Estrogen induces IDO expression via TGF-β in chorionic villi and decidua during early stages of pregnancy

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Abstract. Indoleamine 2,3-dioxygenase (IDO) is one of the most important proteins protecting the embryos from the mother's immune system during pregnancy; however, little is known about the regulation of expression of this protein at the maternal-fetal interface. In the current study, chorionic villi and decidua were collected from women at early stages of pregnancy. Samples of chorionic villi and decidua were cultured in medium containing different concentrations of 17β-estradiol and estriol respectively, with or without fulvestrant. Western blot analysis and/or immunofluorescent staining were used to detect the expression of transforming growth factor β (TGF-β) and IDO in chorionic villi and decidua tissues. Both TGF-β and IDO were expressed in chorionic villi and decidua. The expression levels of these two proteins increased the most in samples of chorionic villi and decidua cultured in medium containing 17β-estradiol at the concentration of 10 ng/ml, or estriol at the concentration of 1 μg/ml. This increase could be reversed when fulvestrant was added in the medium at the concentration of 10 μg/ml. IDO expression increased in a dose-dependent manner in tissue samples cultured in medium containing TGF-β. The results of the current study revealed that administration of estrogen at doses similar to those observed in healthy pregnant women may upregulate the expression of IDO by TGF-β, suggesting that estrogen may prevent allogeneic fetal rejection and may be used as an immunomodulator.

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Abbreviations: E1, estrone; E2, 17β-estradiol; E3, estriol; IDO, indoleamine 2,3-dioxygenase; IFN-γ, interferon-γ; pDC, plasmacytoid dendritic cell; Th, T helper; TGF-β, transforming growth factor-β; Tregs, regulatory T cells

Key words: estrogen, indoleamine 2,3-dioxygenase, transforming growth factor-β, pregnancy, chorionic villi, decidua

Introduction

Although a fetus attains only half of its genetic material from the mother, maternal-fetal tolerance allows implantation of the fetus within the womb and the establishment of a temporary immune system during fetal development (1,2). The underlying molecular mechanism remains unclear; however, three hypotheses of maternal immunological tolerance have been proposed: i) Physical separation of fetal and maternal tissues; ii) antigenic immaturity of fetal tissues; and iii) immunological inertness (tolerance) of maternal tissues towards fetal alloantigens (3). However, these hypotheses do not fully explain the underlying mechanisms. It has been hypothesized trophoblast cells from the outer layer of a blastocyst act as an interface layer and provide nutrients and a constant blood supply to the embryo. Furthermore, trophoblast cells develop to form an important part of the placenta, particularly through their interactions with decidua and placental villi (4).

Estrogen is vital for the female reproductive development and the three major naturally occurring forms are: Estrone (E1), 17 β-Estradiol (E2), Estriol (E3). During pregnancy, E2 and E3 serum levels have been reported to increase 100 and 1,000 times, respectively, while estrogen markedly decreases the levels of regulatory T-cells (Tregs) following parturition (5). During pregnancy, placenta cells produce E3. Estrogen also has a function in the immune system; however, its underlying molecular mechanism remains to be fully understood.

Indoleamine 2,3-dioxygenase (IDO) is an essential enzyme in tryptophan catabolism. It degrades amino acid L-tryptophan into kynurenine, which depletes tryptophan and subsequently suppresses T-cell activity (6,7). The molecular mechanisms protecting the fetus include trophoblast cells, major histocompatibility complex, T cell apoptosis, suppression of cell proliferation and IDO-mediated tryptophan catabolism (8). It has been demonstrated that IDO is expressed by human trophoblasts and decidual cells, and is activated at the maternal-fetal interface (9-11). Toxic effects of tryptophan metabolites are responsible for inhibition of T cell proliferation and Tregs, and it has been suggested that IDO may be associated with the T helper (Th)1/Th2 cell balance (6,12,13). Both direct and indirect analyses have reported that IDO is associated with human maternal fetal interface and the immune system (14,15).

Transforming growth factor β (TGF-β) is a multifunctional signal transducer, which is associated with cell apoptosis, cell cycle and differentiation, and has also been implicated in tumor growth (16). A previous study reported that IDO expression increased in mouse plasmacytoid dendritic cells (pDCs) treated with TGF-β (17). Furthermore, in chorionic villi and decidua tissues of healthy pregnant women, both TGF-β and IDO are expressed at high levels and TGF-β expression is positively correlated with IDO expression (11). Thus, the current study hypothesized that TGF-β may upregulate IDO expression, and that increased levels of estrogen may induce IDO expression in chorionic villi and decidual tissues by upregulating TGF-β expression in pregnant women.

Materials and methods

Participants and samples. A total of 40 healthy pregnant women who underwent legal termination of pregnancy at the Affiliated Hospital of Guizhou Medical University (Guiyang, China) between April 2015 and October 2017 were recruited in the present study. The mean age of participants was 27.56±6.27 years, while gestational age was 57.23±7.42 days. All participants presented with normal embryonic development determined by ultrasonic examination, cases with abnormalities of the reproductive system were identified and excluded (18). The exclusion criteria were as follows: Participant with history of endometriosis, and chronic diseases associated with chronic hypertension, kidney disease and diabetes. The development of a healthy fetus was confirmed via ultrasonic examination, with no detectable uterine abnormality. All participants provided voluntary consent to pregnancy termination.

Tissue culture. Decidua and chorionic villi tissues were identified by morphology. Chorionic villi and decidua tissue samples collected under aseptic conditions were immediately placed in 0.1 M sterile PBS (pH 7.2), transferred to ice within 10 min and subsequently washed twice with PBS. Tissue samples were either preserved at -80˚C prior to western blot analysis or cut into small pieces (≤1 mg wet weight) for tissue culture. The samples prepared for tissue culture were processed within 40 min of collection. The chorionic villi and decidua pieces were washed twice with F-12 nutrient mixture (F-12)/DMEM (Invitrogen; Thermo Fisher Scientific, Inc.), centrifuged at 1,200 x g for 5 min at room temperature within less than 40 min, and cultured in F-12/DMED supplemented with 10% FBS (Gibco, Thermo Fisher Scientific, Inc) and 1% penicillin-streptomycin (Invitrogen; Thermo Fisher Scientific, Inc.) in a CO_2 incubator (Thermo Fisher Scientific, Inc.) at 37˚C with 5% CO_2.

Treatment of villi and decidua. Villi and decidua cultures were plated in six-well plates at a density of $5x10^5$ cells/well (Corning Inc.) and cultured in phenol red-free DMED/Ham's F-12 (Gibco; Thermo Fisher Scientific, Inc.) supplemented with 10% FBS (Gibco; Thermo Fisher Scientific, Inc.) and 1% penicillin-streptomycin (Invitrogen; Thermo Fisher Scientific, Inc.) at 37˚C with 5% CO_2. Villi and decidua cells were prepared for immunofluorescence (IF) and a part of western blotting experiments. TGF-β1 powder (R&D Systems, Inc.) was dissolved in PBS at three concentrations (0.01, 0.5 and 0.1 ng/ml, respectively) and incubated with the chorionic villi and decidua tissue sections for 48 h at 37˚C with 5% CO_2. Fulvestrant was purchased from Selleck Chemicals, diluted in DMED/Ham's F-12 and stored at 4˚C. E2 and E3 were diluted in ethyl alcohol (all Sigma-Aldrich; Merck KGaA). An equal concentration of ethyl alcohol alone was used in the control group. Sections of chorionic villi or decidua were incubated in medium containing 10 ng/ml E2 or 1 μg/ml E3, prior to addition of 0.1, 1 and 10 μg/ml fulvestrant for 12 h at 37˚C with 5% CO_2.

IF staining. For immunofluorescence staining, villi and decidua cells were treated with 10 mg/ml E2, 1 μg/ml E3 or 1 μg/ml fulvestrant for 48 h, then fixed in PBS supplemented with 4% paraformaldehyde (Sigma-Aldrich; Merck KGaA) for 15 min, prior to treatment in PBS supplemented with 0.5% Triton X-100 for 15 min. Cells were incubated with PBS containing 4% bovine serum albumin (Gibco; Thermo Fisher Scientific, Inc.) at room temperature for 30 min and subsequently washed three times with PBS (5 min/wash) Tissue samples were incubated with the following primary antibodies: IDO rabbit anti-human (1:1,000; cat. no. 86630; Cell Signaling Technology, Inc.) and TGF-β rabbit anti human (1:500; cat. no. 3709; Cell Signaling Technology, Inc.) overnight at 4˚C. Following the primary antibody incubation, membranes were incubated with isothiocyanate-conjugated donkey anti-rabbit FITC (1:1,000; F8070; Beijing Solarbio Science & Technology Co., Ltd.) and Cy® 3 Immunoglobulin G donkey anti-rabbit (1:1,000; S1050; Beijing Solarbio Science & Technology Co., Ltd.) secondary antibodies for 1 h at room temperature. All antigen were retrieval Once store at 4˚C, staining cells were wash with PBS reagent 3 times every 5 mins. Nuclei were stained with DAPI (cat. no. D1306; Invitrogen; Thermo Fisher Scientific, Inc.) for 3 min at room temperature, mounted onto slides and confocal dishes, and observed under a confocal microscope (LSM 710; Carl Zeiss AG). Images were captured using a 63X numerical aperture objective.

Western blotting. Western blot analysis was performed as previously described (10). Briefly, total protein was extracted from chorionic villi tissues, decidua tissues or cultured isolated cells using ice-cold RIPA (Sigma-Aldrich; Merck KGaA). Tissue samples or isolated cells were treated with

protease inhibitors at 4˚C for 30 min (Sigma Aldrich) and proteins were isolated followed by centrifugation at 11,000 x g for 20 min at 4˚C (repeated twice). Proteins were separated via SDS-PAGE on 10 or 15% gel. The separated proteins were subsequently transferred onto PVDF membranes (PerkinElmer, Inc.) at 200 mA for 1.5 h. After blocking for 1 h in 5% non-fat milk, the membranes were incubated with the following primary antibodies: Anti-human IDO monoclonal antibody (1:3,000; cat. no. 86630; Cell Signaling Technology, Inc.), TGF-β polyclonal antibody (1:3,000; cat. no. 3709; Cell Signaling Technology, Inc.), or human GAPDH polyclonal antibody (1:6,000; cat. no. GTX100118; GeneTex, Inc.) for 3 h at room temperature. Membranes were washed three time with tris-buffered saline with Tween-20. Following the primary antibody incubation, membranes were incubated with horseradish peroxidase-labeled donkey anti-rabbit immunoglobulin G antibody (Cell Signaling Technology, Inc.) for 1 h at room temperature. Protein bands were visualized using the ECL kit (PerkinElmer, Inc.) followed by autoradiography (GenoSwns1880, 3300018-7Q; Clinx Science Instruments Co. Ltd.). All experiment were performed in triplicate.

Statistical analysis. All statistical analyses were performed using GraphPad (version 6.0; GraphPad Software, Inc.) and SPSS (23.0; IBM, Corp.) and presented as the mean ± standard error of the mean. Two independent groups were compared using Pearson's correlation analysis. One-way ANOVA followed by Student-Newman-Keuls post hoc test or Turkey's test were used to assess protein expression following western blot analysis. $P<0.05$ was considered to indicate a statistically significant difference.

Results

IDO and TGF-β expression in chorionic villi and decidua tissues at early stages of pregnancy. The western blot analysis demonstrated that IDO (Fig. 1A) and TGF-β (Fig. 1B) were expressed in both villi and decidua tissue samples. IF staining indicated that TGF-β was predominantly expressed in the cytoplasm of villi and decidua cells as indicated by red fluorescence, while IDO was identified in both the cytoplasm and nucleus of villi and decidua cells as indicated by green fluorescence (Fig. 1C and D). Furthermore, villi and decidua cells exhibited a similar cell size and IDO and TGF-β expression levels.

E2 and E3 induce IDO expression in chorionic villi and decidua. In order to confirm whether estrogen affected IDO expression, the chorionic villi and decidua sections were cultured in the medium containing 100, 10 or 1 ng/ml E2 or 10, 1 and 0.1 μg/ml E3 for 48 h. Western blot analysis demonstrated that IDO expression increased in chorionic villi (Fig. 2A, C, E and G) and decidua (Fig. 2B, D, F and H) cultured in the medium containing E2 (Fig. 2A-D) or E3 (Fig. 2E-H) compared with the control group. The greatest increase in IDO expression was detected in sections of chorionic villi and decidua cultured in the medium containing 10 ng/ml of E2 or in villi and decidua cultured in 1 μg/ml of E3.

E2 and E3 induce TGF-β expression in chorionic villi and decidua. The effect of estrogen on TGF-β expression was determined in the chorionic villi and decidua sections. Western blot analysis illustrated that TGF-β expression increased in chorionic villi and decidua tissues cultured in the medium containing 10 ng/ml E2 (Fig. 2I) or 1 μg/ml E3 (Fig. 2J) compared with control group.

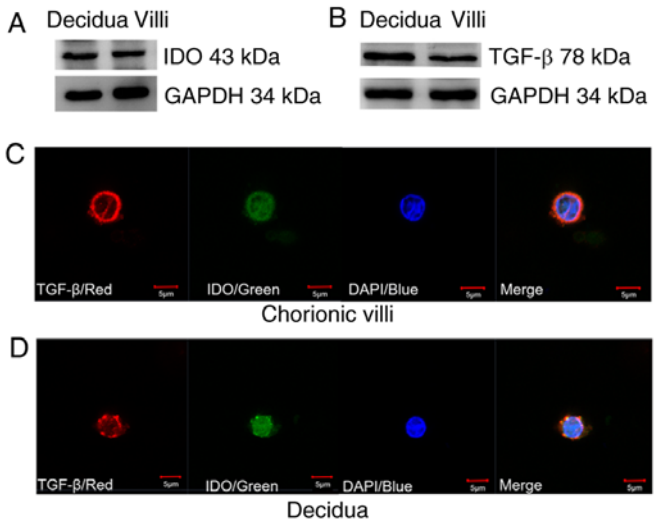

Figure 1. Expression of IDO and TGF-β in chorionic villi and decidua. The expression of (A) IDO and (B) TGF-β in chorionic villi and decidua detected by western blot analysis. Data are representative of three independent experiments. The expression of IDO (green fluorescence) and TGF-β (red fluorescence) detected by immunofluorescence staining in (C) chorionic villi and (D) decidua cells. Cell nuclei were stained by DAPI (blue fluorescence). Data are representative of three independent experiments. Magnification, x63. IDO, indoleamine 2,3-dioxygenase; TGF-β, transforming growth factor β.

E2 and E3 induce IDO expression via TGF-β in chorionic villi and decidua. To elucidate the underlying molecular mechanism by which estrogen induces IDO expression, chorionic villi and decidua cells were incubated in medium containing 100, 10 and 1 ng/ml E2 or 10, 1 and 0.1 μg/ml E3 for 48 h. Western blot analysis indicated that both IDO and TGF-β expression levels increased in 10 ng/ml of E2 and 1 μg/ml of E3 groups compared with the other treatment concentrations, and that TGF-β expression was positively associated with IDO levels in sections of chorionic villi (Fig. 3C and E; R=0.736 and 0.756) and decidua (Fig. 3H and J; R=0.567 and 0.714) cultured in the medium containing E2 (Fig. B-C) or E3 (Fig. D-E). The greatest increase in IDO and TGF-β expression levels was observed in sections of chorionic villi (Fig. 3A, B and D) and decidua (Fig. 3F, G and I) cultured in the medium containing 10 ng/ml E2 (Fig. 3A, B, F and G) or 1 μg/ml E3 (Fig. 3A, D, F and I). The results demonstrated that the TGF-β expression was positively associated with IDO expression in cultured chorionic villi and decidua in the presence of estrogen, suggesting that estrogen may simultaneously upregulate TGF-β and IDO expression.

In order to determine whether TGF-β induced the expression of IDO, sections of chorionic villi and decidua were incubated in the medium containing 0.01, 0.5 and 0.1 ng/ml TGF-β for 48 h. Western blot analysis demonstrated that IDO expression increased in a dose-dependent manner in both chorionic villi (Fig. 3K and M) and decidua (Fig. 3L and N) cultured in the medium containing TGF-β. Taken together,

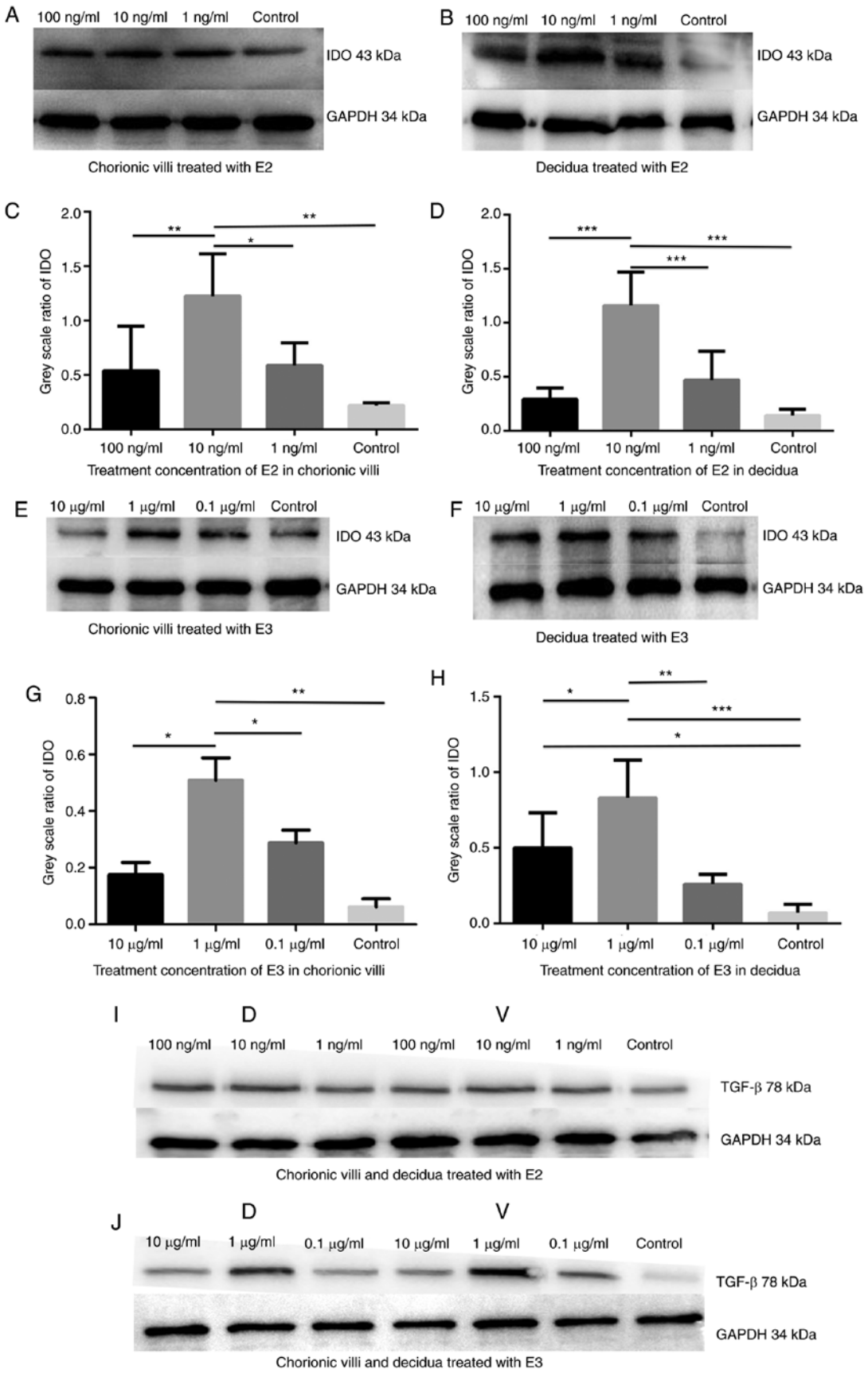

Figure 2. E2 and E3 induce IDO and TGF-β expression in chorionic villi and decidua, as detected by western blot analysis. Western blots presenting the expression of IDO in (A) chorionic villi and (B) decidua treated with different concentrations of E2. Semi-quantitative analysis of IDO expression in (C) chorionic villi and (D) decidua treated with E2. Western blots presenting the expression of IDO in (E) chorionic villi and (F) decidua treated with different concentrations of E3. Semi-quantitative analysis IDO expression in (G) chorionic villi and (H) decidua treated with E3. TGF-β expression in chorionic villi and decidua cultured in the medium containing (I) E2 or (J) E3. Data are presented as the mean ± SE and representative of three independent experiments. Grey scale ratio was calculated using the GAPDH band. *P<0.05, **P<0.01 and ***P<0.001. IDO, indoleamine 2,3-dioxygense; E2, 17β-estradiol; E3, estriol; TGF-β, transforming growth factor β; V, chorionic villi; D, decidua.

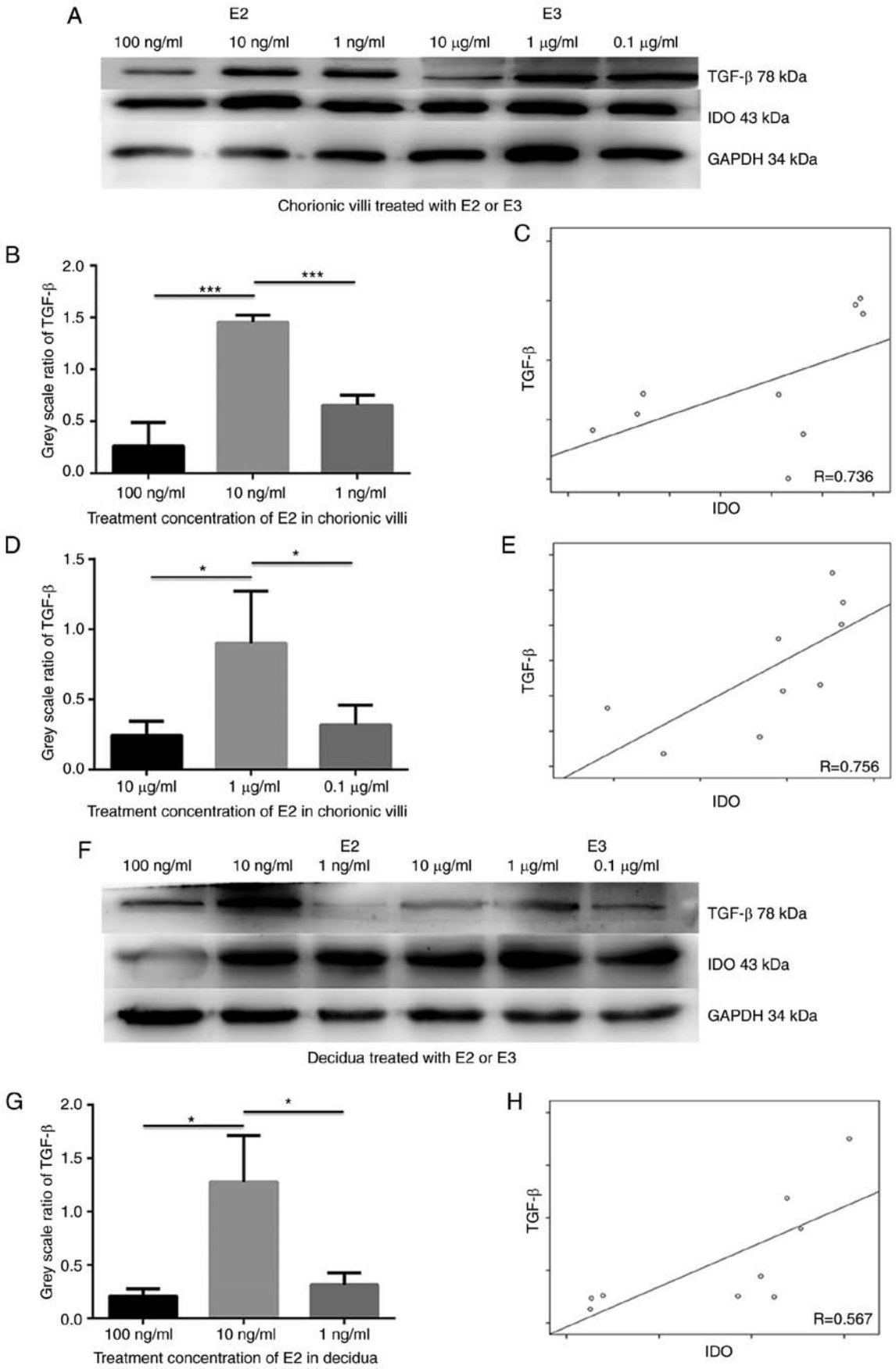

Figure 3. E2 and E3 induces IDO expression by TGF-β in chorionic villi and decidua, as detected by western blot analysis. (A) Western blots presenting the expression of IDO and TGF-β in chorionic villi following treatment with E2 or E3 for 48 h. (B) Semi-quantitative analysis of TGF-β levels in chorionic villi following treatment with E2. (C) Correlation between IDO and TGF-β levels following treatment with E2 in chorionic villi. (D) Semi-quantitative analysis of TGF-β levels in chorionic villi following treatment with E3. (E) Correlation between IDO and TGF-β levels following treatment with E3 in chorionic villi. (F) Western blots presenting the expression of IDO and TGF-β in decidua following treatment with E2 or E3 for 48 h. (G) Semi-quantitative analysis of TGF-β levels in decidua following treatment with E2. (H) Correlation between IDO and TGF-β levels following treatment with E2 in decidua.

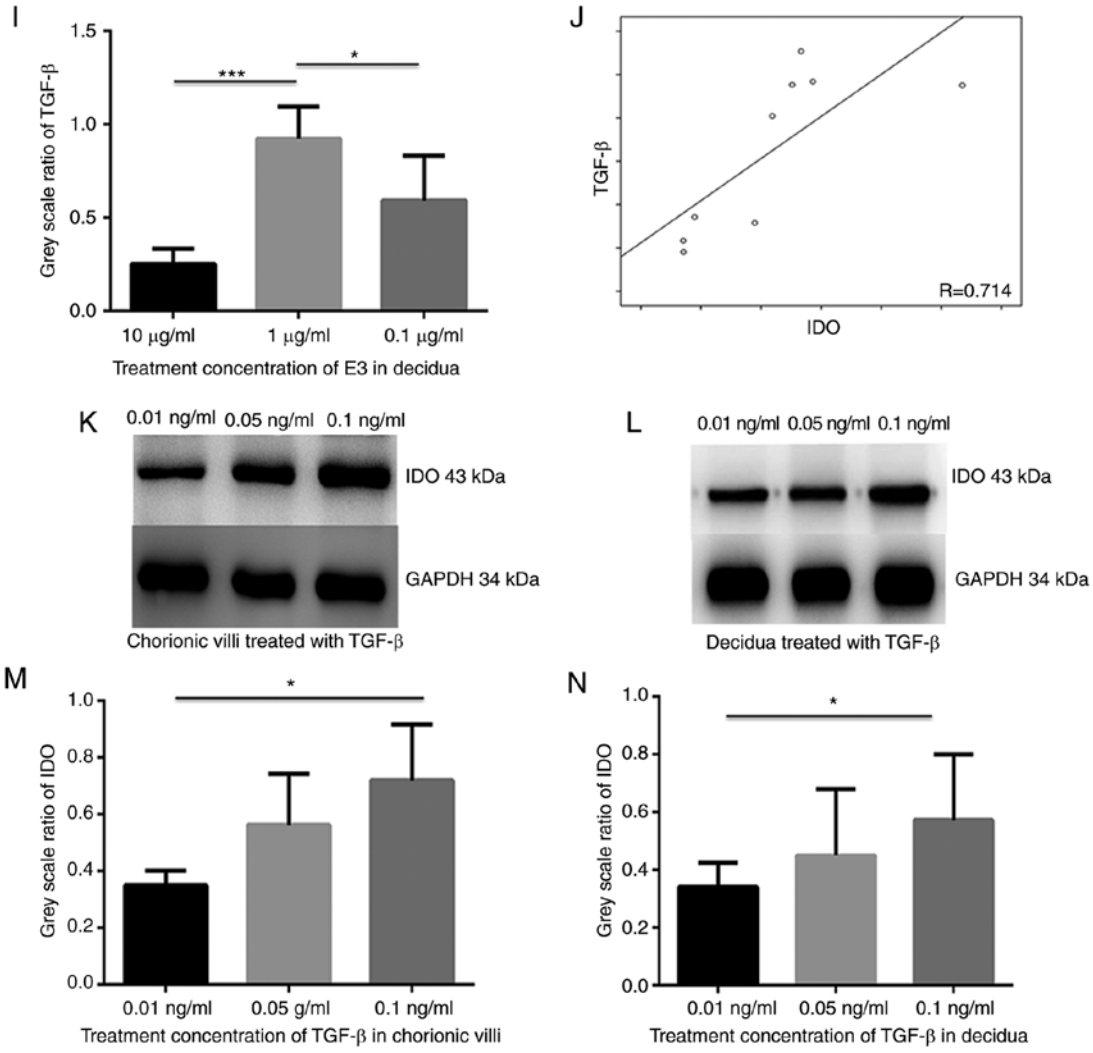

Figure 3. Continued. (I) Semi-quantitative analysis of TGF-β levels in decidua following treatment with E3. (J) Correlation between IDO and TGF-β levels following treatment with E3 in decidua. IDO expression in (K) chorionic villi and (L) decidua following treatment with different concentration of TGF-β. Semi-quantitative analysis of IDO expression levels in (M) chorionic villi and (N) decidua treated with different concentrations of TGF-β. Data are presented as the mean ± SE and representative of three independent experiments. Grey scale ratio was calculated using the GAPDH band. *P<0.05 and ***P<0.001. IDO, indoleamine 2,3-dioxygenase; E2, 17β-estradiol; E3, estriol; TGF-β, transforming growth factor β

these results suggest that TGF-β may upregulate the expression of IDO and that estrogen may induce IDO expression via TGF-β.

Fulvestrant decreases estrogen-depended upregulation of IDO by inhibiting TGF-β expression in chorionic villi and decidua. To validate whether estrogen induced IDO expression via TGF-β in chorionic villi and decidua, chorionic villi or decidua cells were incubated in medium containing 10 ng/ml E2 or 1 μg/ml E3, prior to addition of 0.1, 1 and 10 μg/ml fulvestrant for 12 h. Western blot analysis demonstrated that both IDO and TGF-β expression decreased with the increased fulvestrant dosage in chorionic villi (Fig. 4A-H) and decidua (Fig. 5A-H), cultured in the medium containing 10 ng/ml E2 and 0.1, 1 or 10 μg/ml fulvestrant (Figs. 4A and B, and 5A and B), 1 μg/ml E3 added with 0.1, 1 or 10 μg/ml fulvestrant (Figs. 4E and F, and 5E and F), respectively. For IF staining, chorionic villi or decidua were treated with 10 ng/ml E2 (Figs. 4C and 5C) or 10 ng/ml E2 and 1 μg/ml fulvestrant (Figs. 4D and 5D), 1 μg/ml E3 (Figs. 4G and 5G) or 1 μg/ml E3 and 1 μg/ml fulvestrant (Figs. 4H and 5H), respectively. The greatest decrease in IDO and TGF-β expression was observed in sections of chorionic villi (Fig. 4A and E) and decidua (Fig. 5A and E) cultured in the medium containing 10 ng/ml of E2 and 10 μg/ml fulvestrant (Figs. 4A and 5A), or in medium containing 1 μg/ml of E3 and 10 μg/ml fulvestran (Figs. 4E and 5E). Overall, the results demonstrated that fulvestrant had the ability to decreased estrogen-depended upregulation of IDO, partly by inhibiting TGF-β expression.

Discussion

IDO is considered the main protein that protects embryos from the maternal immune system. During pregnancy, IDO is secreted by placental syncytiotrophoblasts, cytotrophoblasts, decidual cells and maternal monocyte-macrophages, which inhibit the T-lymphocyte reaction and mediate the immune tolerance to the fetus via tryptophan depletion and defective tryptophan catabolism (19). Previous studies have predominantly focused on investigating IDO expression and

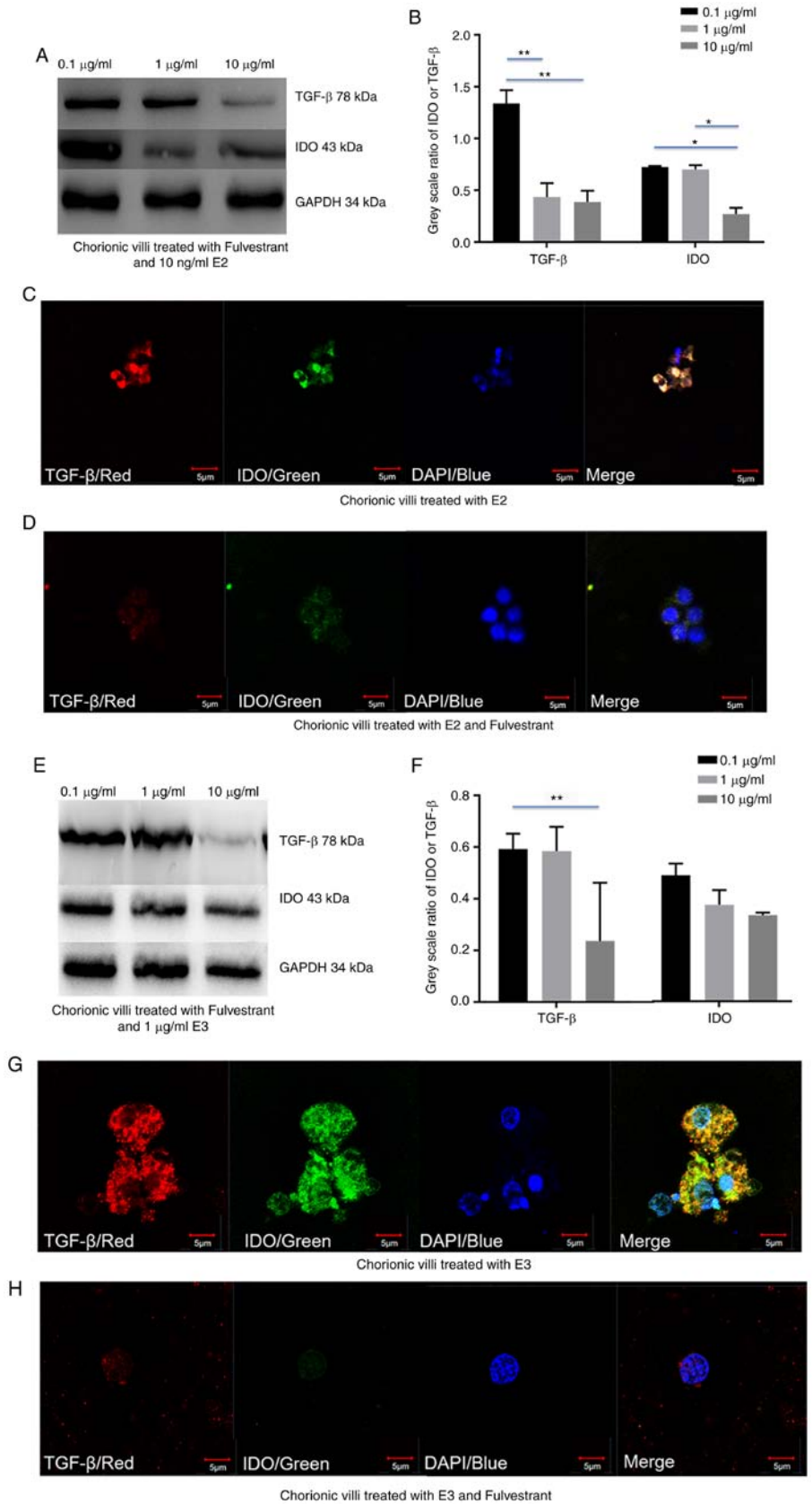

Figure 4. Fulvestrant inhibits estrogen-depended upregulation of IDO in chorionic villi. (A) Western blots presenting the expression of IDO and TGF-β in chorionic villi following treatment with 10 ng/ml E2 and 0.1, 1 and 10 μg/ml fulvestrant. (B) Semi-quantitative analysis of TGF-β and IDO levels in chorionic villi following treatment with E2 and fulvestrant. Immunofluorescent staining of IDO and TGF-β in cultured chorionic villi treated with (C) E2 only and (D) E2 and fulvestran. (E) Western blots presenting the expression of IDO and TGF-β in chorionic villi following treatment with 1 μg/ml E3 and 0.1, 1 and 10 μg/ml fulvestrant. (F) Semi-quantitative analysis of TGF-β and IDO levels in chorionic villi following treatment with E3 and fulvestrant. Immunofluorescent staining of IDO and TGF-β in cultured chorionic villi treated with (G) E3 only and (H) E3 and fulvestrant. Data are presented as the mean ± SE and representative of three independent experiments. Grey scale ratio was calculated using the GAPDH band. The expression of IDO (green fluorescence) and TGF-β (red fluorescence) was detected by immunofluorescence staining (magnification x63). Cell nuclei were stained by DAPI (blue fluorescence). *P<0.05 and **P<0.01. IDO, indoleamine 2,3-dioxygenase; E2, 17β-estradiol; E3, estriol; TGF-β, transforming growth factor β.

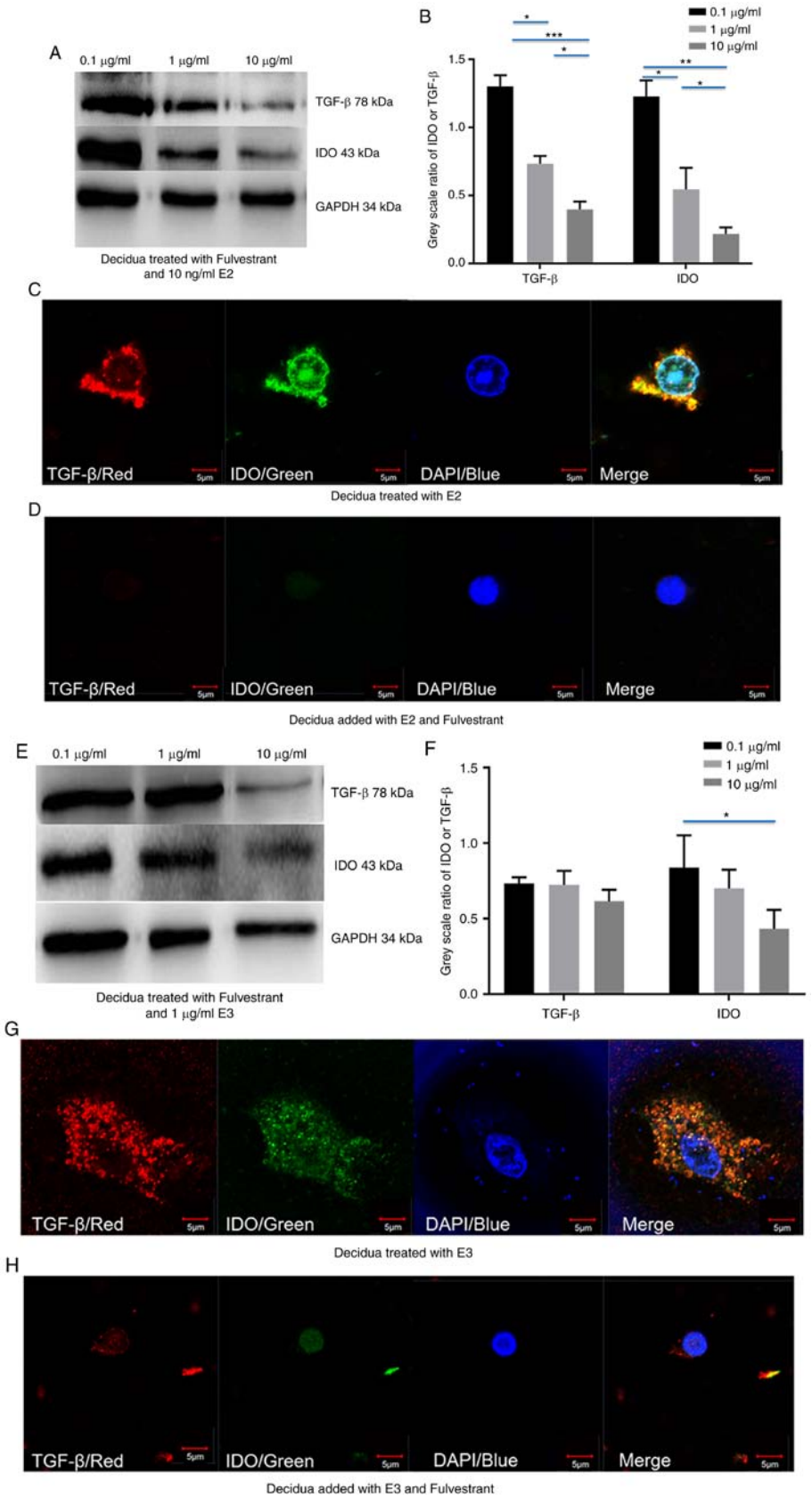

Figure 5. Fulvestrant inhibits estrogen-depended upregulation of IDO in decidua. (A) Western blots presenting the expression of IDO and TGF-β in decidua following treatment with 10 ng/ml E2 and 0.1, 1 and 10 μg/ml fulvestrant. (B) Semi-quantitative analysis of TGF-β and IDO levels in decidua following treatment with E2 and fulvestrant. Immunofluorescent staining of IDO and TGF-β in cultured decidua treated with (C) E2 only and (D) E2 and fulvestran. (E) Western blots presenting the expression of IDO and TGF-β in decidua following treatment with 1 μg/ml E3 and 0.1, 1 and 10 μg/ml fulvestrant. (F) Semi-quantitative analysis of TGF-β and IDO levels in decidua following treatment with E3 and fulvestrant. Immunofluorescent staining of IDO and TGF-β in cultured decidua treated with (G) E3 only and (H) E3 and fulvestrant. Data are presented as the mean ± SE and representative of three independent experiments. Grey scale ratio was calculated using the GAPDH band. The expression of IDO (green fluorescence) and TGF-β (red fluorescence) was detected by immunofluorescence staining (magnification, x63). Cell nuclei were stained by DAPI (blue fluorescence). *P<0.05, **P<0.01 and ***P<0.001. IDO, indoleamine 2,3-dioxygenase; E2, 17β-estradiol; E3, estriol; TGF-β, transforming growth factor β.

function (20). It has been reported that interferon-γ (IFN-γ) secreted by infiltrating leukocytes may increase IDO expression in endometrial stromal cells (21); however, little is known about the regulation of IDO expression at the maternal-fetal interface.

Estrogen affects the development of reproductive capabilities, and high E2 and E3 secretion levels have been observed during the gestation period. Estrogen-mediated IDO expression in monocyte-derived dendritic cells has been reported to limit T-cell proliferation and Th1/Th2 cytokine production in patients with multiple sclerosis (22). During pregnancy, the mean cord serum concentration of E2 is ~7.5 ng/ml (range, 4-13 ng/ml) and the mean cord serum concentration of E3 is 0.3 μg/ml (range, 0.2-0.5 μg/ml) (23). It has been reported that during pregnancy, estradiol may enhance the production of interleukin (IL)-10 and maintain Th2 cytokine expression (24). The results of the present study indicated that E2 and E3 may induce IDO expression, and demonstrated that IDO expression increased most notably when E2 and E3 were administered at concentrations similar to those normally observed in pregnant women. However, further studies are required to fully elucidate the molecular mechanism by which estrogen induces IDO expression at the maternal-fetal interface. The IDO expression induced by IFN-γ may explain the immunoregulatory effects of this enzyme in acute inflammation; however, animal experiments indicated that long-term IDO expression in noninflammatory contexts is driven by TGF-β, as demonstrated in mouse pDCs treated with TGF-β (17).

Our previous study demonstrated that TGF-β expression was positively associated with IDO expression in chorionic villi and decidua tissues of healthy pregnant women (11). In the present study, IDO expression increased in chorionic villi and decidua cultured in the medium containing TGF-β. Thus, the current results support the hypothesis that TGF-β may upregulate the expression of IDO in chorionic villi and decidua.

TGF-β is closely associated with tissue remodeling events and reproductive processes, and is known to be abundantly expressed in the endometrium, decidua and chorionic villi. This protein is a vital regulator of endometrial and placental development and functions. TGF-β affects several modulatory effects on endometrium, such as preparation events for implantation, interactions with preimplantation embryos, and promoting pre- and post-implantation embryo development. TGF-β is also known to have several modulatory effects on trophoblast cells, such as inhibition of proliferation and invasiveness, and stimulation of differentiation by inducing multinucleated cell formation (25,26)

It has been reported that estrogen can upregulate TGF-β expression. A previous study demonstrated that estrogen effected TGF-β1 expression in mouse endometrium by stimulating its expression in the stroma cells and inhibiting its expression in glandular epithelium (27). Another study reported that E2 expression significantly increased in response to TGF-β1 secretion in cultured dermal fibroblasts following wound healing (27). Certain studies reported that estrogen reduced TGF-β expression in endometrial carcinoma; however, these studies were based on a different abnormal cell growth mechanism of endometrial cancer cells and hormone release (28,29). The results of the present study demonstrated that TGF-β may increase the expression of IDO in a dose-dependent manner, and that TGF-β was highly expressed in chorionic villi and decidua tissues of normal pregnant women.

During pregnancy, ovaries induce placenta cell-mediated production of high concentrations of estrogen and progesterone. Abnormally low levels of estrogen and progesterone can lead to a miscarriage, while abnormally high levels may disturb the function of ovaries and cause cancer or early menopause. The mean cord serum concentration of E2 is ~7.5 ng/ml (range, 4-13 ng/ml) and the mean cord serum concentration of E3 is 0.3 μg/ml (range, 0.2-0.5 μg/ml) during pregnancy (30). For our study, the 10 ng/ml E2 and 1 μg/ml E3 is more suitable for increased TGF-β and IDO expression, one of the causes is by simulation of pregnancy women uterus micro-environment *in vitro*, for another possibility is about the toxicological effect of estrogen and progesterone added in cultured tissues. Since high concentrations of estrogen and high TGF-β expression levels were simultaneously observed in chorionic villi and decidua tissues, it unlikely that estrogen downregulates TGF-β expression. Fulvestrant functions as an antagonist of estrogen and is used to treat postmenopausal patients with hormone receptor-positive advanced breast cancer (31). The results of the current study showed that estrogen could upregulate TGF-β expression in chorionic villi and decidua tissues cultured in medium containing E2 or E3. Furthermore, this effect was reversed by fulvestrant, an inhibitor of estrogen receptor.

The results of the present study demonstrated that IDO was expressed in chorionic villi and decidua tissues, and that TGF-β may upregulate IDO expression in these tissues. Furthermore, the results indicated that estrogen during pregnancy may induce maternal-fetal tolerance by upregulation of IDO via TGF-β, E2 and E3. The current study had certain limitations. A wider range of E2 and E3 does will be necessary to confirm the colclusions. Furthermore, Pearson's correlation analysis of the association between the maternal E1, E2 and E3 levels in chorionic villi and decidua tissues and peripheral blood TGF-β/IDO expression levels was not performed. A future study will explore the downstream and upstream signaling pathways of TGF-β, and other molecules that may be involved in estrogen signaling pathways, such as Smad3 and Stat3. The protein expression levels of TGF-β and IDO receptors, and the receptors of estrogen and progesterone should also be determined in future studies.

In conclusion, the current results indicated that there may be an association between estrogen and TGF-β and IDO expression levels in chorionic villi and decidua tissues. Inhibition of estrogen signaling decreased TGF-β and IDO expression levels, suggesting that estrogen may upregulate IDO expression via TGF-β. The current findings may be used in future clinical research to support the understanding of the molecular mechanism of maternal-fetal immunological tolerance. The current results may also be used for the development of effective treatment for autoimmune diseases, including transplant rejection and immune infertility (32,33).

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Availability of data and materials

The data sets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

JW, GH, SZ, ZC, ZX, LW and FS conceived and designed the study. JW and PL analyzed the data. JW, ZC and MY performed the experiments. YW, LY, YT, HZ and JW recruited patients, collected the chorionic villi and decidua samples, and analyzed patient information. JW and GH wrote the manuscript. SZ was responsible for the acquisition of funding. All authors agreed with manuscript results and conclusions and approved the final manuscript.

Ethics approval and consent to participate

The current study was conducted according to the principles expressed in the Declaration of Helsinki. The study was approved by the Ethics Committee of the Affiliated Hospital of Guizhou Medical University (Guizhou, China). All patients who have undergone voluntary termination of pregnancy provided written informed consent for the collection of samples and subsequent analysis.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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