Deletion of the orphan nuclear receptor COUP-TFII in uterus leads to placental deficiency

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COUP-TFII (NR2F2), chicken ovalbumin upstream promoter–transcription factor II, is an orphan nuclear receptor of the steroid/thyroid hormone receptor superfamily. The *Coup-tfII*-null mutant mice die during the early embryonic development because of angiogenesis and heart defects. To analyze the physiological function of COUP-TFII during organogenesis, we used the cre/loxP system to conditionally inactivate COUP-TFII in the ovary and uterus. Homozygous adult female mutants with specific inactivation of the *Coup-tfII* gene in uterine stromal and smooth muscle cells have severely impaired placental formation, leading to miscarriage at days 10–12 of pregnancy. Deletion of the *Coup-tfII* gene resulted in an increase in trophoblast giant cell differentiation, a reduction of the spongiotrophoblast layer, and an absence of labyrinth formation causing an improper vascularization of the placenta. This study describes an important maternal role of COUP-TFII in regulating the placentation. The endometrial COUP-TFII might modulate the signaling between the uterus and the extraembryonic tissue for the proper formation of the placenta.

placenta | reproduction | trophoblast giant cell

In the steroid/thyroid hormone receptor superfamily (1–3), a majority of newly added members have unknown ligands. Therefore, these receptors are classified as orphan receptors. Like the other members of the nuclear receptor superfamily, the orphan receptors are involved in development, differentiation, and homeostasis processes (4, 5). One of the best characterized orphan nuclear receptor is COUP-TF (chicken ovalbumin upstream promoter–transcription factor), which comprises two members, COUP-TFI/EAR3 (NR2F1) and COUP-TFII/ARP-1 (NR2F2). Because of the absence of ligands, the physiological function of these receptors was dissected by using knockout technology. Null mutant mice for COUP-TFI, which is highly expressed in the central nervous system and peripheral nervous system, display defects in neurogenesis, axon guidance, and arborization (6–9). In contrast, null mutant mice for COUP-TFII, which is highly expressed in mesenchymal cells during organogenesis, show defects in angiogenesis and heart development (10).

Interestingly, the COUP-TFII heterozygote female mice present a reduced fertility, which might be linked to a reduced production of progesterone and a reduced decidual response (11). This finding strengthens the idea of a role of COUP-TFII in reproduction and probably during the implantation process. Previously we generated *lacZ* knockin mice (12), which allowed us to analyze precisely the expression pattern of COUP-TFII during development and organogenesis. At embryonic day 12.5, COUP-TFII is expressed in the mesenchyme surrounding the Müllerian and Wolffian ducts, as well as in the mesenchymal compartment of the undifferentiated gonad (Fig. 1*a*). In the adult female, COUP-TFII expression can be seen in the theca cells of the ovary but not in the granulosa cells and oocytes (Fig. 1*b*). In the reproductive tract, COUP-TFII is expressed in the smooth muscle compartment throughout the oviduct and in the oviductal epithelium of the ostium (Fig. 1*c*). In the uterus, COUP-TFII is strongly expressed in the stroma of the endometrium and the myometrium and very weakly, if at all, in the epithelium of both the lumen and the glands (Fig. 1*d*). Unfortunately, the early embryonic lethality of the COUP-TFII knockout mice precludes us from investigating the function of COUP-TFII in developing organs and in adults. Therefore, we decided to conditionally inactivate the *Coup-tfII* gene using the *Cre* recombinase expressed under the control of the type II receptor for AMH/MIS (anti-Müllerian hormone/Müllerian inhibiting substance), named AMHR2. AMHR2 is specifically expressed in the mesenchyme of the developing Müllerian ducts, in the fetal ovaries (13), and in the testes, oviducts, and uteri postnatally (N. A. Arango and R.R.B., unpublished data). The analysis of phenotypes displayed by the COUP-TFII conditional knockout mice reveals an important role for COUP-TFII during placentation. Mutant female mice have normal reproductive behaviors and can be pregnant but have severely impaired placental formation, leading to miscarriage. This finding provides evidence for the involvement of the uterus in the development of the placenta.

Results and Discussion

To specifically study the physiological function of COUP-TFII in the female reproductive tract, we used the cre/*lox*P system. The type II receptor for AMH/MIS (anti-Müllerian hormone/Müllerian inhibiting substance), or AMHR2, is specifically expressed in the mesenchymal compartment of the developing and adult genital tract (13). Therefore, we crossed *Amhr2*-cre mice (13) with mice carrying a conditional null allele with a *lac*Z reporter of COUP-TFII (12) to generate *Amhr2*$^{+/Cre}$; *Coup-tfII*$^{flox/flox}$ mice. The analysis of β-galactosidase activity in *Amhr2*$^{+/Cre}$; *Coup-tfII*$^{+/flox}$ mice showed that COUP-TFII is inactivated in the mesenchyme of the developing Müllerian ducts, the fetal ovaries (data not shown), and ovaries, oviducts, and uteri postnatally (Fig. 2 *a–h*). Importantly, we noted that COUP-TFII is specifically inactivated in the stromal compartment of the endometrium (Fig. 2 *e* and *f*). During pregnancy, decidual cells strongly expressed COUP-TFII (Fig. 2 *g* and *h*).

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The authors declare no conflict of interest.

Abbreviation: TGC, trophoblast giant cell.

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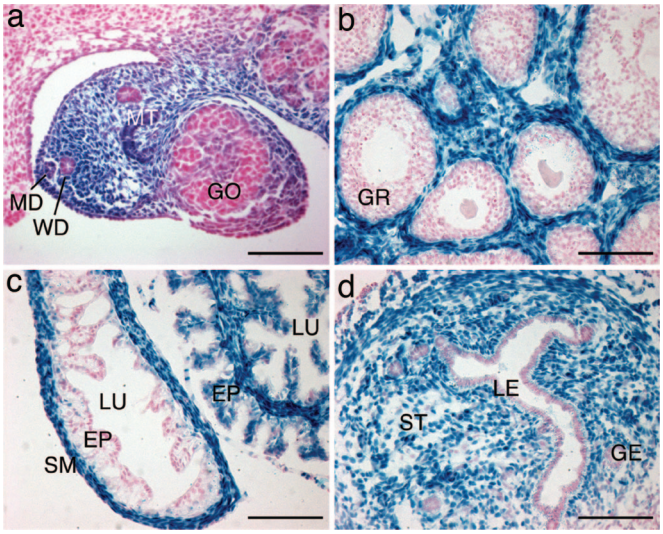

Fig. 1. COUP-TFII expression in the female reproductive tract using *Coup-tfII*$^{+/Z}$ knockin mice. Cryostat sections were incubated with X-Gal to reveal the expression of β-galactosidase. (*a*) COUP-TFII is expressed in the mesenchymal cells surrounding the Müllerian and Wolffian ducts, as well as in the undifferentiated gonad of a 12.5-day embryo. (*b–d*) In a 3-week-old female mouse, COUP-TFII is expressed in the stroma cells of the ovary (*b*), in the smooth muscle cells of the oviduct and the epithelial cells of the ostium (*c*), and in all compartments of the uterus. Note that the staining is weaker in the epithelial cells compared with the mesenchymal cells (*d*). (Scale bars: 100 μm.) EP, epithelium; GE, glandular epithelium; GO, gonad; GR, granulosa cells; LE, luminal epithelium; LU, lumen; MD, Müllerian duct; MT, mesonephric tubules; SM, smooth muscle layer; ST, stroma; WD, Wolffian duct.

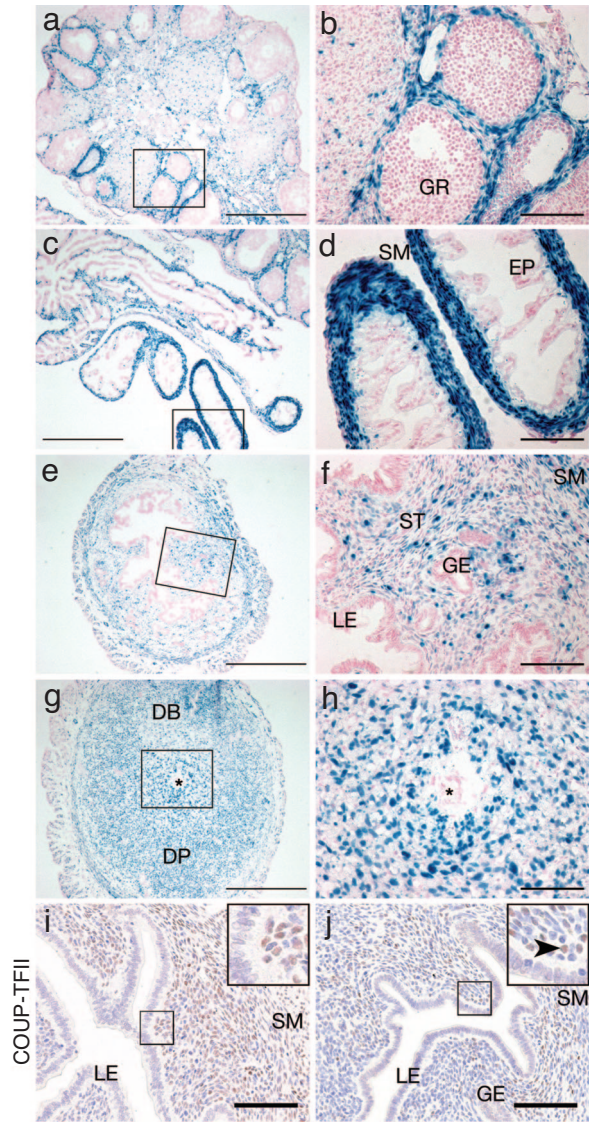

Fig. 2. Analysis of COUP-TFII expression in *Amhr2*$^{+/cre}$; *Coup-tfII*$^{+/flox}$ mature female mice. (*a–f*) In an 8-week-old female mouse at estrus, β-galactosidase expression was observed in the mesenchymal compartment of the ovary (*a* and *b*), the smooth muscle cell layer of the oviduct (*c* and *d*), and the stromal and smooth muscle compartments of the uterus (*e* and *f*). Note that no staining was observed in the luminal and glandular epithelium of the uterus (*f*). (*g* and *h*) At day 5 of pregnancy, decidual cells showed a strong β-galactosidase activity. Asterisk marks a 4.5-day embryo. As observed by immunostaining, COUP-TFII expression is almost undetectable in the epithelial compartment but is highly expressed in the nuclei of both the stromal and myometrial cells in control mice (*i*). However, COUP-TFII is partially expressed in the uterine stroma of the mutant mice (*j*, arrowhead). (*b*, *d*, *f*, and *h*) Higher magnifications of the respective marked areas in *a*, *c*, *e*, and *g*. (Scale bars: 500 μm in *a*, *c*, *e*, and *g* and 100 μm in *b*, *d*, *f*, and *h–j*.) DB, decidua basalis; DP, decidua parietalis; EP, epithelium; GE, glandular epithelium; GR, granulosa cells; LE, luminal epithelium; SM, smooth muscle layer; ST, stroma.

Although *lacZ* staining is a good indicator of the deletion of one copy of the *Coup-tfII* gene, the efficient deletion of both copies cannot be determined by this method. Therefore, we verified the inactivation of the *Coup-tfII* gene by immunohistochemistry using a COUP-TFII antibody (14). As expected, the expression of COUP-TFII is almost undetectable in the epithelium compartment in either control or mutant uteri. Surprisingly, COUP-TFII expression remains detectable in some cells of the stromal compartment, indicating that the *Coup-tfII* gene is only partially deleted in the stromal compartment of the mutant uterus (Fig. 2 *i* and *j*).

As shown in Fig. 3*a*, the conditional mutant mice were not obtained at the expected Mendelian frequency because of embryonic lethality. Ectopic expression of Amhr2-Cre, leading to vascular and heart defects as shown in COUP-TFII-null mice, is probably responsible for this lethality. Indeed, embryos with hemorrhage and edema were observed (data not shown). Moreover, the frequency of mice displaying either *Amhr2*$^{+/+}$; *Coup-tfII*$^{flox/flox}$ or *Amhr2*$^{+/Cre}$; *Coup-tfII*$^{+/flox}$ genotypes is smaller than expected because of the hypomorphic floxed *Coup-tfII* mice. Indeed, *Coup-tfII*$^{flox/flox}$ female mice are hypofertile (data not shown). The anatomical examination of the conditional mutant females showed that the uterine horns are shorter than those from the control females (Fig. 3 *b* and *c*). Histological analysis revealed that the ovary appears normal with the presence of all type of follicles (Fig. 3 *d* and *e*), and the uterus presents a normal endometrium but a disorganized circular smooth muscle layer (Fig. 3 *f* and *g*), as shown by immunohistochemistry using an anti-smooth muscle actin antibody (Fig. 3 *h* and *i*). A similar disorganization of the smooth muscle layer was also observed in conditional COUP-TFII mutant stomach (12), suggesting an important role for COUP-TFII in the maturation or maintenance processes. During pregnancy, the myometrium undergoes spontaneous weak contractions, and during parturition it enters into an active phase of strong contractions. Therefore, in COUP-TFII conditional mutant females, labor might be affected.

We next mated 11 conditional mutant female mice as well as six control female mice with B6SJL-F_1 wild-type males for a period of 35 days (controls) to 1 year. The results showed that only three females gave birth to four litters of one to two pups (total of six). Therefore, we analyzed the conditional mutant females for reproductive behavior and functions. The onset of

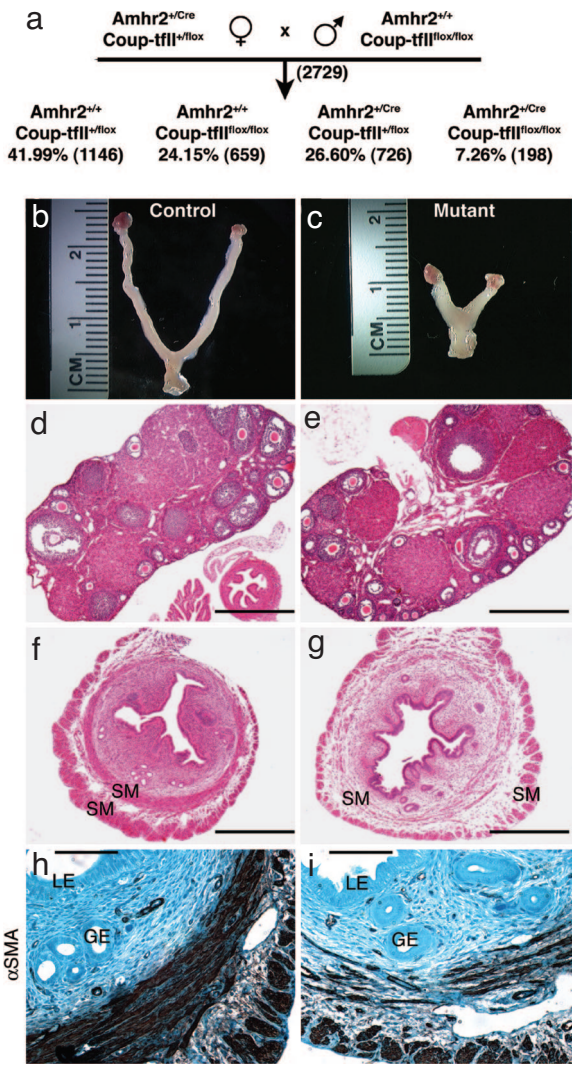

Fig. 3. Shorter uterine horns and disorganized smooth muscle layer in mutant female mice. (*a*) Because of ectopic expression of the cre recombinase, 7.26% (198 mice) of *Amhr2*$^{+/cre}$; *Coup-tfII*$^{flox/flox}$ mice were born. (*b* and *c*) Uterine horns from a mutant female mouse (*c*) appear shorter than those of the control mouse (*Amhr2*$^{+/+}$; *Coup-tfII*$^{+/flox}$) (*b*). (*d* and *e*) Section through the ovary did not show any differences between control (*Amhr2*$^{+/Cre}$; *Coup-tfII*$^{+/flox}$) mice (*d*) and mutant mice (*e*). (*f–i*) In uterus, the smooth muscle layer appears disorganized in mutant mice (*g*) as revealed by anti-smooth muscle actin immunostaining (*i*). (Scale bars: 500 μm in *d–g* and 100 μm in *h* and *i*.) GE, glandular epithelium; LE, luminal epithelium; SM, smooth muscle layer.

mating as assessed by the time of appearance of the first copulatory plug did not present any significant differences (data not shown). The analysis of vaginal smears during a period of 4 weeks showed that mutant mice present regular estrus cycles as compared with control mice (data not shown). During the mating with wild-type males, we observed that the mutant female mice were plugged regularly every 12–14 days but had vaginal bleeding 10–12 days after the presence of a copulatory plug. As expected the control female mice were plugged and had normal pregnancies. These data along with a normal pseudo pregnancy test suggest that the production of endogenous estradiol is not affected in mutant mice (15, 16).

Because COUP-TFII is deleted in the mesenchymal cells of both the ovaries and uterus, we asked whether the ovaries and/or the uterus are responsible for this failure to maintain the pregnancy. We performed ovary transfer from conditional mutant females to wild-type females and vice versa. As presented in Table 1, the wild-type female mice carrying *Amhr2*$^{+/Cre}$; *Coup-tfII*$^{flox/flox}$ ovaries had normal litter sizes with pups derived from the donor ovary. Therefore, the mutant ovary is fully functional. On the other hand, *Amhr2*$^{+/Cre}$; *Coup-tfII*$^{flox/flox}$ female mice receiving a wild-type ovary were unable to produce pups as observed before ovary transfer (Table 1). This result was not due to an experimental failure, because control mice gave birth to wild-type pups. Moreover, histological analysis confirmed the presence of normal ovaries (data not shown). Altogether these data indicate that the mutant ovary is functional and the uterus is responsible for the reproductive defect.

Table 1. Ovary transfer

Ovary genotype	Uterus genotype	*n*
Amhr2$^{+/+}$; *Coup-tfII*$^{+/flox}$	B6129-F$_1$	6/6
Amhr2$^{+/+}$; *Coup-tfII*$^{flox/flox}$	B6129-F$_1$	5/5
Amhr2$^{+/Cre}$; *Coup-tfII*$^{+/flox}$	B6129-F$_1$	3/3
Amhr2$^{+/Cre}$; *Coup-tfII*$^{flox/flox}$	B6129-F$_1$	8/9
B6129-F$_1$	*Amhr2*$^{+/+}$; *Coup-tfII*$^{+/flox}$	2/2
B6129-F$_1$	*Amhr2*$^{+/+}$; *Coup-tfII*$^{flox/flox}$	1/2
B6129-F$_1$	*Amhr2*$^{+/Cre}$; *Coup-tfII*$^{+/flox}$	1/1
B6129-F$_1$	*Amhr2*$^{+/Cre}$; *Coup-tfII*$^{flox/flox}$	1*/6

Amhr2$^{x/x}$; *Coup-tfII*$^{x/x}$ ovaries were transferred into two B6129-F$_1$ female mice (counted as one transfer), and *Amhr2*$^{x/x}$; *Coup-tfII*$^{x/x}$ female mice without their own ovaries received one or two B6129-F$_1$ ovaries. Mice were mated with B6SJL-F$_1$ male mice for a period of 1–6 months. *n* corresponds to the number of females with litters coming from the transferred ovary per total transferred female mice.

*One *Amhr2*$^{+/Cre}$; *Coup-tfII*$^{flox/flox}$ with wild-type ovaries gave birth to one pup in a 6-month period.

Next we examined whether embryo implantation occurs in the conditional mutant uterus. We mated 38 control females and 48 mutant females with wild-type males and checked for the presence of implantation sites and embryos at different time points after the presence of a copulatory plug. Among these mutant females, 64.6% were pregnant (63.2% for the control females) and presented a significant reduced number of implantation sites per pregnant female (5.3 ± 2.4 and $n = 31$ for mutants versus 8.8 ± 2.2 and $n = 24$ for the control females; $P < 0.000001$). Similarly, in *Coup-tfII*$^{+/-}$ mice a reduced fertility was observed with a significant decreased number of pups per litter (11). Interestingly, our conditional mutant mice *PR*$^{+/Cre}$; *Coup-tfII*$^{flox/flox}$, which show an efficient deletion of the *Coup-tfII* gene in the stromal compartment, are infertile and exhibit defects in implantation (I.K., D.-K. Lee, F.G.P., J. Jeong, K. Lee, J. P. Lydon, F.J.D., M.-J.T., and S.Y.T., unpublished data). This observation suggests that the inefficient deletion of *Coup-tfII* in the uterine stroma of *Amhr2*$^{+/Cre}$; *Coup-tfII*$^{flox/flox}$ leads to a hypomorphic phenotype. Analysis of mutant females at various stages of gestation allowed us to determine that embryonic development occurs normally until embryonic day 7.5 (data not shown). At day 9 of pregnancy, most embryos were reabsorbed and a few were growth-retarded (Fig. 4 *a–c*). Progesterone levels were measured at different times during pregnancy, and no significant variation was observed between the control and mutant mice (Fig. 4 *d* and *e*). Therefore, the hormonal status does not appear responsible for the defect in mutant mice. Histological analyses at different stages of pregnancy reveal a defect in placenta formation at day 9. Because embryos are not mutants, the uterus is more likely responsible for the placental failure. Moreover, COUP-TFII-null mutant embryos (10) do not affect the placenta development (F. A. Pereira, personal communication). The placentas of conditional mutant mice developed normally until 8 days of pregnancy (Fig. 4 *f–i*). As in the control uterus, the ectoplacental cone and the extraembryonic

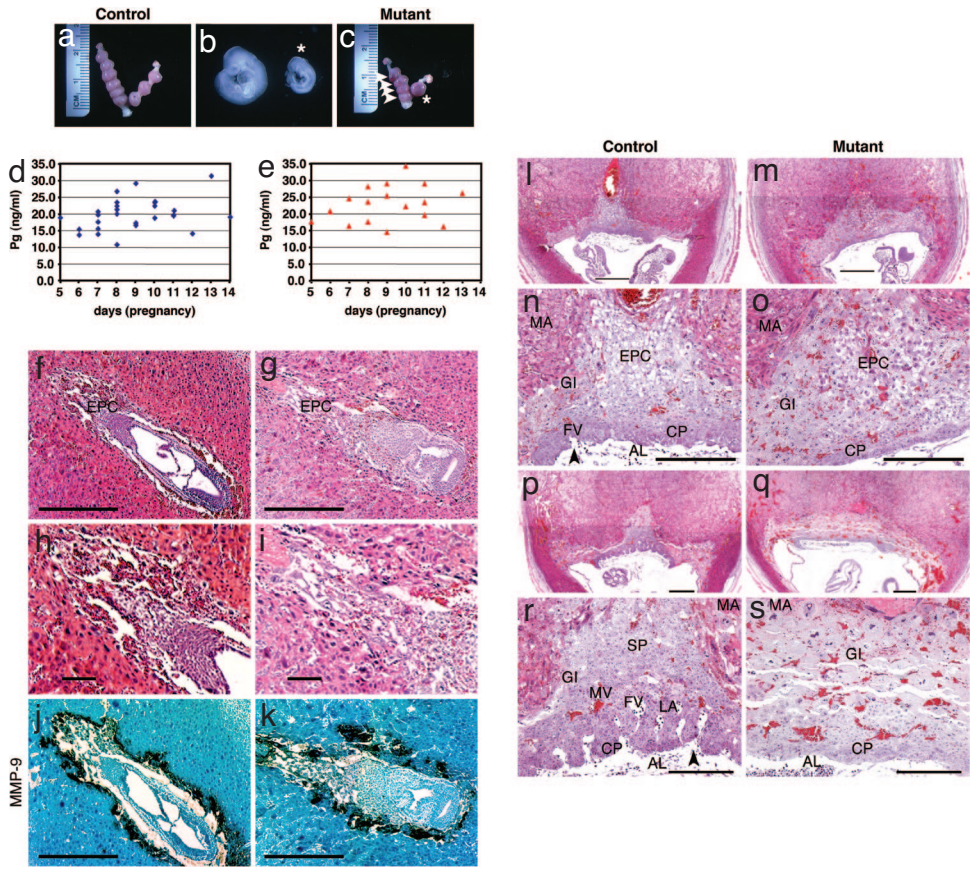

Fig. 4. Placental defect in adult female mice deleted of *Coup-tfII*. (*a–c*) At day 9 of pregnancy, the control uterus (*Amhr2*$^{+/+}$; *Coup-tfII*$^{flox/flox}$) (*a*) contains a normal number of well developed embryos (*b*, left). *Coup-tfII* mutant uterus presents a reduced number of decidual sites (*c*) with the absence of embryo (arrowhead) or the presence of growth-retarded embryo (asterisk in *b* and *c*). (*d* and *e*) The defect in maintaining a normal pregnancy in *Coup-tfII* mutant mice is not due to a reduced production of progesterone. (*f–i*) At day 8 of pregnancy, the development of the embryonic and extraembryonic structures appears normal in both control (*f* and *h*) and mutant (*g* and *i*) mice. (*h* and *i*) Higher magnifications of *f* and *g*, respectively. (*j* and *k*) The production of matrix metalloproteinase 9 by trophoblast cells appears normal in control (*j*) and mutant (*k*) uteri at day 8 of pregnancy. (*l–o*) Control (*l* and *n*) and mutant (*m* and *o*) uteri at day 9 of pregnancy. The number of secondary giant cells is increased in mutant uterus, and the formation of fetal blood vessels from the allantois is absent. The arrowhead in *n* points to a fetal blood vessel in control uterus. (*n* and *o*) Higher magnifications of *l* and *m*, respectively. (*p–s*) Control (*p* and *r*) and mutant (*q* and *s*) uteri at day 10 of pregnancy. Secondary giant cells form multiple layers in mutant uterus, and the formation of fetal blood vessels is clearly absent. The arrowhead in *r* points to a fetal blood vessel in control uterus. (*r* and *s*) Higher magnifications of *p* and *q*, respectively. Control in *f*, *h*, *j*, *l*, *n*, *p*, and *r* is *Amhr2*$^{+/+}$; *Coup-tfII*$^{+/flox}$. (Scale bars: 500 μm in *f*, *g*, *j–m*, *p*, and *q* and 100 μm in *h*, *i*, *n*, *o*, *r*, and *s*.) AL, allantois; CP, chorionic plate; EPC, ectoplacental cone; FV, fetal blood vessel; GI, giant cells; LA, labyrinthine trophoblast; MA, maternal deciduum; MV, maternal vein; SP, spongiotrophoblast cells.

ectoderm develop normally in mutant uterus, and no alteration in the secretion of matrix metalloproteinase 9 by trophoblast giant cells (TGC) was observed (Fig. 4 *j* and *k*). As development proceeds, the allantois makes contact with the chorion (17). At day 9 of pregnancy, the development of the placenta is abnormal and hemorrhages are more frequent. Secondary TGC differentiation appears to increase because more cells are present (Fig. 4 *l–o*). By day 10, the placental deficiency is clearly evident. Many layers of secondary TGC are present in mutant animals compared with control animals and the labyrinth fails to develop (Fig. 4 *p–s*). The absence of a placental vascular network results from a failure of the fetal blood vessels to grow from the allantois. Expression of several markers of trophoblast differentiation was then examined by RNA *in situ* hybridization at day 10 of pregnancy (Fig. 5). Placental lactogen 1 (18), a marker of TGC, was detected in multiple layers in mutant animals (Fig. 5 *c* and *d*). tpbp/4311 (19), a marker for the ectoplacental cone and the spongiotrophoblast, is expressed in few scattered cells in mutant female mice compared with the control (Fig. 5 *e* and *f*). These data suggest an increase in TGC differentiation and an altered differentiation of ectoplacental cone cells into spongiotrophoblast cells. TUNEL and phosphohistone H3 immunohistochemistry assays did not show any differences in apoptosis or proliferation, respectively, between control and mutant mice at this stage (data not shown).

The orphan nuclear receptor COUP-TFII in the uterine stroma plays an essential role in the normal placental formation. The deletion of *Coup-tfII* from the mesenchymal cells of the endometrium and the myometrium results in the differentiation of the TGC, the reduction of the spongiotrophoblast cell number, hemorrhages, embryonic death, and miscarriages. Many genes expressed in the trophectoderm layer are known to be involved in the differentiation of TGC from the trophoblast stem cells, but little is known about the involvement of the uterus in placental development (17, 20, 21). Interestingly, the phenotype observed in *Amhr2*$^{+/Cre}$; *Coup-tfII*$^{flox/flox}$ female mice resembles those observed in ERRβ-null mice (22). Because ERRβ is expressed in a subset of chorionic cells, COUP-TFII expressed in the endometrial cells might alter its expression through activation of signaling molecules. FGF/Nodal signaling suppresses TGC differentiation (17, 20), so we can hypothesize that COUP-TFII could indirectly activate this signaling pathway. In retinoic acid-treated mice an increase in TGC differentiation was observed (23), suggesting that COUP-TFII might interfere with that signaling pathway. Finally, although no clear role

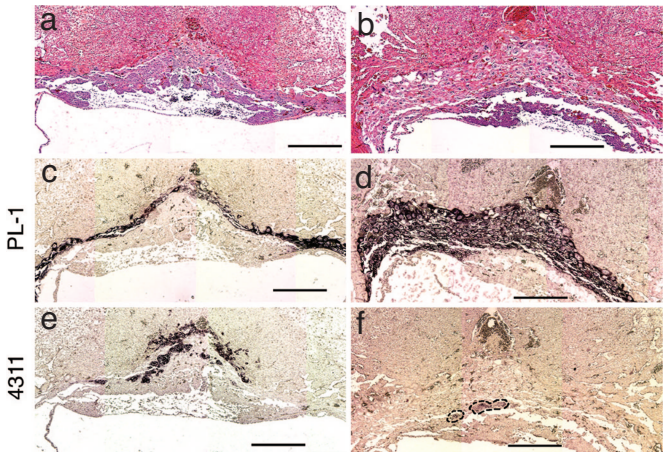

Fig. 5. Expression of trophoblast cell-type-specific markers in the placenta at day 10 of pregnancy. Shown is hematoxylin and eosin staining of control (*Amhr2*$^{+/+}$; *Coup-tfII*$^{+/flox}$) placenta (*a*) compared with the *Coup-tfII* mutant placenta (*b*). *In situ* hybridization with the TGC marker placental lactogen 1 (PL-1) (*c* and *d*) or the spongiotrophoblast marker 4311 (*e* and *f*) showed a marked increase in the number of TGC and a decrease in the spongiotrophoblast cell number. In *f*, 4311-positive cells are circled with a dashed line. (Scale bars: 500 μm.)

in placentation has been established for *Socs3*, *Socs3*-null mice have an increased TGC differentiation, which could result from an increased expression of maternal leukemia inhibitory factor (LIF) (24). Therefore, it is possible that COUP-TFII might act as a negative regulator of the LIF signaling. So far, *in situ* hybridization or immunohistochemistry analyses did not show significant differences to define whether Fgf4 or LIF are involved in the TGC differentiation in COUP-TFII conditional mutant mice (data not shown). This suggests that COUP-TFII function in the placenta development is perhaps more complicated.

Approximately two-thirds of early pregnancy loss presents a defective placentation, and ≈50% of cases of women with recurrent miscarriage remain unexplained (25). The causes include luteal phase defect and endometrial receptivity, immunological factors, environmental factors, placental microthrombosis and necrosis, and fetal chromosomal anomalies. Because COUP-TFII is an orphan nuclear receptor, it is a putative target for environmental agents, which could affect the placental formation. Most of our understanding in placental development comes from the study of gene deletions in extraembryonic ectoderm (17, 20, 21). Here we describe an important maternal function in placental formation and identify a factor involved in TGC differentiation, COUP-TFII, which may be a new target for therapeutic drugs in human reproduction.

Materials and Methods

Animals. *Coup-tfII*/*lacZ* knockin mice and floxed *Coup-tfII* mice were generated in our laboratory and have been previously described (12). *Amhr2*-cre embryos were transferred from the M. D. Anderson Cancer Center facility to the Baylor College of Medicine facility. All wild-type mice (B6SJL-F_1 male and B6129-F_1 female) were purchased from Taconic Farms (Germantown, NY).

Ovary Transfer. We followed the procedure described previously (26). One ovary from a 6-week-old B6129-F_1 female mouse was cut in half, and both halves were transferred to an *Amhr2*$^{+/cre}$; *Coup-tfII*$^{flox/flox}$ mouse. Moreover, the mutant ovaries were transferred to two 6-week-old B6129-F_1 female mice. The same procedure was accomplished by using the control mice *Amhr2*$^{+/+}$; *Coup-tfII*$^{+/flox}$, *Amhr2*$^{+/+}$; *Coup-tfII*$^{flox/flox}$, or *Amhr2*$^{+/cre}$; *Coup-tfII*$^{+/flox}$. Two weeks after surgery, the mice were mated with B6SJL-F_1 male mice for a period of 2–6 months. Each litter was genotyped to characterize the origin of the pups. When two litters came from the transferred ovary, the mating was stopped and the experiment was considered a success.

***LacZ* Staining.** A previous procedure (27) was followed except that the reproductive tracts were fixed for 2 h, the tissues were sectioned at 7 μm, and the staining time was 4.5 h.

Histological Analysis and *in Situ* Hybridization. Reproductive tracts were fixed in 4% paraformaldehyde for 18 h. After dehydration in ethanol and embedding, the tissues were sectioned at 5 μm and stained with hematoxylin and eosin. *In situ* hybridization using a digoxigenin-labeled probe was performed according to Roche (Indianapolis, IN) and a previous protocol with few modifications (28). After the steps of prehybridization, hybridization, and wash, the sections were prepared for immunodetection. Sections were incubated twice for 10 min in 1× washing buffer, 10 min in maleic buffer, 30 min in 1× blocking buffer, 2 h with an anti-DIG-AP antibody in 1× blocking buffer, twice for 10 min in 1× washing buffer, and 10 min in 1× detection buffer. The color was detected by incubating the sections in a NBT/BCIP solution in 1× detection buffer. The reaction was stopped with TE (pH 8.0), and the sections were counterstained with methyl green (Vector Laboratories, Burlingame, CA).

Immunohistochemistry. Sections (5 μm) were hydrated, and then the antigen was unmasked. The endogenous peroxidase activity was quenched, and a blocking solution (MOM kit; Vector Laboratories) was applied. Sections were incubated overnight at 4°C with a mouse monoclonal anti-smooth muscle actin antibody (clone 1A4, 1:2,000; Sigma, St. Louis, MO) or a mouse monoclonal anti-COUP-TFII (1:1,000; Perseus Proteomics, Tokyo, Japan). After several washes with PBS, the secondary horse anti-mouse antibody (Vector Laboratories) was added onto the sections. The signal was amplified by using the ABC kit (Vector Laboratories), the NovaRed substrate for peroxidase (Vector Laboratories) was applied, and the sections were counterstained with methyl green or hematoxylin.

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1. Beato M (1989) *Cell* 56:335–344.
2. Laudet V (1997) *J Mol Endocrinol* 19:207–226.
3. Mangelsdorf DJ, Thummel C, Beato M, Herrlich P, Schutz G, Umesono K, Blumberg B, Kastner P, Mark M, Chambon P, Evans RM (1995) *Cell* 83:835–839.
4. Tsai MJ, O'Malley BW (1994) *Annu Rev Biochem* 63:451–486.
5. Tsai SY, Tsai MJ (1997) *Endocr Rev* 18:229–240.
6. Qiu Y, Pereira FA, DeMayo FJ, Lydon JP, Tsai SY, Tsai MJ (1997) *Genes Dev* 11:1925–1937.

7. Yamaguchi H, Zhou C, Lin SC, Durand B, Tsai SY, Tsai MJ (2004) *Dev Biol* 266:238–251.
8. Zhou C, Qiu Y, Pereira FA, Crair MC, Tsai SY, Tsai MJ (1999) *Neuron* 24:847–859.
9. Zhou C, Tsai SY, Tsai MJ (2001) *Genes Dev* 15:2054–2059.
10. Pereira FA, Qiu Y, Zhou G, Tsai MJ, Tsai SY (1999) *Genes Dev* 13:1037–1049.
11. Takamoto N, Kurihara I, Lee K, Demayo FJ, Tsai MJ, Tsai SY (2005) *Mol Endocrinol* 19:2299–2308.
12. Takamoto N, You LR, Moses K, Chiang C, Zimmer WE, Schwartz RJ, DeMayo FJ, Tsai MJ, Tsai SY (2005) *Development (Cambridge, UK)* 132:2179–2189.
13. Jamin SP, Arango NA, Mishina Y, Hanks MC, Behringer RR (2002) *Nat Genet* 32:408–410.
14. Lee CT, Li L, Takamoto N, Martin JF, Demayo FJ, Tsai MJ, Tsai SY (2004) *Mol Cell Biol* 24:10835–10843.
15. Wuttke W (1976) *Rev Physiol Biochem Pharmacol* 76:59–101.
16. Yi SE, LaPolt PS, Yoon BS, Chen JY, Lu JK, Lyons KM (2001) *Proc Natl Acad Sci USA* 98:7994–7999.
17. Rossant J, Cross JC (2001) *Nat Rev Genet* 2:538–548.
18. Colosi P, Swiergiel JJ, Wilder EL, Oviedo A, Linzer DI (1988) *Mol Endocrinol* 2:579–586.
19. Lescisin KR, Varmuza S, Rossant J (1988) *Genes Dev* 2:1639–1646.
20. Cross JC (2005) *Placenta* 26(Suppl A):S3–S9.
21. Simmons DG, Cross JC (2005) *Dev Biol* 284:12–24.
22. Luo J, Sladek R, Bader JA, Matthyssen A, Rossant J, Giguere V (1997) *Nature* 388:778–782.
23. Yan J, Tanaka S, Oda M, Makino T, Ohgane J, Shiota K (2001) *Dev Biol* 235:422–432.
24. Takahashi Y, Carpino N, Cross JC, Torres M, Parganas E, Ihle JN (2003) *EMBO J* 22:372–384.
25. Jauniaux E, Burton GJ (2005) *Placenta* 26:114–123.
26. Nagy A, Gertsenstein M, Vintersten K, Behringer R (2003) *Manipulating the Mouse Embryo: A Laboratory Manual* (Cold Spring Harbor Lab Press, Cold Spring Harbor, NY), pp 565–598.
27. Rivera-Perez JA, Wakamiya M, Behringer RR (1999) *Development (Cambridge, UK)* 126:3811–3821.
28. Komminoth P (1992) *Diagn Mol Pathol* 1:142–150.