Regulation of tryptophan 2,3-dioxygenase by HOXA10 enhances embryo viability through serotonin signaling

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Doherty LF, Kwon HE, Taylor HS. Regulation of tryptophan 2,3-dioxygenase by HOXA10 enhances embryo viability through serotonin signaling. Am J Physiol Endocrinol Metab 300: E86–E93, 2011. First published October 19, 2010; doi:10.1152/ajpendo.00439.2010.—Tryptophan 2,3-dioxygenase (TDO) is expressed in endometrium and catalyzes tryptophan, a precursor in the biosynthesis of serotonin. Tryptophan metabolism is an important mechanism for regulation of serotonin levels. Preimplantation mouse embryos are known to express serotonin receptors, specifically the 5-HT1D and 5-HT7 serotonin receptor subtypes. Here we demonstrate that Hoxa10 regulates endometrial TDO expression and improves embryo viability through increased serotonin production. Transfection of pcDNA-Hoxa10 to the murine uterus increased total TDO expression. In vitro, epithelial cell TDO expression was decreased after transfection with Hoxa10. Decreased glandular TDO in response to Hoxa10 may augment serotonin production by increasing tryptophan availability. Conversely, stromal TDO expression increased with constitutive Hoxa10 expression. In mice, epithelial serotonin was increased in response to constitutive expression of Hoxa10. Embryo quality was impaired after treatment with Hoxa10 antisense. Blockade of serotonin receptors 1D and 7 also resulted in impaired embryo development, indicating an essential role for Hoxa10 induction of TDO and subsequent serotonin production in embryo development. Transfection of pcDNA-TDO also decreased the number of T cells in the endometrial stroma. We have shown a novel mechanism by which Hoxa10 regulates endometrial TDO expression. In the endometrial stroma, Hoxa10 increases TDO mRNA, which may increase tryptophan catabolism, allowing for immune tolerance at the time of embryo implantation. In endometrial glands, Hoxa10 decreases TDO mRNA leading to increased serotonin that in turn acts to promote normal embryo development.

tryptophan degradation; implantation; serotonin

THE INDUCTION OF RECEPTIVITY in the mammalian endometrium requires specific molecular and structural alterations in response to hormonal signals. These changes occur within the glandular epithelium and the endometrial stroma to create a favorable environment for embryo implantation. The specific genes responsible for inducing endometrial receptivity, as well as their regulation and function at the time of implantation, remain incompletely characterized.

Hoxa10 is a member of the homeobox (Hox) gene family, which encodes transcription factors that function to control embryonic development and to regulate gene expression within the endometrium during the menstrual cycle (39). Hox genes expressed within the reproductive tract regulate normal developmental patterning during the embryonic period and continue to function in the adult. Hoxa10 is expressed by the endometrial glands and stroma throughout the menstrual cycle and its expression is regulated by estradiol and progesterone (36, 38). Mice with targeted disruption of either Hoxa10 or Hoxa11 are infertile due to abnormal endometrial receptivity but are able to produce viable embryos (6, 16, 32). There are no known human mutations of the Hoxa10 or Hoxa11 genes; however, women with implantation defects, including endometriosis, polycystic ovarian syndrome, hydrosalpinx, and submucosal myomas, have diminished expression of these genes (9, 12, 30, 37). Endometrium from women using contraception have also shown to have decreased levels of Hoxa10 (41). The associations between altered Hoxa10 expression and implantation defects are clear. The specific mechanisms by which Hoxa10 functions in endometrial receptivity are not fully characterized.

Hoxa10 functions as a transcription factor and several of its target genes have recently been identified. Hoxa10 acts as a transcriptional repressor of Emx2, which is expressed during the embryonic period in the developing female reproductive tract as well as within the adult endometrium (43). Endometrial β3-integrin subunit gene expression is directly upregulated by Hoxa10 (13). cDNA microarray has recently been used to broadly search for potential downstream targets of Hoxa10 (24, 45). When Hoxa10 was overexpressed, several target genes were found to have significantly increased expression. Of these, the largest increase was in the tryptophan 2,3-dioxygenase (TDO) gene that increased eightfold in response to constitutive expression of Hoxa10 (45).

TDO is one of two mammalian tryptophan degrading enzymes; the second enzyme is indolamine 2,3-dioxygenase (IDO; Ref. 42). Tryptophan is an essential amino acid that is an immediate precursor in the biosynthesis of serotonin. Serotonin synthesis is dependent on tryptophan availability; thus increased tryptophan degradation decreases serotonin (27, 40). Inhibition of TDO activity, leading to an increase in available tryptophan, increases serotonin levels (31). TDO is ubiquitously expressed and has been found in many tissues including liver, brain, skin, and uterus. TDO demonstrates a menstrual cycle dependent expression pattern in the endometrium and is induced in decidualized stromal cells at the time of embryo implantation (35). IDO is expressed by trophoblasts but not by endometrial cells (4). Tryptophan degradation occurs within the murine embryonic and extra-embryonic tissues, peaking on day 6.5 postcoitum. This activity coincides with high expression levels of TDO in the endometrium (26, 34).

T-cell proliferation is inhibited by tryptophan degradation. Tryptophan degradation by IDO is necessary for embryo implantation (28). Inhibition of IDO function leads to embryo rejection by T lymphocytes. IDO is expressed by trophoblasts, after TDO expression in the endometrium (21, 34). Mice with targeted disruption of Hoxa10 have an accumulation of T
lymphocytes within the endometrial stroma (46). This suggests a link between HOXA10 and regulation of immune tolerance, possibly through TDO.

Serotonin [5-hydroxytryptamine (5-HT)] is a hormone and neurotransmitter with diverse effects. Its actions have been best characterized within the central nervous system, vasculature, and gastrointestinal tract. Serotonin has also been identified within the female reproductive tract in oocytes, uterus, oviducts, placenta, and ovarian follicular fluid (1, 2, 7, 8, 10, 18, 25). At least 15 serotonin receptors, grouped into 7 families due to their signaling mechanisms, have been cloned (20). In mammals, multiple serotonin receptor subtypes are located within the reproductive tract (15). Preimplantation mouse embryos express the serotonin receptors 5-HT1D and 5-HT7 (3, 4, 22). In vitro exposure of embryos to serotonin at 1 μM reduced mean cell number, while lower concentrations did not alter embryo cell numbers (17). Maternally derived serotonin, due to their signaling mechanisms, have been cloned (20). In vitro exposure of embryos to serotonin at 1 μM concentration produced a similar effect on cell number. The role of serotonin in normal embryo development is uncertain, but its presence is necessary for normal embryo development. Female mice with targeted disruption of tryptophan hydroxylase 1, the rate-limiting enzyme in serotonin synthesis, produce offspring with abnormal central nervous system development, regardless of the genotype of the fetus (11). These studies show that regulation of maternally derived serotonin levels is necessary for normal embryo development.

TDO expression is upregulated in uteri of mice overexpressing Hoxa10: we therefore sought to examine the cell-specific regulation of TDO by HOXA10. Because of the importance of available tryptophan in serotonin synthesis, we examined serotonin localization within the endometrium in mice with altered expression of HOXA10. Finally, we examined embryos of mice treated with serotonin receptor antagonists to determine the direct effects serotonin on embryo viability.

MATERIALS AND METHODS

Cell culture and transfection of cell lines. The human endometrial stromal cell line HESC, the primary third trimester human deciduod cell (HDC), and the human endometrial adenocarcinoma cell line Ishikawa were used. HESC cells are a well-characterized telomerased immortalized human endometrial stromal cell line that is phenotypically and morphologically similar to parent cells (14, 19, 29). Third trimester human deciduod cells were extracted from women undergoing repeat cesarean sections at term (23). HESC and HDC cells were maintained in a DMEM solution (Sigma, St. Louis, MO) supplemented with 10% charcoal stripped calf serum, 1% penicillin/streptomycin solution, and 1% sodium pyruvate. Ishikawa cells were maintained in a MEM solution (Sigma) supplemented with 10% charcoal stripped calf serum, 1% penicillin/streptomycin solution, and 1% sodium pyruvate.

HOXA10 cDNA cloned into the pcDNA3.1(+) vector (Invitrogen, Carlsbad, CA) and the pcDNA3.1(+) vector alone have been described previously (5). HESC, HDC, and Ishikawa cells, grown to 60–70% confluence, were transfected using Lipofectamine 2000 (Invitrogen) with either pcDNA3.1(+) or HOXA10 cDNA (1 μg/ml) or empty pcDNA3.1(+) vector (1 μg/ml). Human deciduod cells were also transfected with pcDNA3.1(+) or HOXA10 antisense.

In vivo gene transfection. Nulliparous CD1 male and female mice (8–12 wk old; Charles River, Wilmington, MA) were mated and examined every 12 h until the presence of a vaginal plug. Presence of a vaginal plug designated day 1 postcoitus. Twenty-four hours after detection of the vaginal plug, 13 female mice were anesthetized with 200–400 μl of a 10% ketamine-5% xylazine solution intraperitoneally in accordance with the Animal Care and Use Committee guidelines. Peritoneal closure was performed with a single running suture of 4–0 Polysorb suture (Covidien Syneture, Mansfield, MA). Skin was closed with interrupted sutures using the same suture. Postoperative care was carried out according to the Yale Animal Care and Use Committee guidelines.

In vivo serotonin antagonist treatment. Nulliparous female CD1 mice (8–12 wk old) were treated with 5 IU of pregnant mare’s serum gonadotropin (Sigma). Forty-eight hours later, ovulation was triggered with 5 IU of human chorionic gonadotropin (Sigma). Animals were then mated with male CD1 mice and examined 12 h later for the presence of a vaginal plug. The presence of a vaginal plug designated day 1 postcoitus 48 h after detection of a vaginal plug, 9 pregnant female mice were anesthetized, and laparotomy was performed as described above. M2 medium (Sigma) containing 1 mM concentration of both the 5-HT 1D receptor antagonist GR127935 and the 5-HT 7 receptor antagonist SB269970 (Sigma) was used for timed intrauterine injection. Three mice were treated with 25 μl of the M2 solution containing 1 mM of serotonin receptor antagonists injected into the base of each uterine horn. Three control mice were treated with 25 μl of M2 medium alone. Abdominal closure and postoperative care were performed as above.

Quantitative RT-PCR. Total RNA was isolated from cultured cells 48 h after transfection using the RNeasy kit (Qiagen, Valencia, CA) according to the manufacturer’s protocol. Total RNA was extracted from murine uteri by using Trizol reagent in accordance with the
manufacturer’s guidelines (Life Technologies, Gaithersburg, MD). Five hundred nanograms of total RNA were reverse transcribed in 20 μl of reaction mixture using the iScript cDNA synthesis kit (Bio-Rad, Hercules, CA). Quantitative real-time RT-PCR reactions were prepared using the iQ SYBR Green Supermix (Bio-Rad). The conditions for PCR were optimized analyzing the melting curves of the products acquired. Amplification efficiency was assessed after varying primer annealing temperatures, primer concentrations, template concentrations, and cycle number. All products were analyzed in the linear phase of amplification. Each PCR reaction consisted of the following: 1 μl of cDNA template, 1 μl of forward primer (1 μM), 1 μl of reverse primer (1 μM), 9.5 μl of nuclease-free H2O, and 12.5 μl of iQ SYBR Green Supermix. TDO intron-spanning primers were designed using PerlPrimer version 1.1.14. HOXA10 and β-actin primers have been previously described (33). TDO human primers were forward 5′-CTTAGTAAAGGTGAAGACGG-3′ and reverse 5′-GTCCATAAAGAAGTCAGCA-3′. TDO mouse primers were forward 5′-CATGGCTGGAAAGACACCT-3′ and reverse 5′-TCTTTCTTCCAGGCTTTCA-3′.

The Bio-Rad iCycler iQ system (Bio-Rad) was used to quantify fluorescence of PCR products during amplification. RT-PCR reactions were performed for 1 cycle at 95°C for 3 min, followed by 40 cycles at 95°C for 15 s and 59°C for 20 s. Melting curve data were collected for analysis. Relative gene expression was determined by analyzing data using the 2−ΔΔCT method to adjust for expression of β-actin (22). All experiments were conducted in triplicate. Samples without cDNA template were used as negative controls.

Serotonin immunohistochemistry. Serotonin (5-HT) localization was evaluated by immunohistochemistry using a goat monoclonal antibody to Serotonin (20079; Immunostar, Hudson, WI). Specimens were embedded in paraffin, cut into serial 5-μm sections, and mounted on slides. The sections were deparaffinized and dehydrated in xylene and ethanol. After a 5-min rinse in distilled water, an antigen-presenting step was performed by steaming the slides in 0.01 M sodium citrate buffer for 20 min and cooling for 20 min. The slides were then rinsed for 5 min in PBS-Tween (PBST), and sections were circumscribed with a hydrophobic pen. Endogenous peroxidase activity was blocked with 3% hydrogen peroxide. After incubation with 1.5% normal horse blocking serum in PBST for 1 h, the sections were incubated overnight at 4°C with primary antibody (anti-Serotonin in a 1:20,000 dilution). Normal goat IgG (Santa Cruz Biotechnology, Santa Cruz, CA) was used as a negative control. The sections were then rinsed in PBST and incubated with biotinylated horse anti-goat secondary antisera (Vector Laboratories, Burlingame, CA) for 60 min. Slides were then rinsed in PBST and incubated in avidin and biotinylated peroxidase (ABC elite, Vector Laboratories) for 15 min. After being rinsed in PBST, the slides were then incubated in diaminobenzidine for 5 min. Slides were then rinsed in water. Hematoxylin was used for counterstaining. Slides were then rehydrated via a series of ethanol and xylene washes, and coverslips were mounted using Permount.

CD3 immunohistochemistry. Specimens were imbedded in paraffin, cut into 5-μm sections, and mounted on slides. The sections were deparaffinized and dehydrated in xylene and ethanol. After a 5 min rinse in distilled water, an antigen-presenting step was performed by steaming the slides in 0.01 M sodium citrate buffer for 20 min and cooling for 20 min. The slides were then rinsed for 5 min in PBST, and sections were circumscribed with a hydrophobic pen. Endogenous peroxidase activity was blocked with 3% hydrogen peroxide. After incubation with 1.5% normal goat blocking serum in PBST for 1 h, slides were incubated overnight at 4°C with a 1:200 dilution of mouse CD3 antibody (sc-20047; Santa Cruz Biotechnology). Normal mouse IgG (Santa Cruz Biotechnology) was used as a negative control. Slides were then rinsed in PBST and incubated in 3.5 μg/ml of biotinylated...
goat anti-mouse (Vector Laboratories) for 1 h at 4°C. After being rinsed in PBST, the slides were incubated in ABC elite (Vector Laboratories) for 15 min at room temperature. Slides were then washed with PBST and incubated for 5 min in diaminobenzidine (Vector Laboratories). Slides were then rinsed in water. Hematoxylin was used for counterstaining. Slides were then dehydrated via a series of ethanol and xylene washes, and coverslips were mounted using Permount. Three sections per uterus were examined. Three high-of ethanol and xylene washes, and coverslips were mounted using Permount. Three sections per uterus were examined. Three high-powered fields were examined per section. The number of CD3+ cells per high-power field (×600) were counted and averaged for each animal (n = 8).

Statistical analysis. Quantitative PCR and CD3+ staining results were compared using Student’s t-test via SPSS software (version 12.0.1). Embryo number was compared using the Mann-Whitney U-test via SPSS software (version 12.0.1). Embryo quality (reflected as percent blastocysts) results were compared using independent samples t-test via SPSS software (version 12.0.1). P < 0.05 was considered statistically significant.

RESULTS

TDO expression in murine uteri. We (45) have previously shown that TDO expression increases eightfold in whole uteri of mice overexpressing Hoxa10. We confirmed this microarray data by examining TDO expression in whole uteri of mice treated with Hoxa10/pcDNA3.1 (+), Hoxa10 antisense/pcDNA3.1 (+), and controls [empty pcDNA3.1 (+)]. Mice treated with Hoxa10-plasmid in vivo gene transfection showed a 3.8-fold increase in uterine TDO mRNA expression compared with controls (P < 0.05). TDO mRNA expression was not significantly altered in mice treated with Hoxa10 antisense. (Fig. 1A).

TDO expression in endometrial cell lines. Because whole uterine tissue represents multiple cell types, we sought to quantify TDO expression in individual cell lines. To confirm regulation of TDO by HOXA10, Ishikawa cells, HESC, and decidual cells were transfected with a HOXA10-expressing plasmid. Decidual cells were also transfected with HOXA10 antisense. Successful transfection of cells with the HOXA10-containing vector was confirmed by examination of relative Hoxa10 expression using quantitative RT-PCR. In Ishikawa cells, Hoxa10 mRNA levels were increased 120-fold (P < 0.05) in cells transfected with HOXA10-containing plasmid compared with controls. In HESC cells, the increase in HOXA10 expression was 5,853-fold (P < 0.05). In decidual cells transfected with HOXA10-containing plasmid, Hoxa10 expression increased 1100-fold (P < 0.05). Transfection of decidual cells with HOXA10 antisense-plasmid decreased Hoxa10 expression twofold (P < 0.05).

Increased expression of HOXA10 resulted in alterations of TDO expression in Ishikawa Cells, HESC, and decidual cells; however, HOXA10 transfection led to differential effects on TDO expression in these cell lines. In Ishikawa cells, transfection with HOXA10 vector led to significantly lower amounts of TDO expression compared with controls (0.42-fold, P < 0.05; Fig. 1B). In HESC cells, transfection of HOXA10-containing vector led to increased TDO expression compared with controls (5.28-fold, P < 0.05; Fig. 1C). In decidual cells, transfection of HOXA10 vector also increased TDO expression compared with controls (1.89-fold, P < 0.05; Fig. 1D). There was a decrease in TDO expression in decidual cells transfected with HOXA10 antisense; however, this decrease was not statistically significant.

Serotonin is expressed in response to Hoxa10 treatment in vivo. Because TDO activity regulates tryptophan availability for serotonin synthesis, we examined the localization of serotonin within the murine uteri of mice treated with HOXA10-expressing plasmid vectors. Serotonin localization was examined in mice treated with HOXA10/pcDNA3.1 (+) plasmid and controls [treated with pcDNA3.1 (+)]. Our cell culture data showed that increased HOXA10 in endometrial glands (modulated by Ishikawa cells) decreased TDO expression. Because lower TDO increases available tryptophan for serotonin synthesis, our model predicts that overexpression of HOXA10 in endometrial glands would increase serotonin. Serotonin levels were dramatically increased in the luminal and glandular epithelium of mice that were treated with HOXA10 vector compared with control mice. (Fig. 2). There were low levels of

Table 1. Comparison of embryo quality after in vivo gene transfection with pcDNA 3.1 (+)/Hoxa10 antisense cDNA vs. pcDNA 3.1 (+) vector alone

<table>
<thead>
<tr>
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<th>Number of Embryos</th>
<th>Number of Embryos per Mouse (mean, range)</th>
<th>Percent Blastocysts</th>
</tr>
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<tbody>
<tr>
<td>Control</td>
<td>31</td>
<td>7.8 (7–9)</td>
<td>83.9%</td>
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<tr>
<td>Antisense Hoxa10</td>
<td>13</td>
<td>4.3 (3–5)</td>
<td>7.7% (P &lt; 0.05)</td>
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Embryo quality is the percentage of embryos developing to the blastocyst stage. Control is vector alone. Mann-Whitney test was used to determine the significance of differences in embryo number. Independent sample t-test was performed to determine statistical significance of the differences in the percentage of embryos developing to blastocysts.
serotonin in the stroma of both control and Hoxa10-plasmid-treated mice.

Effect of Hoxa10 regulation of serotonin on embryo development. Pregnant mice were transfected in vivo with Hoxa10-antisense. Embryo quality on day 4.5 postcoitum was examined in mice treated with in vivo gene transfection or control. There were fewer total embryos recovered in mice treated with Hoxa10 antisense compared with controls (P < 0.05). There was no significant variation in the number of embryos recovered individual animals in either treatment group. Control mice, transfected with empty pcDNA3.1 vector, had a significantly higher percentage of blastocysts per embryo recovered than mice treated with HOXA10 antisense cDNA/pcDNA3.1(+) transfection (83.9 vs. 7.7%, P < 0.05; Table 1; Fig. 3).

Effect of serotonin receptor blockade on embryo development. Knowing that embryos express 5-HT1D and 5-HT7 receptors, we examined embryos from mice treated with antagonists (the 5-HT1D antagonist GR127935 and the 5-HT7 antagonist SB269970). Embryos were examined on day 4.5 postcoitum in mice treated with the 5-HT 1D receptor and 5-HT 7 receptor antagonists or control media on day 2 postcoitum. Embryo development was assessed by measuring the ability to develop to the blastocyst stage. Control mice (treated with M2 medium alone) had a significantly higher percentage of blastocysts per embryo recovered than mice treated with 5-HT 1D and 5-HT 7 receptor antagonists (75 vs. 20%, P < 0.05). There was a trend towards a decrease in the total number of embryos recovered in mice treated with serotonin receptor antagonists compared with controls. There was no difference in the number of embryos recovered from individual animals in either treatment group. (Table 2; Fig. 4).

Induction of decidual response within the endometrium. Tryptophan degradation at the time of implantation is necessary to avoid T-cell-mediated immune rejection of the implanting embryo. TDO expression increases in HESC and decidual cells in response to HOXA10. TDO functions to catabolize tryptophan. Pseudopregnant mice transfected with TDO expressing plasmid in vivo and controls were subsequently treated with sesame oil to induce a decidual reaction. Immunohistochemistry was performed to examine localization of CD3, a marker present on mature T cells (Fig. 5A). Mice transfected with TDO plasmid showed significantly fewer mean CD3+ cells per high-power field than controls (1.4 vs. 12.1, respectively, P < 0.05).

DISCUSSION

TDO expression is regulated by HOXA10 within the uterus at the time of endometrial receptivity. Here we confirmed the results of our previous microarray, showing increased in TDO mRNA in whole uterine tissue in response to increased Hoxa10. We then used a cell culture model to identify the regulation of TDO expression in individual cell types. TDO was differentially regulated by HOXA10 in epithelial cells (Ishikawa), stromal cells (HESC), and decidual cells. In endo-

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</thead>
<tbody>
<tr>
<td>Control</td>
<td>28</td>
<td>9.3 (7–11)</td>
</tr>
<tr>
<td>Serotonin antagonist</td>
<td>15</td>
<td>5 (4–6)</td>
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Control is M2 medium alone. Mann-Whitney test was used to determine the significance of differences in embryo number. Independent sample t-test was performed to determine statistical significance of the differences in the percentage of embryos developing to blastocysts.

Fig. 3. Embryo morphology after transfection in vivo on day 1.5 postcoitum with pcDNA 3.1(+)/Hoxa10 antisense cDNA vs. pcDNA 3.1(+)-vector alone (control). A: control embryos from mice transfected with empty pcDNA3.1(+) vector. B: embryos from mice transfected with pcDNA 3.1(+)/Hoxa10 antisense cDNA.

Fig. 4. Embryo morphology after in vivo treatment with both 5-HT1D receptor antagonist GR127935 and 5-HT7 receptor antagonist SB269970 in M2 medium vs. mice treated with M2 medium alone. A: control embryos from mice treated with M2 medium alone. B: embryos from mice treated with serotonin antagonists.
metrial glandular cells, constitutive expression of *HOXA10* led to a significant decrease in *TDO* expression. In endometrial stromal cells and decidual cells, elevated *HOXA10* expression led to significant increases in *TDO* expression.

Tryptophan is a necessary substrate for serotonin production, and *TDO* activity regulates tryptophan availability. *TDO* is differentially regulated by *HOXA10* in epithelial and stromal cells. We have shown that increased *Hoxa10* expression decreases *TDO* expression in endometrial epithelial cells. Decreased glandular tryptophan catabolism (mediated by *TDO*) increases available tryptophan for serotonin production. As predicted, constitutive expression of *HOXA10* increased serotonin within the glandular and luminal epithelium in vivo. We further hypothesized that increased glandular serotonin may act to promote embryo development. We (5) have previously shown that litter size is increased in mice treated with in utero transfection of *HOXA10*. Serotonin receptors are expressed in murine preimplantation embryos (3, 15, 17). The importance of maternally derived serotonin has been demonstrated by the fact that mice with targeted disruption of tryptophan hydroxylase 1 produce offspring with abnormal central nervous system development, regardless of the genotype of the fetus (11).

Interestingly, constitutive expression of *HOXA10* in endometrial stromal cells and decidual cells led to increased *TDO* expression, the opposite of the effect seen in epithelial cells. Because *TDO* catabolizes tryptophan, we examined potential roles for stromal tryptophan catabolism in the peri-implantation period. We hypothesize that the increase in *TDO* within the stroma is important for immune tolerance of embryo implantation. T-cell proliferation is inhibited by tryptophan degradation. Before *IDO* expression by trophoblasts, *TDO* is expressed in the endometrium (21, 34). Tryptophan degradation is increased in the early embryonic period, occurs independent of *IDO* activity, and coincides with the time of greatest *TDO* expression (34). These findings are highly suggestive of a role for *TDO* in early embryonic tryptophan catabolism. Our results show that increased expression of *TDO* at the time of implantation significantly decreases the number of CD3+ T cells within the endometrial stroma. Similarly, targeted disruption of *Hoxa10* leads to accumulation of T lymphocytes within the endometrial stroma at the time of implantation (46). The accumulation of T cells seen in *Hoxa10*-deficient mice may be due to decreased stromal tryptophan degradation by *TDO*. This suggests a link between maternal *HOXA10* function and immune tolerance of implantation, which may be mediated through increased *TDO*-mediated tryptophan catabolism in the endometrial stroma.

We found a significant reduction in embryo quality when embryos were exposed to 5-HT1D and 5-HT7 receptor antagonists. Because in utero treatment with serotonin antagonists is a novel technique, we chose to superovulate mice to optimize our chance of successful embryo recovery.

Embryo viability between days 2.5 and 3.5 postcoitum is impaired in *Hoxa10*-deficient mice (32). The timing of embryo demise in these mice correlated with the timing of normal *Hoxa10* expression in the distal oviduct and uterus. *HOXA10*-mediated suppression of glandular *TDO* activity, with a subsequent increase in glandular serotonin, has direct effects on embryo viability; loss of *TDO* activity likely mediates the embryo phenotype seen in *Hoxa10*-deficient mice.

![Fig. 5. CD3+ T-cell response in mice transfected with pcDNA3.1(+)/TDO plasmid vs pcDNA3.1(−) alone. Mice transfected with TDO plasmid or control were subsequently subjected to a decidual stimulus. Immunohistochemistry for CD3, a marker of mature T cells, was then performed. A: representative section showing CD3+ cells in control mice. Scale bar = 50 μm. B: representative section showing CD3+ cells in TDO-plasmid-treated mice. C: comparison of mean number of CD3+ cells in treatment and control mice. *P < 0.05. Error bars represent SE.](image)

![Fig. 6. Model of cell-specific regulation of *TDO* expression by *HOXA10*. Increasing *HOXA10* levels regulate *TDO* expression in opposing ways in endometrial glands and stroma. In the glands, increased *HOXA10* causes a decrease in *TDO* mRNA. Decreased glandular *TDO* activity increases available tryptophan substrate leading to increased serotonin synthesis, which in turn enhances embryo viability. In the stroma, increased *HOXA10* causes increased *TDO* expression. We hypothesize that increased stromal *TDO* increases tryptophan degradation and inhibits T-lymphocyte proliferation.](image)
Embryo implantation is a complex phenomenon, likely dependent on multiple signaling molecules; here we identify an essential role for TDO in this process. HOX10 differentially regulates endometrial TDO expression in the endometrial epithelial and stromal cells. Glandular and stromal tryptophan degradation by TDO may enhance embryo development through both serotonin signaling and immune suppression. Our working model is demonstrated in Fig. 6. During the window of endometrial receptivity, when Hoxa10 levels peak, downregulation of TDO expression within the endometrial glands leads to decreased tryptophan catabolism. With more available tryptophan substrate, there is increased glandular serotonin. Serotonin signaling through the 5-HT1D and 5-HT7 receptors, tryptophan substrate, there is increased glandular serotonin. leads to decreased tryptophan catabolism. With more available tryptophan substrate, there is increased glandular serotonin. Serotonin signaling through the 5-HT1D and 5-HT7 receptors, tryptophan substrate, there is increased glandular serotonin. leads to decreased tryptophan catabolism. With more available.

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DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the author(s).

REFERENCES


