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Foxa2 Is Essential for Mouse Endometrial Gland Development and Fertility[1]

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ABSTRACT

During embryonic development, *Foxa2* is required for the formation of the node and notochord, and ablation of this gene results in defects in gastrulation, neural tube patterning, and gut morphogenesis. *Foxa2* has been shown to be expressed specifically in the glandular epithelium of the murine uterus. To study the uterine function of *Foxa2,* this gene was conditionally ablated in the mouse uterus by crossing mice with floxed *Foxa2* alleles, $Foxa2^{loxP/loxP}$, with the Pgr^{cre} mouse model. $Pgr^{cre/+}$ $Foxa2^{loxP/loxP}$ mice showed significantly reduced fertility. Analysis of the uterus on Day 5.5 of pregnancy showed disrupted blastocyst implantation. $Pgr^{cre/+}$ $Foxa2^{loxP/loxP}$ mice also showed a severe impairment of the uterus to respond to the artificial induction of the decidual response. Morphological examination of the uteri of these mice showed a severe reduction in the number of endometrial glands. The loss of endometrial glands resulted in the reduction of leukemia inhibitory factor (*Lif*) expression. The lack of a decidual response could be partially rescued by an intrauterine injection of LIF before the initiation of the decidual response. This analysis demonstrates that *Foxa2* regulates endometrial gland development and that mice with a loss of endometrial glands cannot support implantation in part due to the loss of LIF, which is a requisite for fertility in the mouse.

decidua, female reproductive tract, Foxa2*, gland development, implantation, uterus*

INTRODUCTION

The uterus consists of heterogeneous cell types that undergo dynamic changes to support embryo development and implantation. The process of implantation consists of attachment and invasion of the uterine luminal epithelium. Successful blastocyst implantation requires the rapid remodeling of the uterine stromal cells in a process termed *decidualization* [1]. Decidualization is a process characterized by morphological and functional changes in the uterine stromal cells that are characterized by endometrial stromal proliferation and differentiation into large epithelioid decidual cells. This process is critical for the establishment of a fetal-maternal interface during implantation.

The uterine luminal epithelium is the initial site of blastocyst attachment, whereas the glandular epithelium is thought to be the principal source of uterine secretions that are required for the establishment and maintenance of pregnancy [2]. The absence of glandular epithelium and the reduced luminal epithelium in the ovine uterine gland ewe knockout model resulted in a reduction of conceptus survival, supporting a fundamental role for the glandular epithelium and their secretions during early pregnancy [3]. The precise difference between luminal and glandular epithelium is not well characterized; however, it is known that they differ biochemically [4, 5]. In the mouse, the endometrial glands, in response to estrogen, express the cytokine leukemia inhibitory factor, *Lif*, which is critical for blastocyst implantation. Although the expression of many genes, including steroid hormone receptors, cytokines, growth factors, and several developmental factors, has been implicated in this process, direct in vivo evidence of gene function has been limited. This is largely due to the fact that the ablation of many of the genes implicated in this process results in early lethality or other developmental consequences that preclude further study. Herein, we investigate the role of a developmentally important gene, *Foxa2*, in the regulation of adult mouse uterine function.

Foxa transcription factors comprise a subfamily of forkhead transcription factors that contain high homology in the winged helix DNA-binding domain [6]. The *Foxa* family has been found to have important roles in multiple stages of mammalian life, beginning with early development, continuing throughout organogenesis, and during adulthood in metabolism and homeostasis [6]. The *Foxa* family includes *Foxa1*, *Foxa2*, and *Foxa3* (previously known as *Hnf3α*, *Hnf3β*, and *Hnf3γ*, respectively) [7]. *Foxa1* and *Foxa2* cooperate to establish competence in the foregut endoderm and are required for normal development of endoderm-derived organs such as the liver, pancreas, lung, and prostate [7–10]. *Foxa1* and *Foxa2* are not expressed in the ovary, and *Foxa2* is only expressed in the glandular epithelium of the uterus [11]. $Foxa2^{-/-}$ mice die at Embryonic Day (E) 10 or 11 due to severe defects in the node, notochord, neural tube, and gut tube formation, making it difficult to investigate the effect of *Foxa2* ablation in the uterus [12]. Herein, we generated a mouse model in which *Foxa2* is conditionally ablated in the uterus to investigate the role of *Foxa2* in endometrial function. The ablation of *Foxa2* resulted in defects in gland formation, decidualization, and fertility. These analyses suggest that *Foxa2* has an important role in uterine function and implantation.

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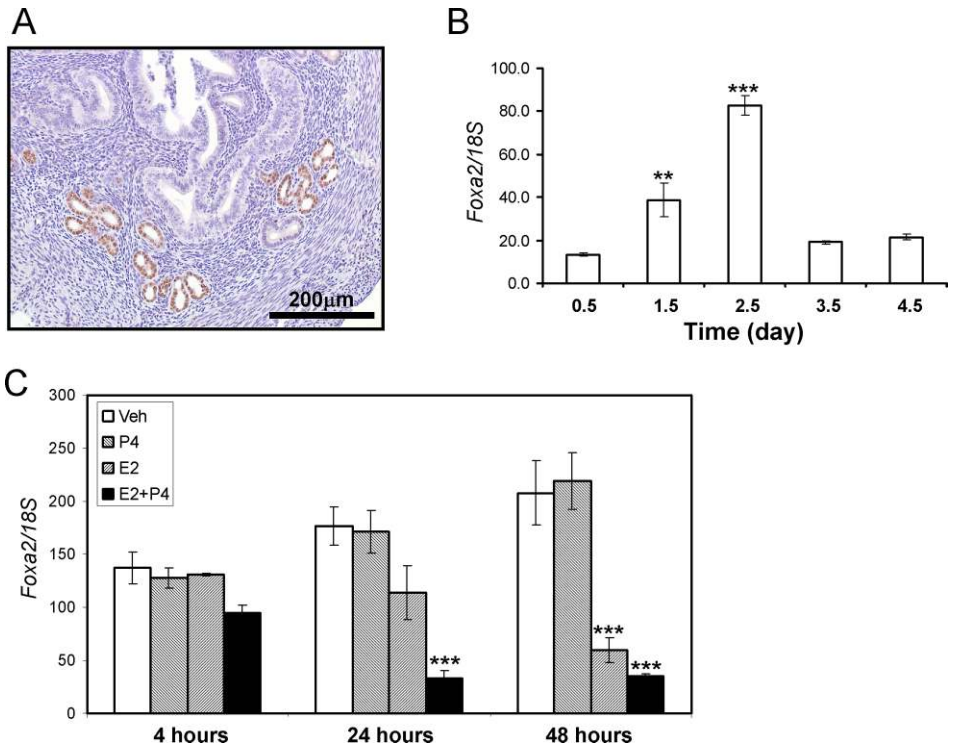

FIG. 1. The expression pattern of *Foxa2* in the murine uterus. **A**) Localization of FOXA2 in the murine uterus. Eight-week-old female mice were killed. Portions of the uterus were fixed in 4% paraformaldehyde, and immunohistochemistry for FOXA2 was performed. Nuclei are lightly counterstained with hematoxylin. **B**) The expression pattern of *Foxa2* by real-time RT-PCR in pseudopregnancy. Total RNA used for the RT-PCR assays was prepared from the pseudopregnant uteri. The expression level of *Foxa2* was measured from Day 0.5 to Day 4.5 in the pseudopregnant uterus. **C**) The expression pattern of *Foxa2* by E2 and P4 in the uterus. Total RNA used for the RT-PCR assays was prepared from wild-type mice that were treated with P4, E2, E2 plus P4, or vehicle (sesame oil) for 4, 24, and 48 h. The results represent the mean ± SEM of three independent RNA sets. $^{**}P < 0.01$; $^{***}P < 0.001$.

MATERIALS AND METHODS

Animals and Tissue Collection

Mice were maintained in the designated animal care facility at Baylor College of Medicine according to the institutional guidelines for the care and use of laboratory animals. Pregnancy samples were obtained by the mating of wild-type C57BL/6 mice, and the day that a vaginal plug was observed was considered Day 0.5 of pregnancy. Uterine tissues were flash frozen at the time of dissection or fixed with 4% paraformaldehyde (vol/vol) and paraffin embedded.

The hormonally induced decidual response has been previously described [13]. Briefly, ovariectomized 129P2/OlaHsd-*Foxa2*^{tm1Khk} (also known as *Foxa2*$^{loxP/loxP}$ [control]) and 129P2/OlaHsd-*Foxa2*^{tm1Khk} × B6;129-*Pgr*$^{tm2(cre)Lyd}$ (also known as *Pgr*$^{cre/+}$ *Foxa2*$^{loxP/loxP}$ [mutant]) mice were treated with three daily injections of 100 ng of estradiol-17 (E2) per mouse (n = 3 per genotype). After 2 days of rest, mice were then treated with three daily injections of 1 mg of progesterone (P4) and 6.7 ng of E2 per mouse by s.c. injection. The uteri were mechanically stimulated by a scratch of the antimesometrial lumen 6 h after the last hormone injection. Mice were given daily s.c. injections of 1 mg of P4 and 6.7 ng of E2 per mouse for 5 days after stimulation to observe the induction of the uterine decidual response.

Immunohistochemistry

Uteri were fixed overnight in 4% paraformaldehyde (vol/vol), followed by thorough washing in 70% ethanol, and tissues were processed, embedded in paraffin, and sectioned. Uterine sections from paraffin-embedded tissue were cut at 5 μm and mounted on silane-coated slides, deparaffinized, and rehydrated in a graded alcohol series. Sections were preincubated with 10% normal goat serum in PBS (pH 7.5) and then incubated with anti-FOXA2 antibodies in 10% normal serum in PBS (pH 7.5). On the following day, sections were washed in PBS and incubated with biotinylated secondary antibody (5 μl/ml; Vector Laboratories, Burlingame, CA) for 1 h at room temperature. Immunoreactivity was detected using the Vectastain Elite ABC kit (Vector Laboratories); the immunoreactivity was visualized as brown staining.

RNA Isolation and Quantitative Real-Time RT-PCR

Total RNA was extracted from uterine tissues using the Qiagen (Valencia, CA) RNeasy total RNA isolation kit. To investigate the effect of *Foxa2* on gene expression changes in the uterus, quantitative real-time RT-PCR analysis was conducted on RNA isolated from mice. Expression levels of *Foxa2* and *Lif* were measured by real-time RT-PCR TaqMan analysis using the ABI Prism 7700 Sequence Detector System according to manufacturer's instructions (Applied Biosystems, Foster City, CA). The RT-PCR was performed using One-Step RT-PCR Universal Master Mix reagent (Applied Biosystems) according to the manufacturer's instructions. Real time probes and primers for *Foxa2*, *Lif*, *Ihh*, *Hoxa10*, *Wnt5a*, *Wnt7a*, *Ptch1*, *Hoxa11*, *Wnt4*, *Bmp2*, *Cebpb*, and *Hbegf* were purchased from Applied Biosystems. Standard curves were generated by serial dilution of a preparation of total RNA isolated from whole mouse uterus. All real-time RT-PCR results were normalized against *18S* RNA or Cdhl using ABI rRNA control reagents (Applied Biosystems).

Rescue of the Decidualization Defect by Recombinant LIF

Foxa2 rescue experiments of proliferation and vascularization were accomplished using recombinant LIF. Ovariectomized mice were treated with three daily injections of 100 ng of E2 per mouse. After 2 days of rest, mice were each treated with daily s.c. injections of 1 mg of P4 and 6.7 ng of E2 per mouse. Six hours after the second injection, 10 μl of 10% bovine serum albumin (BSA) or 10 μl of LIF (100 ng/μl in 10% BSA) was injected intraluminally into one horn of the uterus. Both horns were traumatized by a needle scratch on the antimesometrial lumen 6 h after the third injection. Mice continued to receive daily s.c. injections of 1 mg of P4 and 6.7 ng of E2 per mouse each day following the trauma. Three days after the trauma, the mice were killed, and uteri were collected.

Statistical Analysis

All experimental data are presented as the mean ± SEM. The statistical significance of differences was analyzed using one-way ANOVA, followed by Tukey post hoc multiple range test or Student *t*-test using the Instat software package from GraphPad (San Diego, CA).

RESULTS

Expression of FOXA2 During Early Pregnancy

The spatial expression profile of FOXA2 in the uterus was determined by immunohistochemistry (Fig. 1A). FOXA2 is expressed in the glandular epithelium but not the luminal epithelium, stroma, or myometrium of the mouse uterus. Next, the temporal expression of *Foxa2* was investigated in the mouse uterus during early pregnancy by performing real-time RT-PCR on RNA isolated from the uteri of pseudopregnant

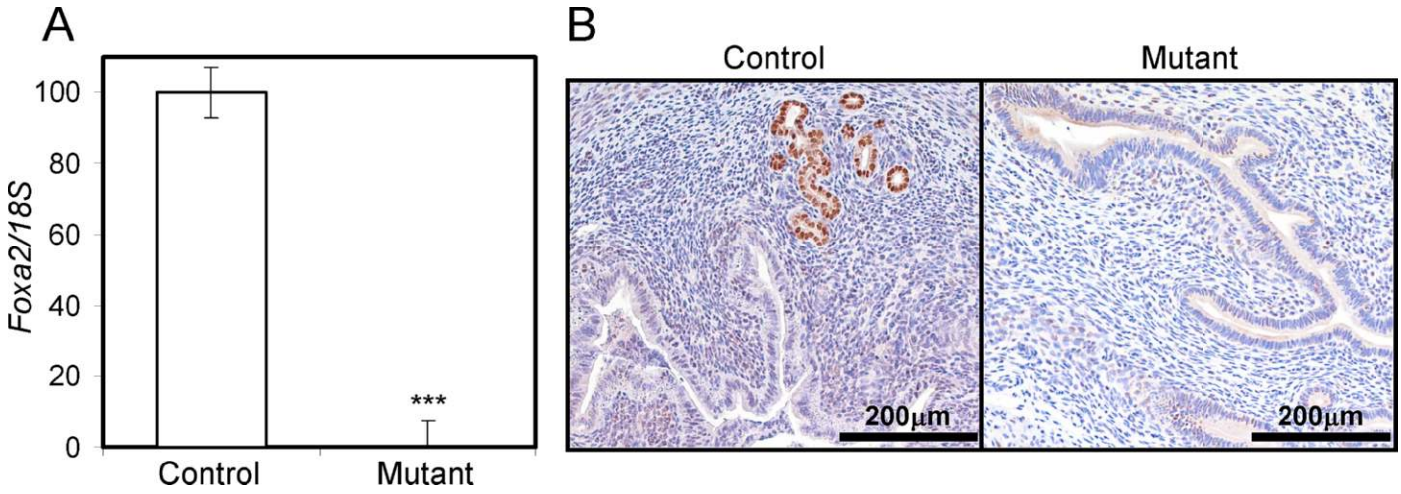

FIG. 2. Analysis of *Foxa2* conditionally ablated in the murine uterus. The expression level of *Foxa2* was measured in the uteri by real-time RT-PCR (**A**) and immunohistochemistry (**B**). Eight-week-old control and mutant mice were killed at Day 2.5. Total RNA used for the RT-PCR assays was prepared from the uteri. The results represent the mean ± SEM of three independent RNA sets. ***$P < 0.001$.

mice. As shown in Figure 1B, *Foxa2* expression gradually increases until its peak at Day 2.5 of pseudopregnancy and then sharply decreases throughout the remainder of pseudopregnancy. Because blastocyst implantation in the mouse uterus occurs at Day 4.5, the expression of *Foxa2* during the preimplantation period implicates it as a potential regulator of implantation.

Steroid Hormone Regulation of Foxa2

Given the temporal regulation of *Foxa2* during the preimplantation period, the effect of ovarian steroid hormone regulation on *Foxa2* expression was examined. Ovariectomized wild-type female mice were injected daily with one of the following hormones: vehicle (sesame oil), P4 (1 mg per mouse), E2 (0.1 μg per mouse), or E2 plus P4 (1 mg of P4 and 0.1 μg of E2 per mouse). Mice were killed at 4, 24, and 40 h after the initial hormone treatment (n = 3 mice per treatment) and subjected to real-time PCR to characterize the effect of steroid hormone treatment on the relative expression of *Foxa2*. As shown in Figure 1C, P4 alone had no effect on *Foxa2* expression. However, E2 repressed *Foxa2* mRNA expression at 48 h but not 4 and 24 h after treatment. The repression of *Foxa2* mRNA by E2 was accelerated by cotreatment with P4, as shown by the significant decrease in expression after 24 h of E2 plus P4 treatment. These results suggest that the steroid hormone treatment inhibits the expression of *Foxa2* in the uterus.

Conditional Ablation of Foxa2 *in the Uterus*

Ablation of *Foxa2* leads to embryonic lethality because of severe defects in gastrulation, neural tube patterning, and gut morphogenesis [12]. To further investigate the role of *Foxa2* in the adult uterus, conditional ablation of *Foxa2* in the uterus was conducted to circumvent the embryonic lethal phenotype. A line of mice in which cre recombinase is under the control of the P4 receptor promoter ($Pgr^{tm2(cre)Lyd}$) was crossed with a mouse line containing the floxed *Foxa2* allele ($Foxa2^{tm1Khk}$) to provide a tissue-specific knockout of *Foxa2* in all *Pgr*-expressing cells, which includes all compartments of the uterus [14, 15]. *Foxa2* gene ablation was validated in the mutant mice by measuring *Foxa2* expression in the control $Foxa2^{loxP/loxP}$ and mutant $Pgr^{cre/+}$ $Foxa2^{loxP/loxP}$ mice at Day 2.5 of pregnancy. *Foxa2* expression was not detectable in the mutant mice compared with RNA and protein from the control mice, as determined by real-time PCR and immunohistochemistry (Fig. 2).

TABLE 1. Fertility defect of mutant mice.

Genotype	No. of mice tested	No. of litters	No. of pups	Mean of pups/litter	Mean of litter/mouse
Control	7	48	309	6.44	6.86
Mutant	13	5	12	1.70	1.70

Fertility and Implantation Defect in $Pgr^{cre/+}$ $Foxa2^{loxP/loxP}$ *Mice*

To determine if *Foxa2* has a role in implantation, female control and mutant mice were mated to wild-type male mice for 6 mo. Control mice exhibited normal fecundity over this period; however, mutant mice had significantly fewer litters, pups per litter, and total pups born (Table 1). Thus, female mutant mice are severely subfertile, demonstrating the importance of *Foxa2* in pregnancy. To determine the cause of the subfertility, we examined whether blastocysts were able to undergo successful implantation. We killed mice on the morning of Day 5.5 of pregnancy and counted the number of implantation sites. Implantation sites were significantly decreased in the mutant mice (2.75 ± 1.25) compared with the control mice (8.20 ± 0.73) (Fig. 3, A and B). Histological examination showed that blastocysts are able to attach to the uterine lumen and initiate a decidual response in mutant mice (Fig. 3C). However, the decidual region and size of the embryos were decreased in the mutant mice compared with control mice. The morphology of the stroma cells showed an abnormal decidual phenotype. Also, the embryos did not exhibit invasion into the uterine stroma through the epithelium. This observation suggests that the mutant uterus is incapable of supporting embryo invasion and normal decidualization.

Defect of Decidualization in $Pgr^{cre/+}$ $Foxa2^{loxP/loxP}$ *Mice*

To confirm that mutant mice exhibit a defect in the ability of uterine stroma cells to undergo a decidual reaction, the ability of their uteri to undergo decidualization after artificial hormonal induction was determined. Ovariectomized mutant

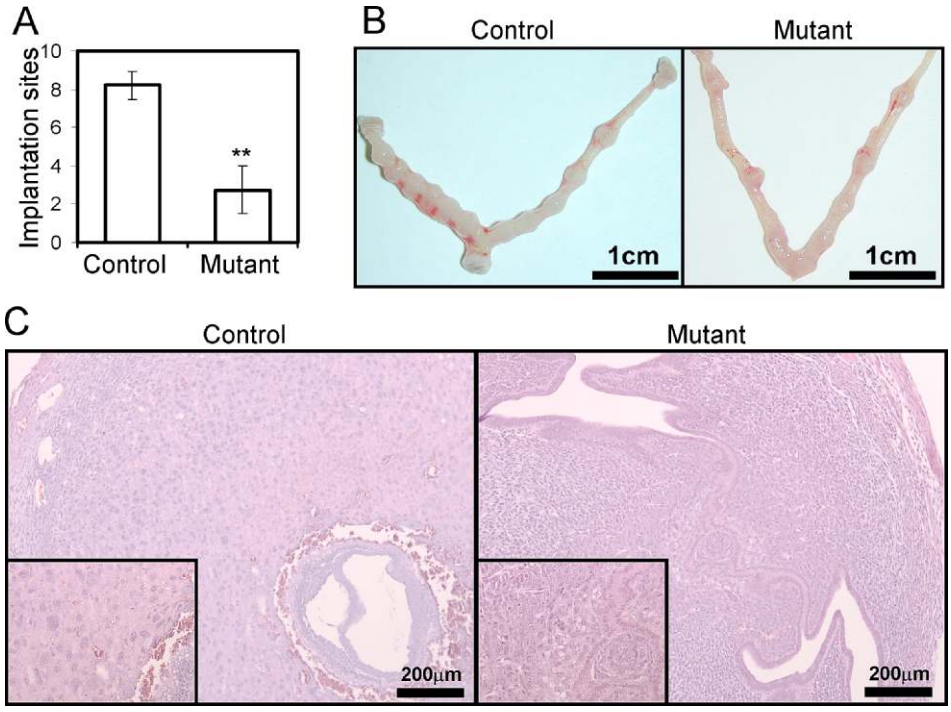

FIG. 3. Implantation defect in the mutant mice. **A**) Implantation sites in the control and mutant uterus at Day 5.5. Control and mutant mice were killed at Day 5.5. The number of implantation sites was counted in the uteri. The results represent the mean ± SEM of five independent mice. $^{**}P < 0.01$. **B**) Gross anatomy of the mutant uteri shows a decrease in the number of implantation sites compared with controls. **C**) Hematoxylin-eosin staining of control and mutant uteri with blastocysts at Day 5.5. Insets are high-power views of attachment sites.

(n = 4) and littermate control (n = 4) mice were treated with E2 and P4 to mimic pregnancy, and the uterus was traumatized to mimic the stimulation of the blastocysts at implantation and to induce decidualization (See *Materials and Methods*). As expected, the uterine horn of control mice exhibited a robust decidual response 5 days after receiving the artificial stimulation. However, mutant mice displayed a reduction in the decidual response (Fig. 4A). Quantification of the decidual response by the measurement of uterine wet weight indicated that the stimulated uterine horn of mutant mice was significantly smaller compared with littermate controls (Fig. 4B). These results demonstrate that the mutant mice exhibit a decidualization defect both during natural pregnancy and in the hormonally induced decidualization reaction.

Defect of Glandular Development in $Pgr^{cre/+}$ $Foxa2^{loxP/loxP}$ *Mice*

To determine if the cause of the subfertility in *Foxa2* mutant mice had a morphological origin, histological analysis of nonpregnant uteri was conducted. Examination of the uteri of control and mutant mice showed a reduction in the number of uterine glands upon ablation of *Foxa2* (Fig. 5A). The number of uterine glands was quantified by counting the number of glands per uterine section, which demonstrated that the reduction in uterine glands was statistically significant (Fig. 5B). One endometrial gland protein, LIF, which is a member of the interleukin 6 family of cytokines and is a secreted glycoprotein, is known to be critical for implantation, as female $Lif^{-/-}$ mice are sterile due to an implantation defect. *Lif* is expressed in the uterine glands in a bimodal fashion with peaks at Day 0.5 and Day 3.5 of pregnancy, and LIF can substitute for the nidatory surge in E2 in regulation of blastocyst implantation in mice [16]. The expression of *Lif* was significantly decreased at Day 3.5 in the mutant mice compared with control mice (Fig. 5C). Examination of other regulators of implantation, *Ihh*, *Hoxa10*, *Wnt5a*, *Wnt7a* (Fig. 5C), *Ptch1*, *Hoxa11*, *Wnt4*, *Bmp2*, *Cebpb*, and *Hbegf* (data not shown), were not significantly altered during the preimplantation period; only *Ptgs2* expression was altered during this period. The expression value of mRNAs was normalized against cadherin 1 (*Cdh1*, also known as E-cadherin) mRNA to eliminate the effect of reduced epithelium due to glandular loss in $Pgr^{cre/+}$ $Foxa2^{loxP/loxP}$. Therefore, the most likely cause of the impaired decidual response in the $Pgr^{cre/+}$ $Foxa2^{loxP/loxP}$ mice is the lack or ablation of *Lif* secretion due to the loss of uterine glands.

Rescue of the Decidualization Defect by Recombinant LIF

To determine if the uterine decidualization defect was due to the lack of LIF secreted by the uterine glands, we investigated whether the administration of recombinant LIF could rescue the decidual defect in the mutant mice. Control and mutant mice were ovariectomized and induced to undergo the decidual reaction as already described. At 24 h before endometrial trauma, 10 μl of vehicle (10% BSA) or recombinant LIF (100 ng/μl in 10% BSA) was injected intraluminally into one uterine horn. The mice were killed 5 days after receiving the decidual trauma. Decidualization was unaffected by administration of exogenous LIF, as seen by the robust decidual response in the control mice. As expected, the mutant stroma showed a decreased decidual response when injected with vehicle, whereas LIF partially restored the decidualization response in mutant mice (Fig. 6, A and B). This result was confirmed by staining for alkaline phosphatase activity, a well-known marker for decidual cells. Robust alkaline phosphatase activity, as seen by dark purple staining, could be seen in both the vehicle- and LIF-treated control mice. Alkaline phosphatase staining was significantly decreased in uteri of mutant mice treated with BSA; however, the staining was intensified in the mutant mice that were administered recombinant LIF (Fig. 6C). Thus, LIF can partially rescue the impaired decidual response in the mutant mice. Taken together, these results indicate that the impaired decidual response observed in *Foxa2* mutant mice is at least in part attributable to a lack of *Lif* induction during implantation.

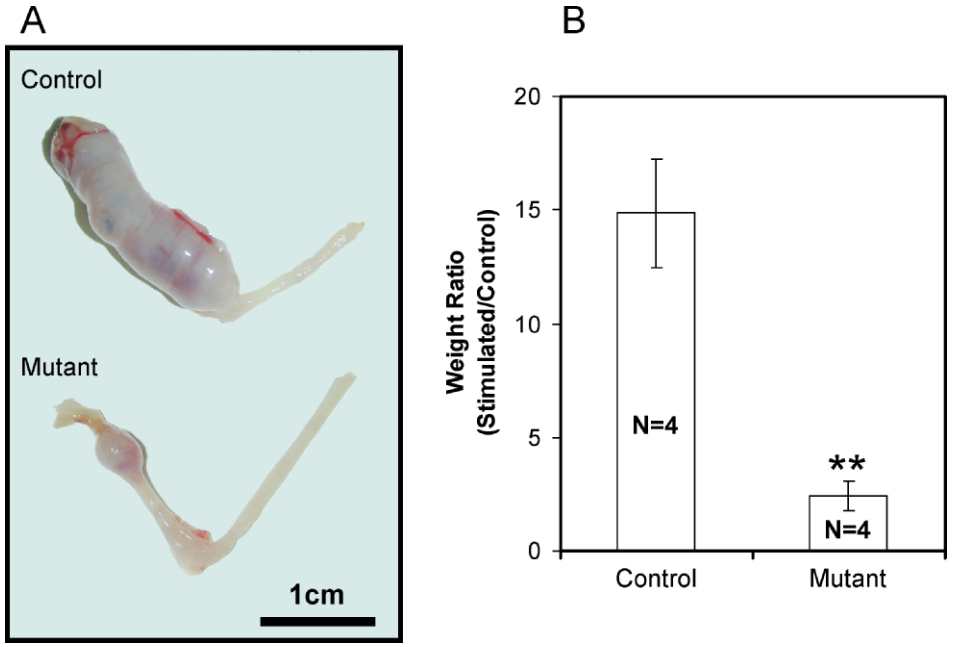

FIG. 4. Decidualization defect in the mutant mice. Six-week-old mice were ovariectomized and 2 wk later were subjected to a hormone regimen and a decidual stimulus. **A**) Gross anatomy of the mutant uteri shows a decrease in size of the decidual horn compared with controls. **B**) Stimulated horn weight:unstimulated horn weight ratio was significantly decreased in the mutant uteri. The results represent the mean ± SEM. $^{**}P < 0.01$.

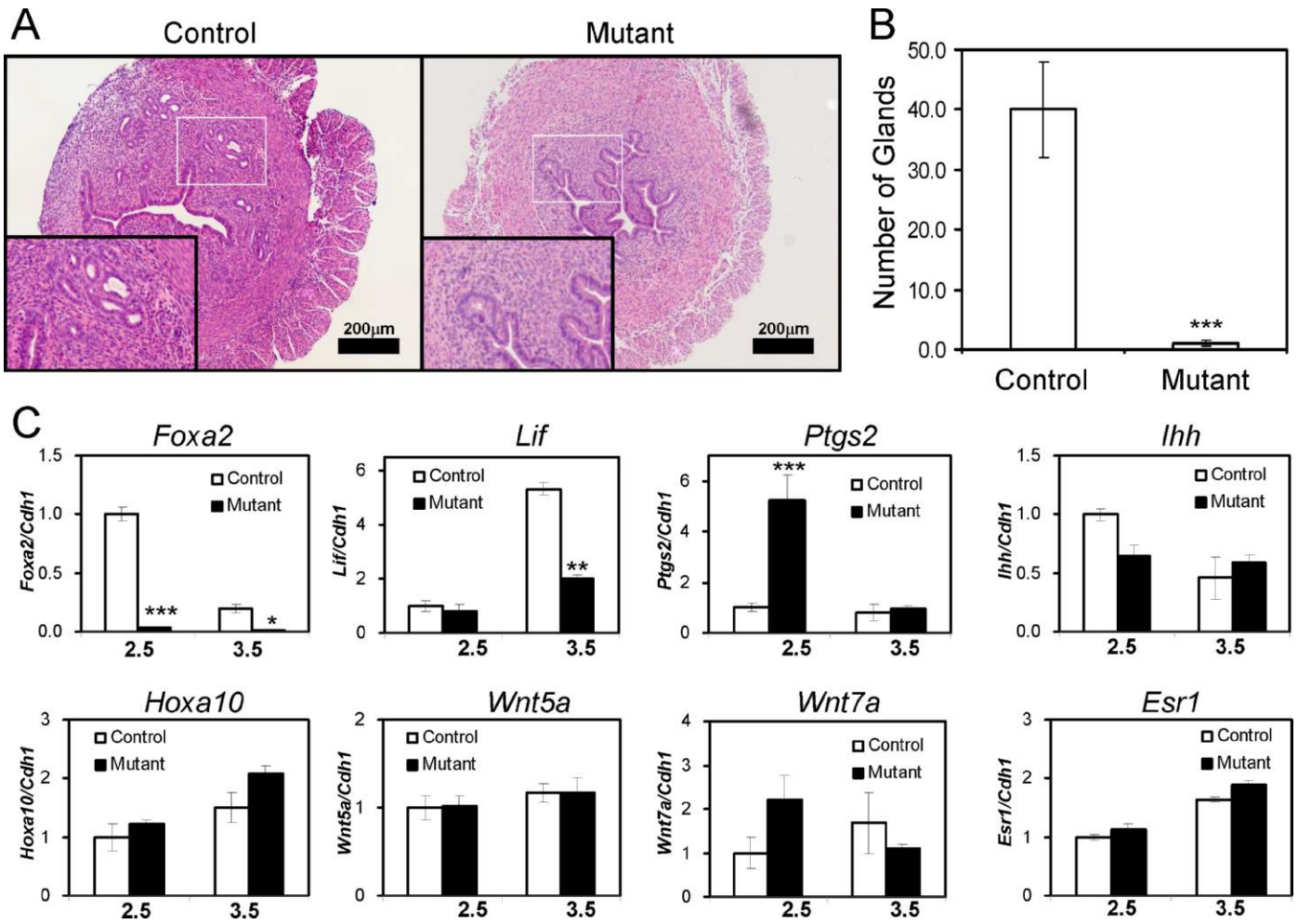

FIG. 5. Defect of gland formation in the mutant mice. **A**) Hematoxylin-eosin staining of uteri from 8-wk-old control and mutant mice. Insets represent high-power views of the boxed region. **B**) The number of glands was counted from the same area of histological slides. The results represent the mean ± SEM of three independent mice. $^{***}P < 0.001$. **C**) The expression of *Lif* at Day 2.5 and Day 3.5 in the mutant mice. The expression levels of *Foxa2*, *Lif*, *Ptgs2*, *Ihh*, *Hoxa10*, *Wnt5a*, *Wnt7a*, and *Esr1* were measured at Day 2.5 and Day 3.5 of the pseudopregnant uterus. Total RNA used for the RT-PCR assays was prepared from the pseudopregnant uteri. The results represent the mean ± SEM of three independent RNA sets. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

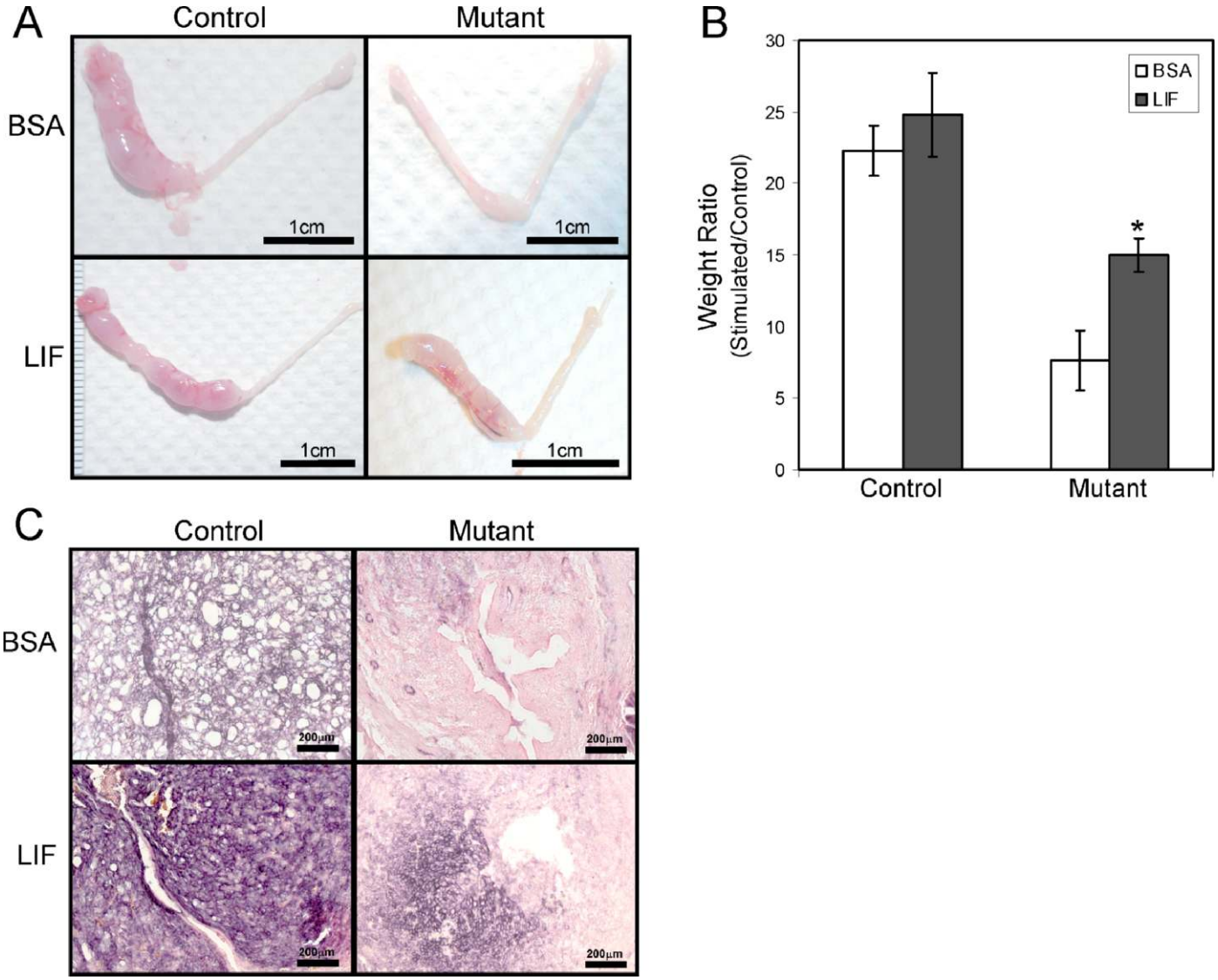

FIG. 6. Partial rescue of the decidualization defect by recombinant LIF administration in the mutant mice. **A**) Gross morphology of the decidual response in the mutant uteri 5 days after the decidual stimulus and treatment of 10% BSA or recombinant LIF in 10% BSA. **B**) Stimulated horn weight:unstimulated horn weight ratio was significantly increased in the mutant uteri treated with recombinant LIF compared with BSA. The results represent the mean ± SEM. $^*P < 0.05$. **C**) Differentiation by alkaline phosphatase staining in the mutant uterus treated with 10% BSA or recombinant LIF in 10% BSA after the decidual trauma. Bars = 1 cm (**A**) and 200 μm (**C**).

DISCUSSION

FOXA2 has important roles in early embryonic development, organogenesis, and glucose homeostasis [6, 9, 12, 17–19]. Among the FOXA proteins, FOXA2 is the only FOXA protein that has been detected in the murine uterus, and this expression is restricted to the glandular epithelium [11]. *Foxa2* knockout mice die at E10 or E11 because of severe defects in node, notochord, neural tube, and gut tube development [12]. However, the function of *Foxa2* in the uterus has remained elusive. To study the role of *Foxa2* in the uterus, we generated mice in which *Foxa2* was ablated ($Pgr^{cre/+}$ $Foxa2^{loxP/loxP}$) in the reproductive tract using previously generated mice with floxed *Foxa2* alleles and the Pgr^{cre} mouse model [14, 15]. The female $Pgr^{cre/+}$ $Foxa2^{loxP/loxP}$ mice were severely subfertile, with an inability of the uterus to support embryo invasion and decidualization. The major morphological phenotype of these mice was the absence of uterine glands. Because the Pgr^{cre} mouse model recombines alleles in all compartments of the uterus, as well as the pituitary, mammary gland, and ovary, we had to determine if the subfertility was due to the uterine defect or was in part due to pituitary or ovarian defects. No ovarian phenotype was detected in these mice. Although a pituitary contribution to this phenotype has not been ruled out, the severe inability of the uterus to support implantation and undergo an experimentally induced decidual response with exogenous steroid hormones indicates that the fertility defect is due in part to a uterine defect. Also in support of a uterine defect as the cause of the subfertility is the lack of uterine glands in this model. As shown in sheep, P4 administration to neonatal sheep resulted in the ablation of uterine glands, which rendered the adult ewes sterile [20].

Postnatal uterine morphogenesis involves the differentiation and development of the endometrial glandular epithelium from the luminal epithelium, as well as the development of the endometrial stroma and inner circular and outer longitudinal layers of the myometrium from the uterine mesenchyme [2, 20]. Little is known about the mechanisms regulating postnatal

uterine morphogenesis and, in particular, endometrial gland development. Within the uterus, the Pgr^{cre} mouse model recombines alleles in both the endometrial epithelial and stromal compartments, as well as in the myometrium. However, given that the expression of *Foxa2* is limited to the glandular epithelium, it is likely that *Foxa2* directly regulates adenogenesis in the mouse uterus. *Foxa2* has an important role in epithelial budding and morphogenesis in many organs, including the pancreas, liver, lung, and prostate [11, 21–25]. Thus, *Foxa2* is likely involved in bud formation and epithelial specification during uterine gland formation. *Foxa2* may have a role in gland specification by forming a gene regulatory network capable of inducing a competence in the luminal epithelium to become glandular epithelium via its ability to alter chromatin. *Foxa* transcription factors are able to open highly compacted chromatin, facilitating the binding of other transcription factors, and this process has been shown to occur for glucocorticoid receptor, androgen receptor, and estrogen receptor 1 [8, 19, 26–29]. One possible signaling pathway that *Foxa2* may regulate that is integral to gland formation is the Wnt/β-catenin pathway. Mouse models in which *Wnt5a*, *Wnt7a*, and catenin beta 1 (*Ctnnb1*) were ablated all displayed a lack of uterine glands [30–33]. *Foxa2* has been shown to regulate the expression of multiple Wnt signaling members, including *Wnt3a*, *Wnt8a*, and *Wnt7b*, the expression of which was decreased in mouse models lacking *Foxa2* [10, 34]. *Foxa2* also transactivates the *Wnt7b* promoter in vitro [35]. The reciprocal interaction between *Foxa2* and Wnt signaling has been noted also in that *Ctnnb1*, a downstream effector of the Wnt pathway, can promote *Foxa2* [36–38]. There is also evidence that *Foxa2* is able to engage in autoregulation by binding to its own promoter to enhance transcription [39]. The lack of altered expression of *Wnt7a* and *Wnt5a* in the preimplantation $Pgr^{cre/+}$ $Foxa2^{loxP/loxP}$ mouse uterus (Fig. 5C) does not rule out the possibility that *Foxa2* regulates adenogenesis through Wnt signaling during the neonatal period, when glands are initially formed. Future studies are needed to determine the potential role of complex interactions between Wnt and *Foxa2* signaling to either initiate or maintain glandular identity.

Because uterine gland development is repressed in the ovine model by treatment with P4, one might speculate that *Foxa2* expression is repressed by P4 in the mouse uterus [20]. However, we determined that P4 alone has no effect on *Foxa2* expression but that E2 and cotreatment of E2 plus P4 repress *Foxa2* expression (Fig. 1C). Therefore, treatment of the neonatal mouse with E2 plus P4 may repress *Foxa2* and attenuate endometrial glandular development.

Endometrial glands and their secretions are critical regulators of peri-implantation survival of blastocysts and implantation, as well as the establishment of uterine receptivity in numerous species [40–43]. These glands produce histotrophic factors that nurture the blastocysts and support attachment and, depending on the species, invasion of blastocysts [2, 44, 45]. During normal pregnancy, the presence of an active blastocyst in the uterus is the stimulus for implantation; however, the uterus itself is only receptive to the blastocyst for a limited period of time, known as the ''window of receptivity.'' Deficiencies in uterine receptivity, embryo development, and blastocyst-uterine communication compromise fertility [46, 47]. Several signaling pathways necessary for implantation have been identified [48, 49]. However, the mechanism by which the attachment of a blastocyst to the uterine luminal epithelium is triggered remains unclear. After the attachment of the blastocyst to the luminal epithelium, the surrounding stromal cells undergo decidualization, eventually embedding the embryo into the stroma. While insight into the mechanisms by which this decidualization occurs has been elucidated, much still remains to be known about this vital process [1, 48, 49]. The *Foxa2* mutant mice exhibit a defect in implantation and decidualization. Notably, some of the implanting blastocysts can induce decidualization; however, further development and implantation of blastocysts are defective (Fig. 4C). These results suggest that uterine glands are necessary to produce growth factors and potential histotrophic factors that are critical to prime the endometrial stroma for implantation, as well as to stimulate the embryo trophoblasts to invade the uterus.

The *Foxa2* mutant mouse serves as a model to determine which products of the endometrial glands are responsible for allowing trophoblast invasion of the uterus and the induction of decidualization. One product of these glands that may regulate this function is LIF. LIF is secreted by the uterine glands in response to nidatory estrogen at Day 3.5 [16, 50, 51] and is expressed in the subluminal stroma at the implantation site [52]. $Lif^{-/-}$ mice are unable to undergo blastocyst implantation and decidualization of the stroma [44]. LIF can substitute for E2 action in the termination of artificially delayed implantation and in the reinitiation of blastocyst implantation in mice [16]. Our results show that the expression of *Lif* is significantly decreased at Day 3.5 in the mutant mice compared with control mice (Fig. 3) and that the decidualization defect of the mutant mice was partially rescued by administration of recombinant *LIF* (Fig. 6). These results confirm that *Lif* is a critical product of the endometrial glands that is important for regulating changes in the endometrium during implantation [16]. However, determining if *Foxa2* directly regulates *Lif* expression or if the lack of *Lif* expression is simply the result of loss of endometrial glands is difficult in this model because uterine glands are completely ablated. Transfections and chromatin immunoprecipitation analysis would be required to determine if *Foxa2* is a direct regulator of *Lif* transcription. However, in silico analysis of 2 kilobases of the *Lif* promoter region using the Transcription Element Search System (http://www.cbil.upenn.edu/cgi-bin/tess/tess?RQ=WELCOME) did not detect any *Foxa2*-binding sites (data not shown).

In conclusion, we have demonstrated a role for *Foxa2* in endometrial function using mice with conditional ablation of *Foxa2* in the uterus. Uterine-specific ablation of *Foxa2* results in a defect in uterine gland formation. Furthermore, these mice exhibit both implantation and decidualization defects, demonstrating that *Foxa2* regulation is critical for adult uterine function. The results of this investigation provide significant insights into our understanding of the importance of *Foxa2* in female reproduction.

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