


RESEARCH

Open Access



Cysteine dioxygenase and taurine are essential for embryo implantation by involving in E₂-ER α and P₄-PR signaling in mouse

Di Zhang^{1,2†}, Zhijuan Wang^{3†}, Xuan Luo³, Hongzhou Guo^{1,2}, Guobin Qiu^{1,2}, Yuneng Gong^{1,2}, Hongxu Gao^{1,2} and Sheng Cui^{1,2,4*} 

Abstract

Background Taurine performs multiple physiological functions, and the maintenance of taurine level for most mammals relies on active uptake from diet and endogenous taurine synthesis through its synthesis enzymes, including cysteine dioxygenase (CDO). In addition, uterus tissue and uterus fluid are rich in taurine, and taurine synthesis is regulated by estrogen (E₂) and progesterone (P₄), the key hormones priming embryo-uterine crosstalk during embryo implantation, but the functional interactions and mechanisms among which are largely unknown. The present study was thus proposed to identify the effects of CDO and taurine on embryo implantation and related mechanisms by using *Cdo* knockout (KO) and ovariectomy (OVX) mouse models.

Results The uterine CDO expression was assayed from the first day of plugging (d 1) to d 8 and the results showed that CDO expression level increased from d 1 to d 4, followed by a significant decline on d 5 and persisted to d 8, which was highly correlated with serum and uterine taurine levels, and serum P₄ concentration. Next, *Cdo* KO mouse was established by CRISPER/Cas9. It was showed that *Cdo* deletion sharply decreased the taurine levels both in serum and uterus tissue, causing implantation defects and severe subfertility. However, the implantation defects in *Cdo* KO mice were partly rescued by the taurine supplementation. In addition, *Cdo* deletion led to a sharp decrease in the expressions of P₄ receptor (PR) and its responsive genes *Ihh*, *Hoxa10* and *Hand2*. Although the expression of uterine estrogen receptor (ER α) had no significant change, the levels of ER α induced genes (*Muc1*, *Ltf*) during the implantation window were upregulated after *Cdo* deletion. These accompanied by the suppression of stroma cell proliferation. Meanwhile, E₂ inhibited CDO expression through ER α and P₄ upregulated CDO expression through PR.

Conclusion The present study firstly demonstrates that taurine and CDO play prominent roles in uterine receptivity and embryo implantation by involving in E₂-ER α and P₄-PR signaling. These are crucial for our understanding the mechanism of embryo implantation, and infer that taurine is a potential agent for improving reproductive efficiency of livestock industry and reproductive medicine.

Keywords CDO, E₂, Embryo implantation, P₄, Taurine

[†]Di Zhang and Zhijuan Wang contributed equally to this work.

*Correspondence:

Sheng Cui
cuisheng@yzu.edu.cn

¹ College of Veterinary Medicine, Yangzhou University, Yangzhou, Jiangsu 225009, People's Republic of China

² Jiangsu Co-Innovation Center for Prevention and Control of Important Animal Infectious Diseases and Zoonoses, Yangzhou University,

Yangzhou 225009, People's Republic of China

³ State Key Laboratory of Agrobiotechnology, College of Biological Sciences, China Agricultural University, 100193 Beijing, People's Republic of China

⁴ Institute of Reproduction and Metabolism, Yangzhou University, 225009 Jiangsu, People's Republic of China



Background

High rate of embryo loss in early pregnancy is a major constraint both in livestock industry and human reproduction, whereas much pregnancy wastage is caused by the failure of embryo implantation [1–3]. The implantation of the blastocyst into the maternal uterus is a crucial step in mammalian reproduction [4, 5]. It is generally accepted that embryo implantation depends on blastocyst quality, endometrial receptivity, and the synchronization of their development [6]. In mice, embryo implantation consists of apposition between the trophectoderm layer of blastocyst and the luminal epithelium (LE), attachment and final invasion into the LE [7]. Upon embryo invasion, the uterine stromal cells are rapidly remodeled in the process of decidualization, which is characterized by morphological and functional changes in stromal cells in the form of proliferation and differentiation into large epithelioid decidual cells [8]. However, the cell proliferation and differentiation of specific uterine cell types in early pregnancy are dependent on the coordinated actions of ovarian steroid hormones, including progesterone (P_4) and estrogen (E_2). During mouse pregnancy, an E_2 surge on d 1 stimulates uterine epithelial cell proliferation, and the decline of E_2 level on d 2 leads to apoptosis of a large number of epithelial cells. P_4 , from the newly formed corpora lutea on d 3 [8], initiates uterine stromal cell proliferation. In conjunction with P_4 , an acute E_2 spike on d 4 further stimulates uterine stromal cell proliferation and renders the uterus receptivity for the blastocyst to implant [8–10]. However, it is still largely unknown about the molecular mechanisms of coordinate proliferative events induced by E_2 and differentiative processes directed by P_4 .

Although the receptivity of the uterus during implantation is primed by E_2 and P_4 , their actions on cell proliferation and differentiation are complicated, and the relative molecular mechanisms have been extensively studied. The related molecular and genetic studies indicate that E_2 and P_4 act respectively via estrogen receptor 1 (*Esr1*, ER α) [11] and progesterone receptor (*Pgr*, PR) [12] to govern the embryo-uterine crosstalk during peri-implantation stage by targeting local transcriptional factors, signals or paracrine molecules [13], some of which include *Muc1* [14], *Ltf* [15], *Hox10a* [16, 17], *Hand2* [17] and *IHH* [18, 19]. In addition, uterine epithelium and its secretions are essential for uterine receptivity and embryo implantation. Uterine epithelium includes LE and glandular epithelium (GE), which directly synthesize, secrete or selectively transport a wide variety of substances from serum and transudate, collectively termed histotroph [20], into uterus lumen. Whereas histotroph is complex and comprised of many different substances, such

as leukemia inhibitory factor (LIF), ions, sugars, lipids, proteins and amino acids [20–24], among which taurine is included [25]. It is much interested that taurine concentration in the uterine luminal fluid (UFL) of mouse is much higher during the implantation [25]. But as we know, there is no direct evidence about the functional relations of taurine with uterine receptivity and embryo implantation, although it is reported that *Cdo* knockout (KO) mice exhibit impaired reproductive capacity [26].

Taurine is one of the most abundant non-essential amino acids in most mammals [27], and performs numerous physiological functions, including bile salts synthesis and hepatoprotection [28], energy metabolism [29], maintenance of Ca^{2+} homeostasis [30], anti-oxidative, osmoregulation, anti-inflammatory and anti-apoptotic [31–33]. While the maintenance of the body taurine level mainly relays active uptake from diet and the endogenous taurine synthesis through the sequential actions of its synthesis enzymes, including cysteine dioxygenase (CDO) [34].

CDO is a critical enzyme for taurine synthesis and CDO expression has been detected in liver, adipose tissue, pancreas, kidneys, lungs and reproductive system [35]. *Cdo* KO results in a higher incidence of postnatal mortality, retards postnatal growth and damages male fertility [26, 36]. In addition, CDO is highly expressed in mouse ovary and uterus [37], but the functions of CDO in female reproduction remains unclear. Interestingly, uterus tissue and ULF are rich in taurine, and ULF taurine concentration is increased during embryo implantation [25]. Furthermore, CDO expression in uterus is up-regulated by P_4 , whereas E_2 decreases CDO expression [37]. In addition, our recent study shows that E_2 regulates taurine synthesis through E_2 -ER α -CSD signaling [38]. These make us to hypothesize that CDO may play important roles in E_2 and P_4 primed embryo implantation, possibly through the physiological actions of taurine. The present study was thus proposed to identify the effects of CDO and taurine on embryo implantation and illustrate the related mechanisms by using *Cdo* KO and ovariectomy (OVX) mouse models.

Materials and methods

Animals and treatments

Eight weeks old ICR mice were used in fertility test and other animal experiments. *Cdo* KO mice were generated by using 129 mice (Additional file 1). Mice were raised in controlled temperature (25 ± 1 °C) and humidity (60%–70%) with a 12 h light, 12 h dark cycle. The animal experiments were approved by the Chinese Association for Laboratory Animal Sciences. Virgin female mice were mated with sexually matured males to induce pregnancy (day 1 is the day of vaginal plug checked, d 1). Embryos

were collected from oviducts on d 1. The implantation sites (IS) were visualized by intravenous injection of 0.1 mL 1% Chicago blue dye (Sangon Biotech, Shanghai, China) in saline on d 5 [39]. To investigate the ovarian hormonal influence on uterine CDO expression, wild-type (WT) mice were ovariectomized. Oil, 100 ng/mouse 17β -estradiol (E_2 ; MedChemExpress, NJ, USA) or 2 mg/mouse progesterone (P_4 ; MedChemExpress, NJ, USA) was injected 7 d later [40]. The treated mice were then sacrificed at indicated times for further experiments.

Real-time quantitative PCR (RT-qPCR) and common PCR

Total RNA of the uterus tissues was isolated using the TRIzol reagent (Takara, Dalian, China), purified by DNase I and quantified by spectrophotometry. 1 μ g purified total RNA was used as a template for cDNA synthesis using HiScript Reverse Transcriptase (Vazyme, Nanjing, China) according to the manufacturer's instructions. RT-qPCR was performed using SYBR Green master mix (Vazyme, Nanjing, China) in the StepOnePlus Real-Time PCR System (Applied Biosystems, Foster City, CA, USA) and reactions were done in triplicate. RT-qPCR conditions were as follows: 95 °C for 2 min, followed by 40 cycles of 95 °C for 15 s and 60 °C for 1 min. Relative gene expressions were normalized to endogenous control *Gapdh*. All Primers listed in Table S3 were designed using NCBI.

The genotype identification of the *Cdo* KO mice was performed by common PCR using primers as described in Table S4. Amplifications were carried out on PCR instrument (Bio-Rad, Hercules, CA, USA) using the following protocol: 94 °C for 5 min (one time); 94 °C for 50 s, 65 °C for 30 s, 72 °C for 30 s (35 times); 72 °C for 10 min; and holding at 4 °C.

Western blotting (WB)

The uterus tissues were lysed with RIPA buffer (Beyotime, Shanghai, China) containing 1 mmol/L phenylmethanesulfonyl fluoride (PMSE, Sangon Biotech, Shanghai, China). The protein concentration of each group was determined by using the BCA assay reagent (CoWin Biosciences, Jiangsu, China) according to the manufacturer's recommendations. Equal amounts of 50 μ g proteins were electrophoresed on 12% sodium dodecyl sulfate–polyacrylamide gel (SDS–PAGE), and the bands were transferred to 0.45 μ m polyvinylidene difluoride (PVDF) membrane (Millipore, MA, USA). The membrane was blocked with 5% (w/v) nonfat dry milk in 0.05 mol/L pH 7.4 Tris buffered saline (TBS) for 3 h and incubated with CDO antibody (ab53436, abcam, Cambridge, UK; 1:2000), internal control GAPDH antibody (AM4300, Ambion, TX, USA; 1:10,000), PR antibody (ab2765, abcam, Cambridge, UK; 1:2000) or ER α antibody

(ab32063, abcam, Cambridge, UK; 1:2000) overnight at 4 °C. The PVDF membrane was then washed 3 times for 30 min in TBST (0.1% Tween-20 in TBS) and incubated for 2 h with horseradish peroxidase-conjugated goat anti-rabbit IgG or horseradish peroxidase-conjugated goat anti-mouse IgG (Zhongshan, Beijing, China). After washing for 30 min with 3 changes of TBST, the membrane was treated with the ECL kit (Vazyme, Nanjing, China) and visualized by Tannon gel imager (Tanon, Shanghai, China). The intensity values pertaining to each group were normalized against the optical density of GAPDH corresponding to the same group.

Immunohistochemistry (IHC) and immunofluorescence (IF)

Tissues were fixed in 4% paraformaldehyde, dehydrated via graded ethanol solutions, and then embedded in paraffin to obtain 5 μ m thick sections. IHC was performed as previously described [41]. The sections were incubated with CDO antibody (ab53436, abcam, Cambridge, UK; 1:200), PR antibody (ab2765, abcam, Cambridge, UK; 1:200), ER α antibody (ab32063, abcam, Cambridge, UK; 1:200) or Ki67 antibody (D385, CST, MA, USA; 1:200) diluted in PBS overnight at 4 °C. After washing with PBS for 30 min, the sections were incubated with biotinylated goat anti-rabbit/mouse IgG (31820/31802, Thermofisher, Waltham, MA, USA; 1:200) for 3 h at RT. After washing with PBS for 30 min, the sections were incubated with streptavidin peroxidase complex (SA10001, Thermofisher, Waltham, MA, USA; 1:200) for 30 min at room temperature. Finally, the signals were visualized by incubating the sections with 0.05 mol/L Tris–HCL (pH 6.5) containing 0.06% (w/v) diaminobenzidine (DAB, ZSGB-Bio, Beijing, China) and 0.03% (v/v) H₂O₂. For IF staining, Hand2 (ab200040, abcam, Cambridge, UK; 1:200), Muc1 (ab15481, abcam, Cambridge, UK; 1:200) primary antibodies, and their respective secondary antibodies (Jackson Immuno Research, Philadelphia, PA, USA; 1:200) were used. Nuclear staining was performed using 4',6-diamidino-2-phenylindole (DAPI) dye (0.1 μ g/mL, Beyotime, Shanghai, China). The signals were captured using a microscope (Olympus, Tokyo, Japan).

In vivo production of embryo

Non-superovulated virgin female mice (8–9 weeks) were mated with adult males. The males and females (1:2) were housed overnight and the presence of a vaginal plug in the following morning was regarded as successful mating (d 1 is the day of vaginal plug checked). Mice were killed by cervical dislocation and oviducts were immediately dissected and placed in M2 solution (CaCl₂·2H₂O 1.71 mmol/L, Glucose 5.56 mmol/L,

HEPES 20.85 mmol/L, KCl 4.78 mmol/L, MgSO₄·7H₂O 1.19 mmol/L, NaCl 94.66 mmol/L, NaHCO₃ 4.15 mmol/L, Sodium lactate 23.28 mmol/L, Sodium pyruvate 0.33 mmol/L) at 11:00 on d 2. Embryos punched out from the ampulla were digested with hyaluronidase (HA) followed by three times washing with M2 and cultured in KSOM at 37 °C until the time of embryo transfer.

Embryo transfer

The uterine horn was exposed by flank laparotomy and six expanding embryos were transferred with a minimal amount of medium into the uterine cavity of pseudo-pregnant (d 3) recipients. The uterine horn was then placed back into the abdominal cavity and the incision site was closed. The procedure was repeated in the opposite flank where another six expanding embryos were transferred. The recipients were then placed individually in clean cages to recover from anesthesia in a warm room (28–30 °C).

Fertility test

Cdo KO females and their nesting WT females were caged with adult wild-type males (8–9 weeks) at the rate of male:female=1:2. Vaginal plug was examined the next morning. Female mice with vaginal plug withdrew from the experiment, while the none plugged females were backed to the test after one day's rest. The plugged females were caged alone to observe their pregnancy and parturition.

Measurement of taurine

Uterus, liver, and serum taurine contents were measured by HPLC–UV (HPLC). Firstly, samples were weighed, homogenized and deproteinized using 0.2 mol/L sulfosalicylic acid. After being centrifuged at 14,000 × *g* for 20 min, the supernatants were added into a dual-bed column containing cation exchange resins to remove other amino acids and metabolic precursors of taurine. Secondly, samples were added with 100 μmol/L glutamine as an internal standard. All samples were then filtrated through a 0.22-μm PVDF membrane and saved in –80 °C refrigerator until use. The samples and the standard samples of taurine which were 100, 50, 25, 10, 5, 2 and 1 μmol/L were derivated with OPA (Sigma-Aldrich, St. Louis, MO, USA) solution (20 mg OPA, 2 mL methanol, 80 μL 2-hydroxy-1-ethanethiol, 18 mL 0.1 mol/L borate buffer (pH 9.6)) for 3 min. Then 20 μL sample was automatically injected into a six-port valve to analysis with Waters Symmetry C18 Column (4.6 μm, 150 mm × 5 mm) (Waters, Milford, MA, USA) on a Shimadzu HPLC system (Shimadzu, Kyoto, Japan). The HPLC conditions were: flow A: 100% methanol, flow B: sodium phosphate buffer pH 4.7 containing 50%

methanol. Flow rate was 1.2 mL/min, and the detection wavelength was 340 nm. The duration times were 2.3 min and 4.95 min for the internal standard and taurine.

Radioimmunoassay (RIA)

Serum P₄ and E₂ were analyzed using RIA reagents provided by the Beijing North Institute Biological Technology (Beijing, China). The minimum detectable concentrations were 2 pg/mL for E₂ and 0.2 ng/mL for P₄. For each RIA the intra and inter assay coefficients of variation were respectively less than 15% and 10%.

Statistical analysis

Statistical analysis was performed using GraphPad Prism 6.0. Data from at least three independent samples were expressed as mean ± SEM. Two group comparison studies were performed using Student's *t*-test and one-way analysis of variance (ANOVA) for data comprising three or more groups. *P* < 0.05 was considered to be statistically significant.

Results

Uterine CDO expression and taurine levels during early pregnancy

In order to evaluate the physiological significance of CDO and taurine in adult female mouse, we firstly examined the CDO expression profiles in different organs by RT-qPCR and WB. The results showed that CDO mRNA and protein were highly expressed in mouse uterus (Fig. S1). Next, the uterine CDO mRNA and protein expressions, their relations to the changes of uterine taurine concentrations and serum steroid hormones were analyzed in the duration of early pregnancy, from d 1 to d 8. RT-qPCR results showed that *Cdo* mRNA increased from d 1 to d 4 and reached the maximum on d 4, which followed by a sharp decline on d 5 and persisted on d 6 and d 8 (Fig. 1 A). WB results showed that CDO protein levels were highly correlated with *Cdo* mRNA levels in the duration examined (Fig. 1 B). In addition, IHC staining revealed that CDO was located in uterine epithelial cells, including LE and GE, and some stromal cells (Fig. 1 E). The taurine concentrations in uterus tissue and serum were assayed by HPLC and the results indicated that the uterine taurine levels exhibited an increasing tendency from d 1 to d 4, and reached the maximum on d 4, followed by a steady decline and returned to the similar level of d 1 on d 8 (Fig. 1 C). The serum taurine level also increased from d 1 to d 4, which significantly declined from d 5 to d 8 (Fig. 1 C). In addition, the dynamic patterns of the uterine CDO expression and taurine concentration corresponded to the change of serum P₄ from d 1 to d 6 (Fig. 1 D). These suggest that CDO might play important roles in regulating embryo implantation.

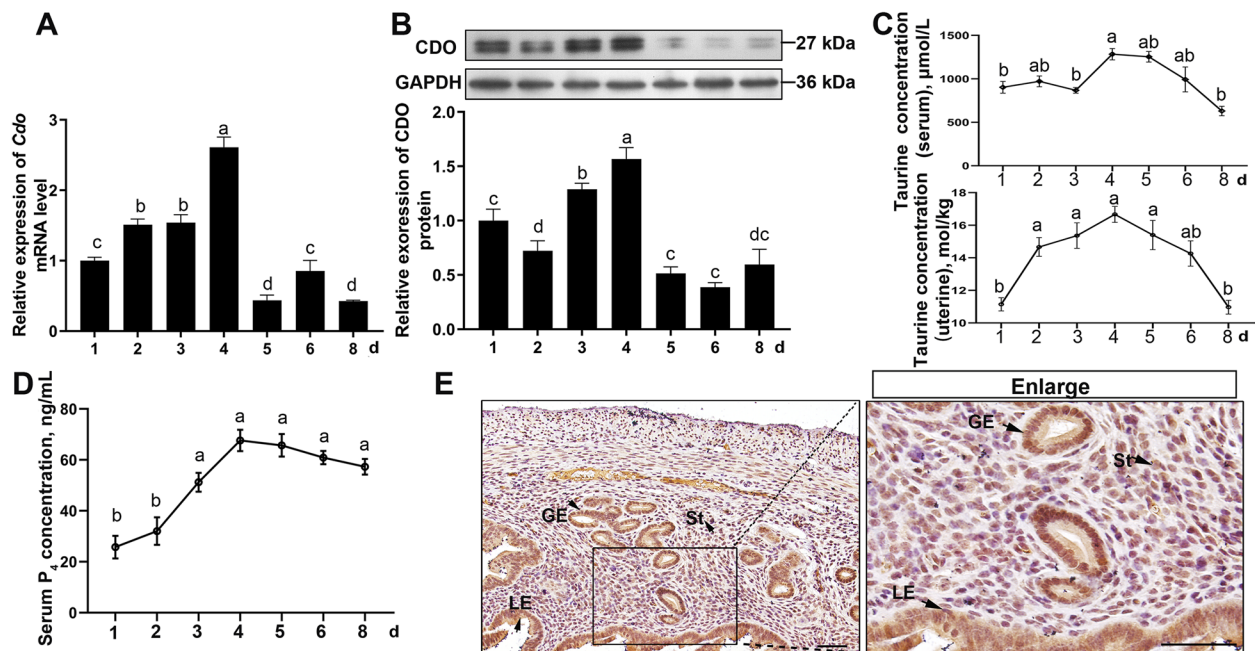


Fig. 1 Uterine *Cdo* mRNA and protein expressions and their relations to taurine levels in uterus from d 1 to d 8 of pregnancy mice. **A**, *Cdo* mRNA expression in mouse uteri detected by RT-qPCR ($n=3$). **B**, *CDO* protein expression in uteri of d 1 to d 8 pregnancy mice assayed by WB. **C**, Serum and uterine taurine concentrations assayed by HPLC ($n=3$). **D**, Serum P_4 concentrations assayed by RIA ($n=5$). **E**, *CDO* IHC staining of uterine cross-sections. Data shown as Mean \pm SEM. Different letters represent significant differences ($P < 0.05$). d, days post coitum; E_2 , estrogen; GE, glandular epithelium; LE, luminal epithelium; P_4 , progesterone; St, stromal cells. Bars: 50 μm

Establishment of *Cdo* KO mouse

To detect the functions of *CDO* in uterus, *Cdo* KO mice were generated by CRISPR/Cas9 technology. Two pairs of single guide RNAs (sgRNAs) (Table S1) targeting exon1 and exon3 of *Cdo* gene respectively (Fig. 2 A), and SpCas9n (D10A) mRNA were co-injected into d 1 embryos (Additional file 1). A *Cdo* KO mutant line with 7489 nucleotide deletion from the first to the third exon was used in the following experiments (Fig. 2 B). The genotypes were identified by PCR that the 245-base pair (bp) strand represent mutant type and the 325 bp strand represent wild type (Fig. 2 C). The knockout efficiency was identified by RT-qPCR, IHC and WB, and the results showed that *CDO* was totally deleted, at least in mouse uterus (Fig. 2D–F). As *CDO* is a key enzyme for taurine synthesis, we detected the changes of taurine levels after *Cdo* knocked out using HPLC. The results showed that the taurine concentrations in the liver, serum and uterus of *Cdo* KO mice decreased by 76.24%, 51.20% and 70.33% respectively than that of WT mice (Fig. 2G–I). These results demonstrate that *CDO* is successfully deleted from mouse genome and the lack of *CDO* leads to taurine deficiency.

Cdo KO causes impairment of embryo implantation and severe subfertility

In order to identify the effects of *Cdo* deletion on the embryo implantation, the serum and uterine

taurine levels of the *Cdo* KO females from d 1 to d 8 were assayed by HPLC. The results showed that *Cdo* KO sharply decreased the taurine levels in the serum and uterus tissue compared with that of WT mice, and there were no significant differences from d 1 to d 8 (Fig. 3A, B). However, there was a rising spike on d 4 in WT mice (Fig. 3A, B).

Further, the effects of *Cdo* deletion on the implantation and fertility were examined. Adult WT and *Cdo* KO females were respectively mated with ICR males, the parturition and pups of each litter were then recorded. The results showed that the plug-positive WT females exhibited normal fecundity, but only 38.8% (7/18) of the plug-positive *Cdo* KO females produced litters (Fig. 3 C), and the litter size (5.429 ± 0.65 pups/litter, $n=7$) was much smaller than that of the WT females (11.29 ± 0.68 pups/litter, $n=7$) (Fig. 3 D).

In order to answer whether the subfertility of *Cdo* KO mice was resulted from the abnormalities in the ovaries or embryos, the embryos were flushed out from oviducts of d 2 *Cdo* KO females, which naturally mated with WT males. The flushed 2-cell embryo numbers did not have significant difference between WT and *Cdo* KO mice (Fig. S2 B), and morphological defect was not observed either (Fig. S2 A). In addition, the histological abnormality was not observed in 10 weeks *Cdo* KO mouse ovaries

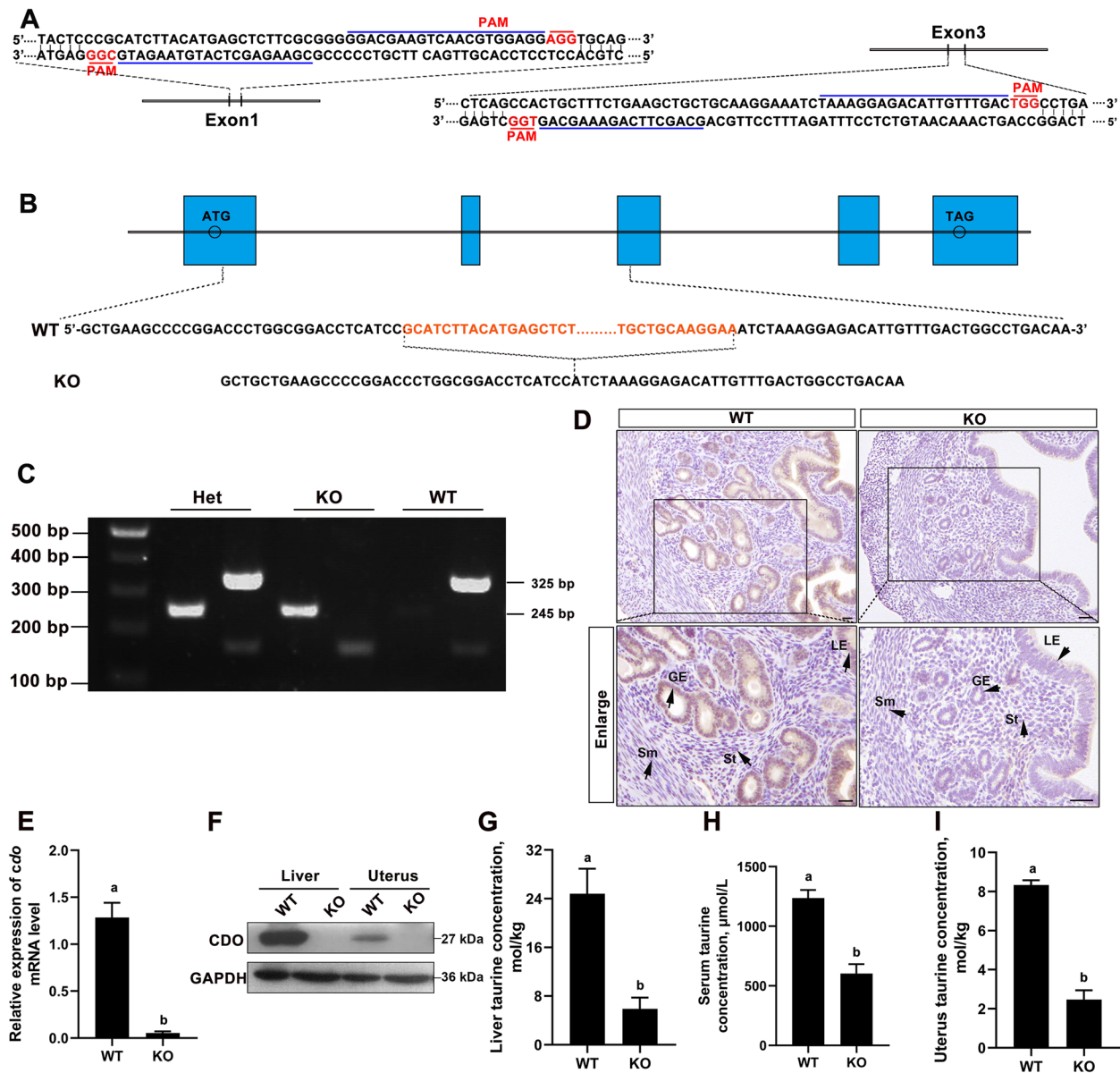


Fig. 2 Generation of *Cdo* KO mouse. **A**, Cas9n target scheme in the first and the third exons of *Cdo* gene. The blue lines indicate sgRNAs' spacer and the red lines indicate PAM (protospacer adjacent motif). **B**, Sanger sequencing of the target region in F0 mice. The *Cdo* KO mice get a miss of 7489 nucleotides. **C**, Genotype identification results using PCR. The 325 bp strand indicates wild type and the 245 bp strand indicates mutant type. **D**, IHC staining of CDO in WT and *Cdo* KO mouse uterus. Bars, 50 μm. **E**, RT-qPCR analysis of *Cdo* mRNA levels in WT and *Cdo* KO mouse uteri. **F**, Representative patterns of WB analysis of CDO expressions. **G-I**, Taurine levels in WT and *Cdo* KO mice liver, serum and uterus detected by HPLC. Data are the Mean ± SEM. ($n \geq 3$). Different letters above columns indicate significant differences ($P < 0.05$). GE: glandular epithelium. LE: luminal epithelium. Sm: smooth muscle cells. St: stromal cells. Scale bars: 50 μm

(Fig. S2 C). Serum P_4 and E_2 levels did not exhibit significant differences between the *Cdo* KO and WT females on d 4 (Fig. S2 D and E).

Further, in order to dissect the cause of the subfertility in *Cdo* KO females, the effect of *Cdo* deletion on the embryo implantation was examined. The plug positive mice were euthanized on d 5 and the numbers of embryos implanted to the uterine epithelium were

identified by employing the Chicago blue dye assay [39]. The implantation sites (IS) were clearly observed in WT females, but only a few or no IS were detected in *Cdo* KO females (Fig. 3 E, G). However, *Cdo* KO females had blastocysts which could be flushed out from uterus (Fig. 3 F).

In order to confirm whether the subfertility of *Cdo* KO females was resulted from the defects of uterus

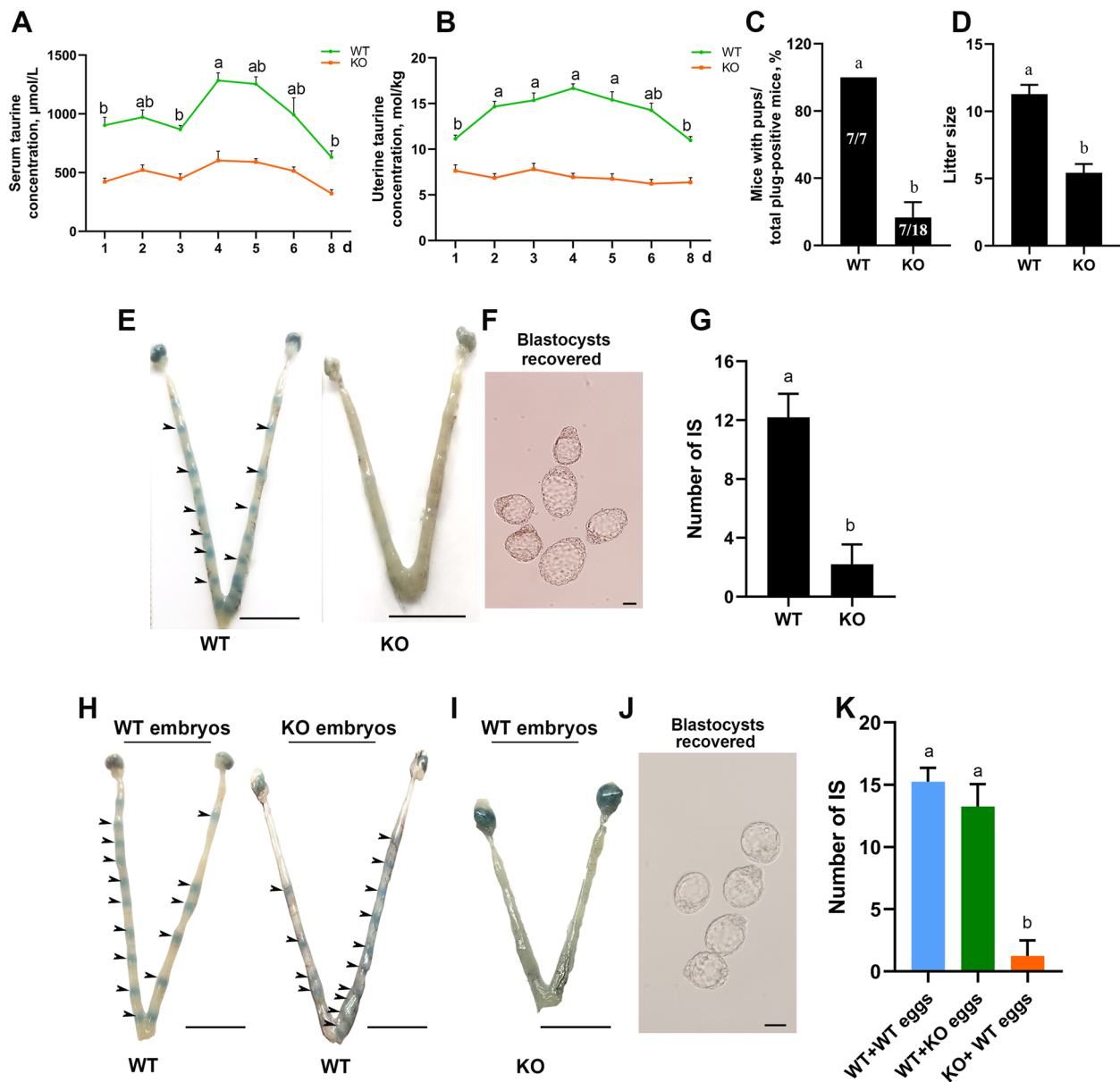


Fig. 3 CDO deletion causes impairment of embryo implantation and severe subfertility. **A** and **B**, Serum (**A**) and uterus (**B**) taurine concentrations of pregnancy *Cdo* KO and WT females from d 1 to d 8, assayed by HPLC ($n = 3$). **C**, Pregnancy rates of WT and KO females. The number within brackets indicate females with pups over total number of plug-positive females. **D**, Average litter sizes of WT and *Cdo* KO females ($n = 7$). **E**, Representative images of d 5 pregnant uteri from WT and *Cdo* KO females. Bars, 1 cm. **F**, Blastocysts flushed from *Cdo* KO uterus. Bar, 50 μ m. **G**, Average numbers of implanted embryos represented by implantation sites ($n = 5$). **H**, Representative patterns of d 5 WT receptive mice uteri transferred with WT and *Cdo* KO embryos. **I**, d 5 KO receptive mice uteri transferred with WT embryos. Arrowheads indicate the location of blastocysts. Bars, 1 cm. **K**, IS numbers of different treated mice uteri ($n = 3$). Data are presented as Mean \pm SEM. Different letters above columns indicate significant differences ($P < 0.05$)

receptive status, *Cdo* KO and WT females were separately mated with *Cdo* KO and WT males to obtain *Cdo* KO and WT embryos. The harvested *Cdo* KO and WT embryos were respectively transferred to WT female uteri. On d 5, IS numbers were assayed and there was no significant difference between the WT females to

which the WT and *Cdo* KO embryos were respectively transferred (Fig. 3 H, K). However, the IS number in *Cdo* KO receptive females was much less than in WT receptive females after both of which accepted WT embryos (Fig. 3 I, K). Meanwhile, blastocysts could be flushed out from *Cdo* KO uterus (Fig. 3 J). These infer

that the failure of embryo implantation and subfertility in *Cdo* KO females are resulted from the defects of uterine receptivity.

***Cdo* KO results in the defects of uterine receptivity by dysregulating PR and ER signaling in mouse**

Our results showed that *Cdo* KO had no significant effects on ovary morphology and functions, including E_2 and P_4 secretions (Fig. S2C–E). As the window of uterine receptivity coincides with the P_4 -mediated down-regulation of ER α activity in uterine LE, we thus assayed the effects of *Cdo* deletion on uterus PR expression on d 4 by RT-qPCR and WB. The results showed that PR mRNA and protein levels in *Cdo* KO uteri respectively decreased by 28.74% and 26.63% compared with that in WT uteri (Fig. 4A–C). IHC results showed that PR staining intensity on LE, GE and stromal cells were much weaker in *Cdo* KO mice than in WT mice (Fig. 4 G). In addition, the uterine mRNA expression levels of the known PR responsive genes *Ihh*, *Hoxa10* and *Hand2* decreased by 56.33%, 35.49% and 45.38% in KO mice than that of the WT mice (Fig. 4 H). Further, *Hand2* IF staining was performed and it was observed that *Hand2* was located only in the stroma cells, but its staining intensity was much weaker in *Cdo* KO mice than that in WT mice (Fig. 4 J), which was consistent with PR IHC staining result (Fig. 4 G).

In addition, *Cdo* deletion had no significant effects on ER α mRNA and protein levels (Fig. 4D–G). Whereas the expression levels of E_2 -responsive genes *Muc1* and *Ltf* elevated over 2.5 and 8 times in *Cdo* KO uterus than that of the WT uterus on d 4 (Fig. 4 I). The further IF staining confirmed the *Muc1* expression in LE and GE cells of *Cdo* KO mouse uteri, but which was not detected in WT mice (Fig. 4 J).

Another important character of uterus receptivity to embryo is a cessation of epithelial cell proliferation and robust proliferation of stroma prior to implantation. We thus detected the cell proliferation in the uterine tissues of *Cdo* KO and WT mice on d 4 by Ki67 IHC staining. The results showed that Ki67 was not detected in LE and GE cells of WT and *Cdo* KO mice uterus, but Ki67 staining intensity in the stroma of WT uteri was much stronger than that of *Cdo* KO uteri. Moreover, Ki67 positive cells were concentrated around the LE in WT uteri (Fig. 4 K), but Ki67 positive cells in *Cdo* KO uteri were irregularly scattered (Fig. 4 K). These indicate that *Cdo* KO results in the defects of uterine receptivity by dysregulating PR and ER signaling in mouse.

P_4 and E_2 are involved in regulating uterine CDO expression

As the dynamic patterns of uterine CDO expression and taurine level were parallel to serum P_4 concentration and

uterine PR expression during early stage of pregnancy (Fig. 1A–D and Fig. 3A, B), we further identified the effects of P_4 and E_2 on uterine CDO expression by using OVX mouse model. RT-qPCR results showed that uterine *Cdo* mRNA levels were decreased by 54.65%, 90% and 93% after 2, 6 and 12 h after 100 ng E_2 treatments in OVX mice (Fig. 5 A). These were further confirmed by the CDO IHC staining results that the CDO staining intensity got much weaker after 12 h E_2 treatment (Fig. 5 C). It was opposite that 2 mg P_4 injection sharply increased uterine CDO mRNA and protein levels, which were several folds higher than that of the controls after 12 h P_4 treatment (Fig. 5 B and C). Further, in order to identify whether the regulating effects of P_4 and E_2 on uterine CDO expressions were through P_4 -PR and E_2 -ER α signaling, OVX mice were respectively pretreated with PR inhibitor RU486 and ER α inhibitor ICI182780, followed by P_4 or E_2 treatment. The uterine CDO expressions were then assayed and the results showed that RU486 blocked the enhancing effect of P_4 on CDO expression (Fig. 5G–I), and ICI182780 restrained the inhibiting effect of E_2 on CDO expression (Fig. 5D–F).

Taurine supplement partly recues the defects of embryo implantation and subfertility caused by *Cdo* KO

As the maintenance of the global taurine level relays on the endogenous taurine synthesis through the action of CDO and the active uptake from the diet, we thus supplied extra taurine in the regular diet of *Cdo* KO mice to confirm whether the defects of uterine receptivity and subfertility caused by *Cdo* KO could be rescued. Adult female *Cdo* KO mice were supplemented with drinking water containing 0.2% (w/v) taurine. The uterine and serum taurine levels were assayed. The results showed that taurine supplement markedly elevated taurine levels in uteri and serum, but all of which were significantly lower than that in wild mice with regular diet (Fig. 6A, B). In addition, the embryo implantation was detected on d 5. The results showed that taurine supplement significantly increased the IS number of *Cdo* KO females, although it was still lower than that of the WT females (Fig. 6C–E). These results demonstrate that taurine supplementation can partly rescue the subfertility of *Cdo* KO females and suggest that CDO and taurine are essential factors for embryo implantation.

Discussion

The present study, for the first time as we know, demonstrates that CDO and taurine play important roles for embryo implantation in mouse. This is supported by our results here that uterine CDO expression and serum taurine level during early stage of mouse pregnancy are parallel to the uterine taurine level, which sharply increases

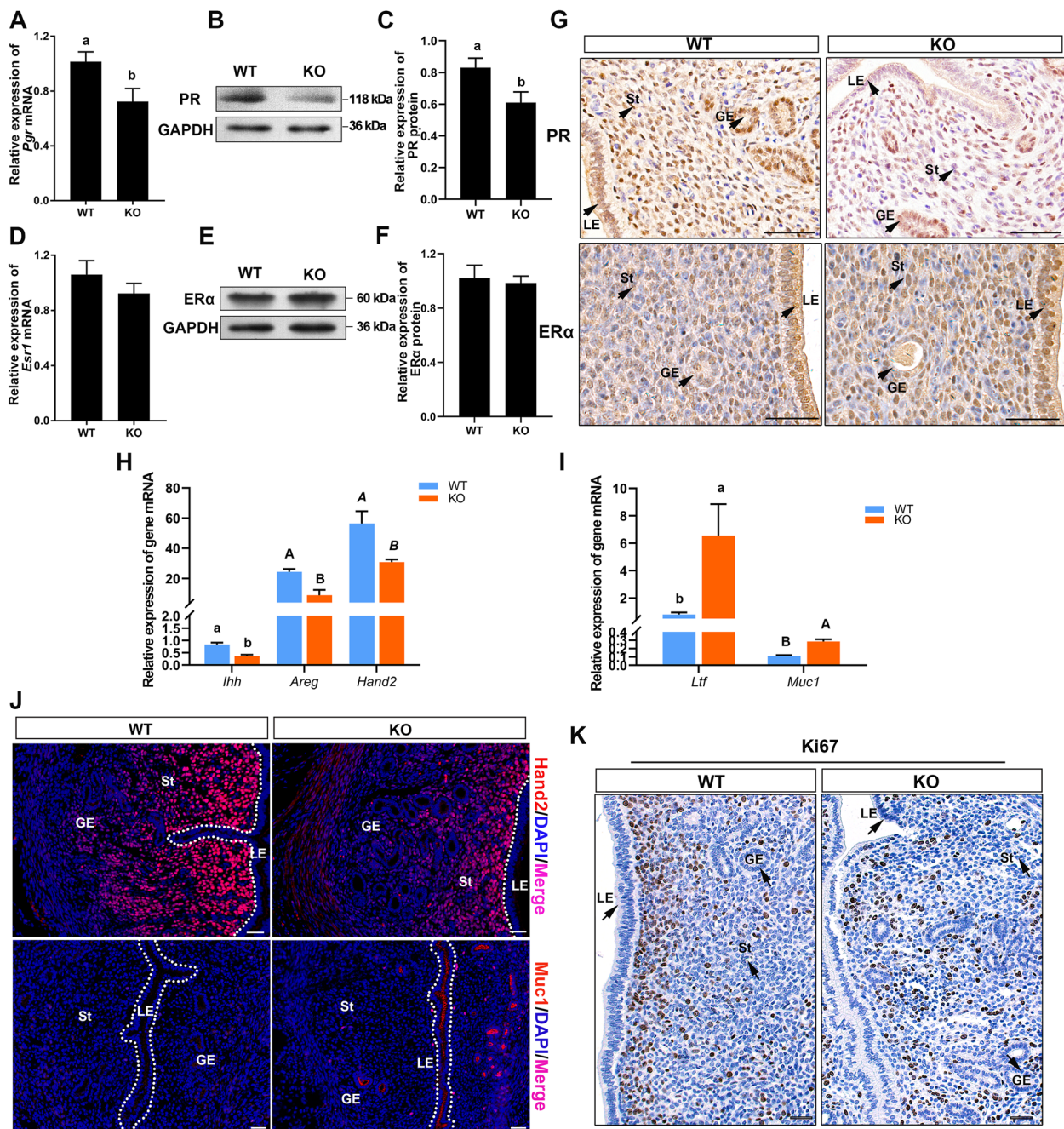


Fig. 4 Effects of *Cdo* deletion on PR and ER signaling transduction and the cell proliferation in the uteri of d 4 pregnancy mice. **A**, RT-qPCR analysis of relative *Pgr* mRNA expression levels. **B** and **C**, WB detection and analysis of PR protein expression. **D**, RT-qPCR analysis of relative *Esr1* mRNA levels. **E** and **F**, WB detection and analysis of ERα protein expression levels. **G**, IHC staining of PR and ERα. Scale bars: 50 μm. **H**, Relative mRNA expressions of *lhh*, *Areg* and *Hand2*. **I**, Relative mRNA levels of *Ltf* and *Muc1*. **J**, IF staining of Hand2 and Muc1. Scale bars: 50 μm. **K**, Ki67 (proliferative cell marker) IHC staining. Scale bars: 50 μm. GE: glandular epithelium. LE: luminal epithelium. St: stroma. Data are presented as Mean ± SEM, $n \geq 4$. Different letters represent significant differences ($P < 0.05$)

and reaches a peak just on d 4, the window of implantation [42], followed by a steadily decline under physiological condition. In addition, serum P_4 , a key factor to

regulate the embryo implantation [7, 43], behaves a similar dynamic pattern with the uterine CDO expression and taurine concentration in the duration examined. Whereas

global *Cdo* KO markedly decreases uterine taurine level and omits the dynamic pattern of uterine taurine, accompanied by the decline of serum taurine level, although *Cdo* KO does not affect the ovary functions to synthesize P_4 and E_2 . These demonstrate that uterus has function to synthesize taurine, whereas *Cdo* deletion causes a severe subfertility in female mice. CDO and endogenous taurine synthesis are thus essential for embryo implantation and the maintenance of animal reproduction. These are also supported by our results here that taurine supplement to the *Cdo* KO mice can partly rescue the reproductive capacity.

Another important finding of this study is that *Cdo* KO causes defects in uterine epithelium receptivity and decline of pregnancy rate. It has been well documented that successful embryo implantation depends on the intimate connection between the blastocyst and maternal endometrial surface [43, 44]. Healthy embryos at the blastocyst stage and receptive maternal endometrium are necessary for the implantation [45]. The results of this study show that *Cdo* deleted embryos do not have morphological defects, and behave normal implantation as that of the WT embryos in WT recipients. However, the implantation and birth rates are dramatically decreased after the WT embryos are transferred to the *Cdo* deleted recipients. These imply that *Cdo* deleted embryos have normal capacity to get implantation. Another important character of uterine receptivity is the cessation in epithelial cell proliferation and robust proliferation in stroma cells just prior to implantation [46, 47]. The results of present study are in agreement with reports [44, 46] that the cell proliferation, marked by Ki67 IHC staining, is undetectable in the uterine epithelium, but the number of Ki67 positive stroma cells significantly decreases in *Cdo* KO mice compared with that of WT mice. These collective data suggest that *Cdo* deletion leads to the abnormalities of uterine receptivity and embryo implantation, and the subfertility of *Cdo* KO mice is primarily of uterine origin.

However, it has been reported that *Cdo* KO females are fertile and carry their pregnancies to term [26], whereas our results here demonstrate that 38.8% of plug-positive *Cdo* KO females are fertile, and the IS number and litter size of *Cdo* KO females are much less than that of WT females. These discordant statements might be resulted from the limited assessment, and no description about the statistics analysis of the IS numbers and birth rates by

Ueki et al. [26]. Another possible explanation is that different mouse strains have been used in our study and the previous report [26].

In addition, the results presented here demonstrate that *Cdo* deletion impairs embryo implantation by dysregulating P_4 and E_2 signaling in mouse uterus. The related molecular and genetic studies indicate that both E_2 and P_4 play functions through their corresponding receptor $ER\alpha$ and PR [48, 49] together with local transcriptional and paracrine factors to govern the complicated embryo-uterine crosstalk [11, 12, 43, 50]. It is much interested that our results here show that CDO involves in regulating embryo implantation by affecting P_4 and E_2 signaling. In support, the uterus PR mRNA and protein levels in *Cdo* deleted mice are significantly decreased, accompanied by decline of *Ihh*, *Hoxa10* and *Hand2* expressions, which are known PR targeting molecules [18, 43, 51]. These are also confirmed by PR and *Hand2* IHC results of this study. However, *Cdo* deletion does not affect $ER\alpha$ mRNA and protein levels, but the mRNA levels of E_2 - $ER\alpha$ responsive genes *Lf* and *Muc1* [14, 15] are increased over 5 and 1.5 time in *Cdo* KO mouse uteri, especially the abnormal expression of *Muc1* in *Cdo* KO LE and GE cells. In addition, CDO or/and taurine might participate in E_2 - $ER\alpha$ signaling by affecting the $ER\alpha$ activity through $ER\alpha$ Ser 118 site phosphorylation [43, 52], although we did not detect it in this study. Collectively, the results presented here demonstrate that CDO is a crucial factor to affect uterine receptivity during embryo implantation by involving in P_4 -PR and E_2 - $ER\alpha$ signaling in mouse uterus, although the relating signaling pathways and mechanisms need to be clarified in future study.

Furthermore, the regulating effects of P_4 and E_2 on uterine CDO are confirmed by using OVX mouse model. The in vivo results show that uterus CDO expression level is decreased over 90% after 12 h E_2 treatment in OVX mouse, which is in agreement with the reports that E_2 inhibits taurine synthesis through estrogen-ERs-CDO/CSAD signaling in liver and uterus [37, 38]. It is inverted that uterine CDO expression level is sharply elevated after P_4 injection in OVX mouse. In addition, it is well known that E_2 and P_4 are key drivers of uterine plasticity throughout the sexual cycle and early stage of pregnancy, we thus propose that both E_2 and P_4 may play roles through their respective receptors to regulate uterine CDO expression and taurine synthesis, which subsequently affect the uterine receptivity and embryo

(See figure on next page.)

Fig. 5 The relations of P_4 -PR and E_2 - $ER\alpha$ signaling to uterine CDO expression. **A** and **B**, Relative *Cdo* mRNA levels in OVX mouse uteri after 0 (control), 2, 6 and 12 h treatments with 100 ng E_2 (**A**) and 2 mg P_4 (**B**). **C**, CDO IHC staining in OVX mouse uteri after 0 (control) and 12 h 100 ng E_2 or 2 mg P_4 treatments. Scale bars: 50 μ m. **D**, **E** and **F**, Relative *Cdo* mRNA (**D**) and protein levels (**E** and **F**) in OVX mouse uteri treated with E_2 and E_2 inhibitor ICI182780 (ICI). **G**, **H** and **I**, Relative *Cdo* mRNA (**G**) and protein levels (**H** and **I**) in OVX mouse uteri treated with 2 mg P_4 and PR inhibitor RU486. Data are presented as Mean \pm SEM. Different letters represent significant differences ($P < 0.05$, $n = 3$)

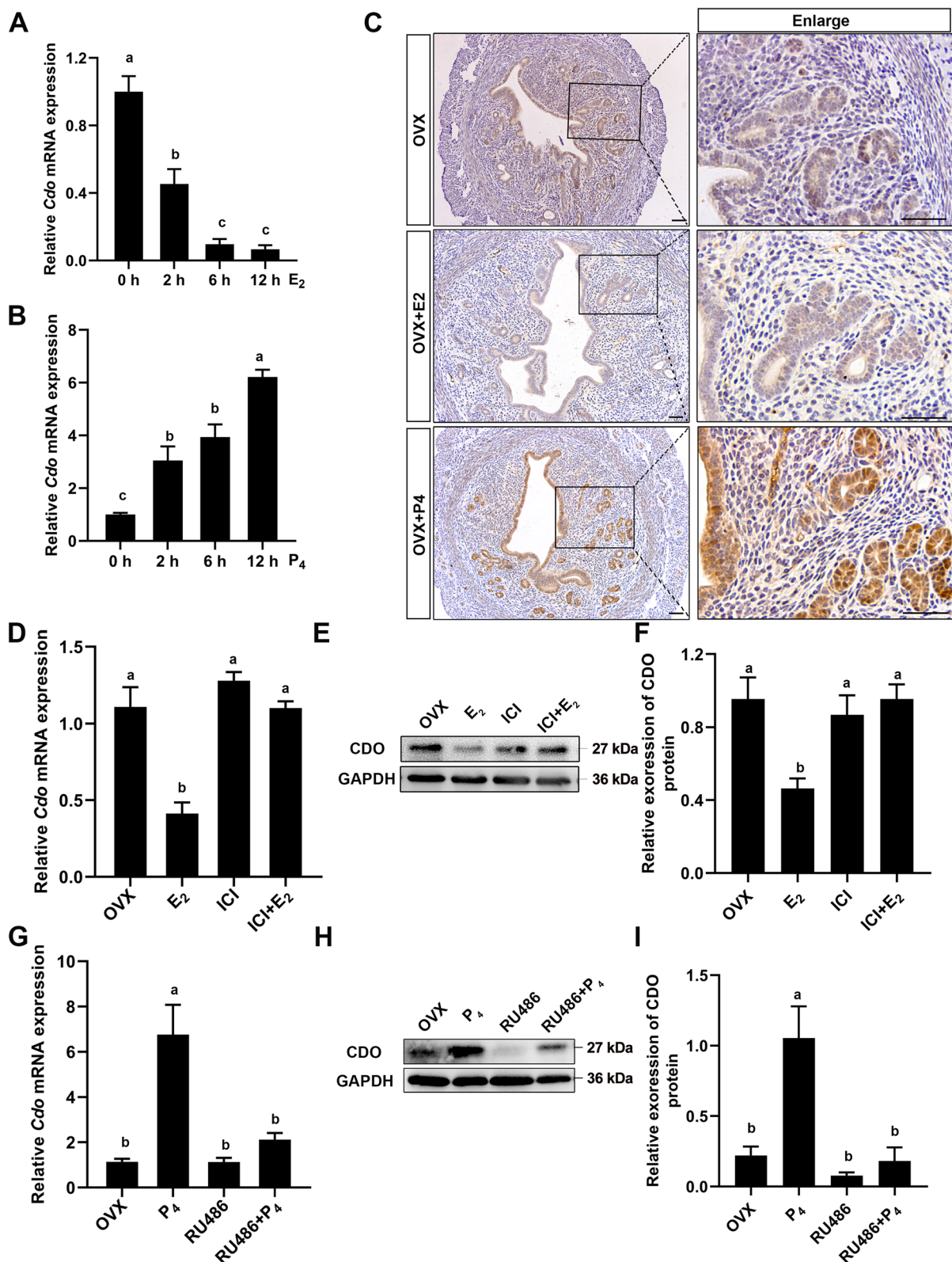


Fig. 5 (See legend on previous page.)

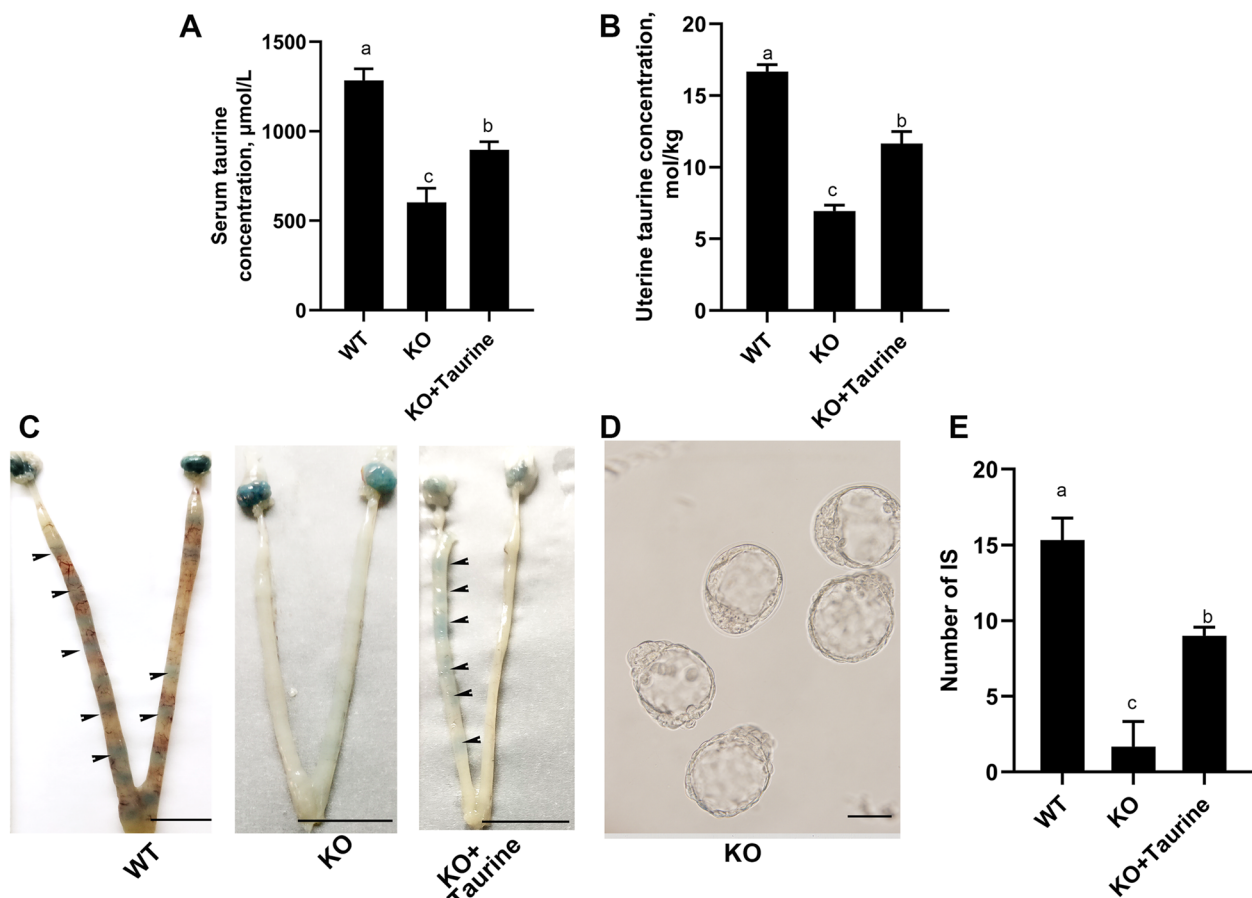


Fig. 6 Taurine supplement increases the taurine levels in serum and uteri, and partly rescues the defects of embryo implantation in the CDO KO mice. **A**, Serum taurine concentration. **B**, Taurine concentration in uterus tissues. **C**, Representative images of implantation sites. Bars: 1 cm. WT: wide type female mice. KO: *Cdo* KO female mice. KO + taurine: *Cdo* KO female mice supplied with taurine. $n = 3$ for each genotype. **D**, Blastocysts flushed out from *Cdo* KO uterus. Bar, 50 μm . **E**, Numbers of implantation sites. Data are presented as Mean \pm SEM. Different letters represent significant differences ($P < 0.05$, $n \geq 3$)

implantation. However, it remains to be elucidated about the interactions or cross-talks among P_4 -PR signaling, E_2 -ER α signaling, CDO and taurine in the duration of embryo implantation.

Finally, the maintenance of taurine levels in uterus and serum relies on the endogenous taurine synthesis through the action of CSAD, CDO, and the active uptake from the diet [34, 35], while *Cdo* deletion impairs embryo implantation and causes severe subfertility as it is showed in this study. Whereas taurine supplementation significantly increases the litter size and parturition rate of *Cdo* KO females (Table S2). These provide the in vivo evidence that CDO and taurine are crucial factors for the maintenance and improvement of animal production, and suggest that taurine may be potential agent for animal production.

In conclusion, the present study demonstrates that taurine and CDO play prominent roles for uterine

receptivity and embryo implantation by involving in E_2 -ER α and P_4 -PR signaling pathways. These are crucial for our understanding the mechanism of embryo implantation, and infer that taurine is a potential agent for improving reproductive efficiency of livestock industry and/or for reproductive medicine. But it still remains to be elucidated about the interactions among P_4 -PR signaling, E_2 -ER α signaling, CDO and taurine in the duration of embryo implantation.

Conclusions

The present study demonstrates that taurine and CDO play prominent roles for the uterine receptivity and embryo implantation by involving in E_2 -ER α and P_4 -PR signaling in early pregnancy stage. These elucidate a new mechanism for taurine regulating embryo implantation. On the other side, abuse of steroid hormones in stockbreeding industry usually gives rise to premature

ovary failure and declines animal fertility. The balancing effect of CDO and taurine between P₄-PR signaling and E₂-ER α signaling shows that taurine is a potential agent for improving reproductive efficiency of livestock industry and reproductive medicine.

Abbreviations

CDO	Cysteine dioxygenase
E ₂	Estrogen
<i>Esr1</i> (ER α)	Estrogen receptor 1
GE	Glandular epithelium
HA	Hyaluronidase
IF	Immunofluorescence
IHC	Immunohistochemistry
LE	Luminal epithelium
OVX	Ovariectomy
P ₄	Progesterone
<i>Pgr</i> (PR)	Progesterone receptor
RIA	Radioimmunoassay
Sm	Smooth muscle cells
St	Stromal cells
WB	Western blotting

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s40104-022-00804-1>.

Additional file 1: Data 1. Generation of *Cdo* knockout (KO) mice.

Additional file 2: Table S1. sgRNA pairs used with SpCas9n to target *Cdo* gene.

Additional file 3: Fig. S1. CDO mRNA and protein expressions in different tissues of female mice.

Additional file 4: Fig. S2. *Cdo* KO mouse ovary has normal morphology and functions.

Additional file 5: Table S2. Taurine supplement on *Cdo* KO female mice fertility.

Additional file 6: Table S3. Primer sequences for RT-qPCR.

Additional file 7: Table S4. Primer sequences for mice genotype identification.

Acknowledgements

This work is supported by the Natural Science Foundation of China (32130098) and the Project Funded by the Priority Academic Program Development of Jiangsu Higher Education Institutions (PAPD).

Authors' contributions

DZ, ZW and SC conceived and designed the research; DZ, ZW, GQ, YG and HG (Gao) performed experiments; DZ, ZW, XL and HZ (Guo) analyzed the results and processed data; DZ and SC interpreted results of experiments and drafted the manuscript. The author(s) read and approved the final manuscript.

Availability of data and materials

The datasets supporting the conclusions of this article are available from the corresponding author upon reasonable request.

Declarations

Ethics approval and consent to participate

Institutional Animal Care and Use Committee (IACUC) at the Yangzhou University approved the experimental protocol of this study.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

Received: 19 July 2022 Accepted: 20 November 2022

Published online: 05 January 2023

References

- Boivin J, Bunting L, Collins JA, Nygren KG. International estimates of infertility prevalence and treatment-seeking: potential need and demand for infertility medical care. *Hum Reprod.* 2007;22(6):1506–12. <https://doi.org/10.1093/humrep/dem046>.
- Clark DA. Is there any evidence for immunologically mediated or immunologically modifiable early pregnancy failure? *J Assist Reprod Genet.* 2003;20(2):63–72. <https://doi.org/10.1023/a:1021788024214>.
- Macaluso M, Wright-Schnapp TJ, Chandra A, Johnson R, Satterwhite CL, Pulver A, et al. A public health focus on infertility prevention, detection, and management. *Fertil Steril.* 2010;93(1):16 e1–10. <https://doi.org/10.1016/j.fertnstert.2008.09.046>.
- Norwitz ER, Schust DJ, Fisher SJ. Implantation and the survival of early pregnancy. *N Engl J Med.* 2001;345(19):1400–8. <https://doi.org/10.1056/NEJMra000763>.
- Wilcox AJ, Weinberg CR, O'Connor JF, Baird DD, Schlatterer JP, Canfield RE, et al. Incidence of early loss of pregnancy. *N Engl J Med.* 1988;319(4):189–94. <https://doi.org/10.1056/NEJM198807283190401>.
- Horne AW, White JO, Lalani EN. The endometrium and embryo implantation. A receptive endometrium depends on more than hormonal influences. *BMJ.* 2000;321(7272):1301–2. <https://doi.org/10.1136/bmj.321.7272.1301>.
- Wang H, Dey SK. Roadmap to embryo implantation: clues from mouse models. *Nat Rev Genet.* 2006;7(3):185–99. <https://doi.org/10.1038/nrg1808>.
- Lee KY, Jeong JW, Tsai SY, Lydon JP, DeMayo FJ. Mouse models of implantation. *Trends Endocrinol Metab.* 2007;18(6):234–9. <https://doi.org/10.1016/j.tem.2007.06.002>.
- Franco HL, Jeong JW, Tsai SY, Lydon JP, DeMayo FJ. In vivo analysis of progesterone receptor action in the uterus during embryo implantation. *Semin Cell Dev Biol.* 2008;19(2):178–86. <https://doi.org/10.1016/j.semcdb.2007.12.001>.
- Lee JH, Kim TH, Oh SJ, Yoo JY, Akira S, Ku BJ, et al. Signal transducer and activator of transcription-3 (Stat3) plays a critical role in implantation via progesterone receptor in uterus. *FASEB J.* 2013;27(7):2553–63. <https://doi.org/10.1096/fj.12-225664>.
- Curtis SW, Clark J, Myers P, Korach KS. Disruption of estrogen signaling does not prevent progesterone action in the estrogen receptor alpha knockout mouse uterus. *Proc Natl Acad Sci U S A.* 1999;96(7):3646–51. <https://doi.org/10.1073/pnas.96.7.3646>.
- Lydon JP, DeMayo FJ, Funk CR, Mani SK, Hughes AR, Montgomery CA Jr, et al. Mice lacking progesterone receptor exhibit pleiotropic reproductive abnormalities. *Genes Dev.* 1995;9(18):2266–78. <https://doi.org/10.1101/gad.9.18.2266>.
- Dey SK, Lim H, Das SK, Reese J, Paria BC, Daikoku T, et al. Molecular cues to implantation. *Endocr Rev.* 2004;25(3):341–73. <https://doi.org/10.1210/er.2003-0020>.
- Surveyor GA, Gendler SJ, Pemberton L, Das SK, Chakraborty I, Julian J, et al. Expression and steroid hormonal control of Muc-1 in the mouse uterus. *Endocrinology.* 1995;136(8):3639–47. <https://doi.org/10.1210/endo.136.8.7628404>.
- Carson DD, Bagchi I, Dey SK, Enders AC, Fazleabas AT, Lessey BA, et al. Embryo implantation. *Dev Biol.* 2000;223(2):217–37. <https://doi.org/10.1006/dbio.2000.9767>.
- Ramathal CY, Bagchi IC, Taylor RN, Bagchi MK. Endometrial decidualization: of mice and men. *Semin Reprod Med.* 2010;28(1):17–26. <https://doi.org/10.1055/s-0029-1242989>.

17. Satokata I, Benson G, Maas R. Sexually dimorphic sterility phenotypes in Hoxa10-deficient mice. *Nature*. 1995;374(6521):460–3. <https://doi.org/10.1038/374460a0>.
18. Matsumoto H, Zhao X, Das SK, Hogan BL, Dey SK. Indian hedgehog as a progesterone-responsive factor mediating epithelial-mesenchymal interactions in the mouse uterus. *Dev Biol*. 2002;245(2):280–90. <https://doi.org/10.1006/dbio.2002.0645>.
19. Simon L, Spiewak KA, Ekman GC, Kim J, Lydon JP, Bagchi MK, et al. Stromal progesterone receptors mediate induction of Indian Hedgehog (IHH) in uterine epithelium and its downstream targets in uterine stroma. *Endocrinology*. 2009;150(8):3871–6. <https://doi.org/10.1210/en.2008-1691>.
20. Kelleher AM, DeMayo FJ, Spencer TE. Uterine Glands: Developmental biology and functional roles in pregnancy. *Endocr Rev*. 2019;40(5):1424–45. <https://doi.org/10.1210/er.2018-00281>.
21. Cheng J, Rosario G, Cohen HV, Hu J, Stewart CL. Tissue-specific ablation of the LIF receptor in the murine uterine epithelium results in implantation failure. *Endocrinology*. 2017;158(6):1916–28. <https://doi.org/10.1210/en.2017-00103>.
22. Filant J, Spencer TE. Endometrial glands are essential for blastocyst implantation and decidualization in the mouse uterus. *Biol Reprod*. 2013;88(4):93. <https://doi.org/10.1095/biolreprod.113.107631>.
23. Filant J, Zhou H, Spencer TE. Progesterone inhibits uterine gland development in the neonatal mouse uterus. *Biol Reprod*. 2012;86(5):146, 1–9. <https://doi.org/10.1095/biolreprod.111.097089>.
24. Stewart CL, Kaspar P, Brunet LJ, Bhatt H, Gadi I, Kontgen F, et al. Blastocyst implantation depends on maternal expression of leukemia inhibitory factor. *Nature*. 1992;359(6390):76–9. <https://doi.org/10.1038/359076a0>.
25. Kelleher AM, Burns GW, Behura S, Wu G, Spencer TE. Uterine glands impact uterine receptivity, luminal fluid homeostasis and blastocyst implantation. *Sci Rep*. 2016;6:38078. <https://doi.org/10.1038/srep38078>.
26. Ueki I, Roman HB, Valli A, Fieselmann K, Lam J, Peters R, et al. Knockout of the murine cysteine dioxygenase gene results in severe impairment in ability to synthesize taurine and an increased catabolism of cysteine to hydrogen sulfide. *Am J Physiol Endocrinol Metab*. 2011;301(4):E668–84. <https://doi.org/10.1152/ajpendo.00151.2011>.
27. Huxtable RJ. Physiological actions of taurine. *Physiol Rev*. 1992;72(1):101–63. <https://doi.org/10.1152/physrev.1992.72.1.101>.
28. Hou Y, Hu S, Li X, He W, Wu G. Amino Acid Metabolism in the Liver: Nutritional and Physiological Significance. *Adv Exp Med Biol*. 2020;1265:21–37. https://doi.org/10.1007/978-3-030-45328-2_2.
29. Wen C, Li F, Zhang L, Duan Y, Guo Q, Wang W, et al. Taurine is Involved in Energy Metabolism in Muscles, Adipose Tissue, and the Liver. *Mol Nutr Food Res*. 2019;63(2):e1800536. <https://doi.org/10.1002/mnfr.201800536>.
30. Zimniak P, Little JM, Radomska A, Oelberg DG, Anwer MS, Lester R. Taurine-conjugated bile acids act as Ca²⁺ ionophores. *Biochemistry*. 1991;30(35):8598–604. <https://doi.org/10.1021/bi00099a015>.
31. Chen W, Guo JX, Chang P. The effect of taurine on cholesterol metabolism. *Mol Nutr Food Res*. 2012;56(5):681–90. <https://doi.org/10.1002/mnfr.201100799>.
32. Lambert IH, Kristensen DM, Holm JB, Mortensen OH. Physiological role of taurine—from organism to organelle. *Acta Physiol (Oxf)*. 2015;213(1):191–212. <https://doi.org/10.1111/apha.12365>.
33. Murakami S, Ono A, Kawasaki A, Takenaga T, Ito T. Taurine attenuates the development of hepatic steatosis through the inhibition of oxidative stress in a model of nonalcoholic fatty liver disease in vivo and in vitro. *Amino Acids*. 2018;50(9):1279–88. <https://doi.org/10.1007/s00726-018-2605-8>.
34. Parsons RB, Ramsden DB, Waring RH, Barber PC, Williams AC. Hepatic localisation of rat cysteine dioxygenase. *J Hepatol*. 1998;29(4):595–602. [https://doi.org/10.1016/s0168-8278\(98\)80155-4](https://doi.org/10.1016/s0168-8278(98)80155-4).
35. Stipanuk MH, Ueki I. Dealing with methionine/homocysteine sulfur: cysteine metabolism to taurine and inorganic sulfur. *J Inher Metab Dis*. 2011;34(1):17–32. <https://doi.org/10.1007/s10545-009-9006-9>.
36. Asano A, Roman HB, Hirschberger LL, Ushiyama A, Nelson JL, Hinchman MM, et al. Cysteine dioxygenase is essential for mouse sperm osmoadaptation and male fertility. *FEBS J*. 2018;285(10):1827–39. <https://doi.org/10.1111/febs.14449>.
37. Guerra DD, Bok R, Breen K, Vyas V, Jiang H, MacLean KN, et al. Estrogen regulates local cysteine metabolism in mouse myometrium. *Reprod Sci*. 2021;28(1):79–90. <https://doi.org/10.1007/s43032-020-00284-6>.
38. Ma Q, Zhao J, Cao W, Liu J, Cui S. Estradiol decreases taurine level by reducing cysteine sulfinic acid decarboxylase via the estrogen receptor- α in female mice liver. *Am J Physiol Gastrointest Liver Physiol*. 2015;308(4):G277–86. <https://doi.org/10.1152/ajpgi.00107.2014>.
39. Aikawa S, Deng W, Liang X, Yuan J, Bartos A, Sun X, et al. Uterine deficiency of high-mobility group box-1 (HMGB1) protein causes implantation defects and adverse pregnancy outcomes. *Cell Death Differ*. 2020;27(5):1489–504. <https://doi.org/10.1038/s41418-019-0429-z>.
40. Kong S, Han X, Cui T, Zhou C, Jiang Y, Zhang H, et al. MCM2 mediates progesterone-induced endometrial stromal cell proliferation and differentiation in mice. *Endocrine*. 2016;53(2):595–606. <https://doi.org/10.1007/s12020-016-0894-9>.
41. Liu J, He Y, Wang X, Zheng X, Cui S. Developmental changes of Islet-1 and its co-localization with pituitary hormones in the pituitary gland of chick embryo by immunohistochemistry. *Cell Tissue Res*. 2005;322(2):279–87. <https://doi.org/10.1007/s00441-005-0021-3>.
42. Strowitzki T, Germeyer A, Popovici Rvon R, Wolff M. The human endometrium as a fertility-determining factor. *Hum Reprod Update*. 2006;12(5):617–30. <https://doi.org/10.1093/humupd/dml033>.
43. Li Q, Kannan A, DeMayo FJ, Lydon JP, Cooke PS, Yamagishi H, et al. The antiproliferative action of progesterone in uterine epithelium is mediated by Hand2. *Science*. 2011;331(6019):912–6. <https://doi.org/10.1126/science.1197454>.
44. Paulson EE, Comizzoli P. Endometrial receptivity and embryo implantation in carnivores—commonalities and differences with other mammalian species. *Biol Reprod*. 2021;104(4):771–83. <https://doi.org/10.1093/biolre/iaob001>.
45. Achache H, Revel A. Endometrial receptivity markers, the journey to successful embryo implantation. *Hum Reprod Update*. 2006;12(6):731–46. <https://doi.org/10.1093/humupd/dml004>.
46. Hirota Y. Progesterone governs endometrial proliferation-differentiation switching and blastocyst implantation. *Endocr J*. 2019;66(3):199–206. <https://doi.org/10.1507/endocrj.EJ18-0431>.
47. Kim JJ, Kurita T, Bulun SE. Progesterone action in endometrial cancer, endometriosis, uterine fibroids, and breast cancer. *Endocr Rev*. 2013;34(1):130–62. <https://doi.org/10.1210/er.2012-1043>.
48. Kawagoe J, Li Q, Mussi P, Liao L, Lydon JP, DeMayo FJ, et al. Nuclear receptor coactivator-6 attenuates uterine estrogen sensitivity to permit embryo implantation. *Dev Cell*. 2012;23(4):858–65. <https://doi.org/10.1016/j.devcel.2012.09.002>.
49. Tranguch S, Wang H, Daikoku T, Xie H, Smith DF, Dey SK. FKBP52 deficiency-conferred uterine progesterone resistance is genetic background and pregnancy stage specific. *J Clin Invest*. 2007;117(7):1824–34. <https://doi.org/10.1172/JCI31622>.
50. Lee KY, DeMayo FJ. Animal models of implantation. *Reproduction*. 2004;128(6):679–95. <https://doi.org/10.1530/rep.1.00340>.
51. Lim H, Ma L, Ma WG, Maas RL, Dey SK. Hoxa-10 regulates uterine stromal cell responsiveness to progesterone during implantation and decidualization in the mouse. *Mol Endocrinol*. 1999;13(6):1005–17. <https://doi.org/10.1210/mend.13.6.0284>.
52. Kato S, Endoh H, Masuhiro Y, Kitamoto T, Uchiyama S, Sasaki H, et al. Activation of the estrogen receptor through phosphorylation by mitogen-activated protein kinase. *Science*. 1995;270(5241):1491–4. <https://doi.org/10.1126/science.270.5241.1491>.