reprod* 19:1222–1230

- Li DD, Gao YJ, Tian XC, Yang ZQ, Cao H, Zhang QL, Guo B, Yue ZP (2013) Differential expression and regulation of Tdo2 during mouse decidualization. *J Endocrinol* 220:73–83
- Logan PC, Ponnampalam AP, Steiner M, Mitchell MD (2013) Effect of cyclic AMP and estrogen/progesterone on the transcription of DNA methyltransferases during the decidualization of human endometrial stromal cells. *Mol Hum Reprod* 19:302–312
- McConaha ME, Eckstrum K, An J, Steinle JJ, Bany BM (2011) Microarray assessment of the influence of the concepts on gene expression in the mouse uterus during decidualization. *Reproduction* 141:511–527
- McKiernan SH, Clayton MK, Bavister BD (1995) Analysis of stimulatory and inhibitory amino acids for development of hamster one-cell embryos in vitro. *Mol Reprod Dev* 42:188–199
- Mei J, Jin LP, Ding D, Li MQ, Li DJ, Zhu XY (2012) Inhibition of IDO1 suppresses cyclooxygenase-2 and matrix metalloproteinase-9 expression and decreases proliferation, adhesion and invasion of endometrial stromal cells. *Mol Hum Reprod* 18:467–476
- Merlo LM, Pigott E, DuHadaway JB, Grabler S, Metz R, Prendergast GC, Mandik-Nayak L (2014) IDO2 is a critical mediator of autoantibody production and inflammatory pathogenesis in a mouse model of autoimmune arthritis. *J Immunol* 192:2082–2090
- Metz R, Rust S, DuHadaway JB, Mautino MR, Munn DH, Vahanian NN, Link CJ, Prendergast GC (2012) IDO inhibits a tryptophan sufficiency signal that stimulates mTOR: a novel IDO effector pathway targeted by D-1-methyl-tryptophan. *Oncimmunology* 1:1460–1468
- Metz R, Smith C, DuHadaway JB, Chandler P, Baban B, Merlo LM, Pigott E, Keough MP, Rust S, Mellor AL, Mandik-Nayak L, Muller AJ, Prendergast GC (2014) IDO2 is critical for IDO1-mediated T-cell regulation and exerts a non-redundant function in inflammation. *Int Immunol* 26:357–367
- Minatogawa Y, Suzuki S, Ando Y, Tone S, Takikawa O (2003) Tryptophan pyrrole ring cleavage enzymes in placenta. *Adv Exp Med Biol* 527:425–434
- Mitchell JA, Hammer RE (1983) Serotonin-induced disruption of implantation in the rat: I. Serum progesterone, implantation site blood flow, and intrauterine pO₂. *Biol Reprod* 28:830–835
- Mitchell JA, Hammer RE, Goldman H (1983) Serotonin-induced disruption of implantation in the rat: II. Suppression of decidualization. *Biol Reprod* 29:151–156
- Rankin JC, Ledford BE, Baggett B (1977) Early involvement of cyclic nucleotides in the artificially stimulated decidual cell reaction in the mouse uterus. *Biol Reprod* 17:549–554
- Saito S, Shima T, Nakashima A, Shiozaki A, Ito M, Sasaki Y (2007) What is the role of regulatory T cells in the success of implantation and early pregnancy? *J Assist Reprod Genet* 24:379–386
- Schutte SC, Taylor RN (2012) A tissue-engineered human endometrial stroma that responds to cues for secretory differentiation, decidualization, and menstruation. *Fertil Steril* 97:997–1003
- Sedlmayr P, Blaschitz A, Wintersteiger R, Semlitsch M, Hammer A, MacKenzie CR, Walcher W, Reich O, Takikawa O, Dohr G (2002) Localization of indoleamine 2,3-dioxygenase in human female reproductive organs and the placenta. *Mol Hum Reprod* 8:385–391
- Stone TW, Darlington LG (2002) Endogenous kynurenes as targets for drug discovery and development. *Nat Rev Drug Discov* 1:609–620
- Suzuki S, Toné S, Takikawa O, Kubo T, Kohno I, Minatogawa Y (2001) Expression of indoleamine 2,3-dioxygenase and tryptophan 2,3-dioxygenase in early concepti. *Biochem J* 355:425–429
- Tawadros N, Salamonsen LA, Dimitriadis E, Chen C (2007) Facilitation of decidualization by locally produced ghrelin in the human endometrium. *Mol Hum Reprod* 13:483–489
- Thackray SJ, Mowat CG, Chapman SK (2008) Exploring the mechanism of tryptophan 2,3-dioxygenase. *Biochem Soc Trans* 36:1120–1123
- Tome Y, Uehara F, Mii S, Yano S, Zhang L, Sugimoto N, Maehara H, Bouvet M, Tsuchiya H, Kanaya F, Hoffman RM (2014) 3-dimensional tissue is formed from cancer cells in vitro on Gelfoam[®], but not on Matrigel[™]. *J Cell Biochem* 115:1362–1367
- Trabanelli S, Očadlíková D, Ciciarello M, Salvestrini V, Lecciso M, Jandus C, Metz R, Evangelisti C, Laury-Kleintop L, Romero P, Prendergast GC, Curti A, Lemoli RM (2014) The SOCS3-independent expression of IDO2 supports the homeostatic generation of T regulatory cells by human dendritic cells. *J Immunol* 192:1231–1240
- Tsuno A, Nasu K, Yuge A, Matsumoto H, Nishida M, Narahara H (2009) Decidualization attenuates the contractility of eutopic and ectopic endometrial stromal cells: implications for hormone therapy of endometriosis. *J Clin Endocrinol Metab* 94:2516–2523
- Wang DF, Minoura H, Sugiyama T, Tanaka K, Kawato H, Toyoda N, Sagawa N (2007) Analysis on the promoter region of human decidual prolactin gene in the progesterone-induced decidualization and cAMP-induced decidualization of human endometrial stromal cells. *Mol Cell Biochem* 300:239–247
- Yano S, Miwa S, Mii S, Hiroshima Y, Uehara F, Yamamoto M, Kishimoto H, Tazawa H, Bouvet M, Fujiwara T, Hoffman RM (2014) Invading cancer cells are predominantly in G0/G1 resulting in chemoresistance demonstrated by real-time FUCCI imaging. *Cell Cycle* 13:953–960
- Zhang S, Lin H, Kong S, Wang S, Wang H, Wang H, Armant DR (2013) Physiological and molecular determinants of embryo implantation. *Mol Aspects Med* 34:939–980