

# Cochaperone immunophilin FKBP52 is critical to uterine receptivity for embryo implantation

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Embryo implantation in the uterus is a critical step in mammalian reproduction, requiring preparation of the uterus receptive to blastocyst implantation. Uterine receptivity, also known as the window of implantation, lasts for a limited period, and it is during this period blastocysts normally implant. Ovarian steroid hormones estrogen and progesterone ( $P_4$ ) are the primary regulators of this process. The immunophilin FKBP52 serves as a cochaperone for steroid hormone nuclear receptors to govern appropriate hormone action in target tissues. Here we show a critical role for FKBP52 in mouse implantation. This immunophilin has unique spatiotemporal expression in the uterus during implantation, and females missing the *Fkbp52* gene have complete implantation failure due to lack of attainment of uterine receptivity. The overlapping uterine expression of FKBP52 with nuclear progesterone receptor (PR) in wild-type mice together with reduced  $P_4$  binding to PR, attenuated PR transcriptional activity and down-regulation of several  $P_4$ -regulated genes in uteri of *Fkbp52*<sup>-/-</sup> mice, establishes this cochaperone as a critical regulator of uterine  $P_4$  function. Interestingly, ovulation, another  $P_4$ -mediated event, remains normal. Collectively, the present investigation provides evidence for an *in vivo* role for this cochaperone in regulating tissue-specific hormone action and its critical role in uterine receptivity for implantation.

mouse | uterus | ovulation | blastocyst | progesterone receptor

Progesterone ( $P_4$ ) is essential for implantation and pregnancy maintenance in all mammalian species studied. In mice,  $P_4$  priming of the uterus is obligatory for estrogen to prepare the uterus to the receptive state conducive to blastocyst implantation.  $P_4$  acting through the nuclear  $P_4$  receptor (PR) modulates uterine physiology and expression of various genes that are required for implantation (1, 2). Numerous defects in mice lacking the *Pgr* gene that encodes PR include failure in ovulation, mammary gland development, and sexual behavior along with uterine hyperplasia and inflammation, reflecting the critical role of  $P_4$  in female reproduction (3). Appropriate functioning of nuclear steroid hormone receptors depends on interactions with the molecular chaperone machinery to maintain a functional state competent for hormone binding and subsequent transcriptional activation. Functionally mature steroid receptor complexes consist of a receptor monomer, a 90-kDa heat shock protein (Hsp90) dimer, the cochaperone p23, and one of four cochaperones that contain a tetratricopeptide repeat (TPR) domain. The TPR cochaperones include two members of the FK506 binding family of immunophilins, FKBP52/FKBP4 and FKBP51/FKBP5, a member of the cyclosporin-binding immunophilin cyclophilin 40 (CyP40) or the protein phosphatase PP5. FKBP52 and FKBP51 are similar to other FKBP family members in that both contain an active peptidylprolyl cis/trans isomerase domain that catalyzes conformational changes in protein substrates (4, 5).

Roles for Hsp90 and p23 in initiating and maintaining receptor competency for hormone binding are well established (6), but the contribution of cochaperones in receptor complexes are not clearly understood. Although FKBP51 and FKBP52 share 60% sequence

identity, have similar x-ray crystallographic structures (7, 8), and display similar peptidylprolyl cis/trans isomerase and Hsp90-binding activities, there are clear functional distinctions between these cochaperones in steroid receptor complexes. There is evidence based on *in vitro* cellular assays that, whereas FKBP52 potentiates the function of glucocorticoid receptors (GR) (9), androgen receptors (10), and PR (5), FKBP51 antagonizes GR and PR functions (11, 12). However, physiological roles of FKBP52 and FKBP51 in an *in vivo* context have not been examined. More specifically, a physiological role for FKBP52 and FKBP51 in  $P_4$ -dependent processes including ovarian and uterine functions remains unknown. Here we show that female mice missing the *Fkbp52* gene have compromised PR functions leading to total failure of the uterus to support blastocyst implantation.

## Materials and Methods

**Mice.** The disruption of the *Fkbp52* gene was achieved by homologous recombination as described (10). Tail genomic DNA was used for genotyping by PCR. Mice on C57BL/6/129SvJ were housed and used in the present investigation in accordance with the National Institutes of Health and institutional guidelines on the care and use of laboratory animals.

**Ovulation, Fertilization, Implantation, and Blastocyst Transfer.** Mice were examined for ovulation, fertilization, and implantation as described (13). To examine ovulation and fertilization, mice were mated with fertile males. On day 2 of pregnancy (day 1 = vaginal plug), oviducts were flushed with Whitten's medium to recover ovulated eggs and to examine the fertilization rate. Implantation sites on days 5 and 6 of pregnancy were visualized by an i.v. injection (0.1 ml per mouse) of a Chicago Blue B dye solution (1% solution in saline) and the number of implantation sites, as demarcated by distinct blue bands, was recorded. For blastocyst transfer, pseudopregnant recipients were generated by mating females with vasectomized males. Day 4 wild-type blastocysts were transferred into day 4 uteri of wild-type, heterozygous or *Fkbp52*<sup>-/-</sup> pseudopregnant recipients, and implantation sites were recorded 24 h later (day 5) by the blue dye method. All mice used were between 2 and 4 months of age.

**Comparative RT-PCR and Southern Hybridization.** Comparative RT-PCR and Southern blotting were performed as described (14).

**$P_4$  Binding Assay.** Ovariectomized wild-type or *Fkbp52*<sup>-/-</sup> mice were injected s.c. with estradiol-17 $\beta$  (1  $\mu$ g per mouse in sesame oil) or vehicle for 14 days. Aliquots of wild-type or *Fkbp52*<sup>-/-</sup> uterine cytosol (200  $\mu$ g of total protein) were incubated overnight at 4°C in the presence of indicated concentrations of

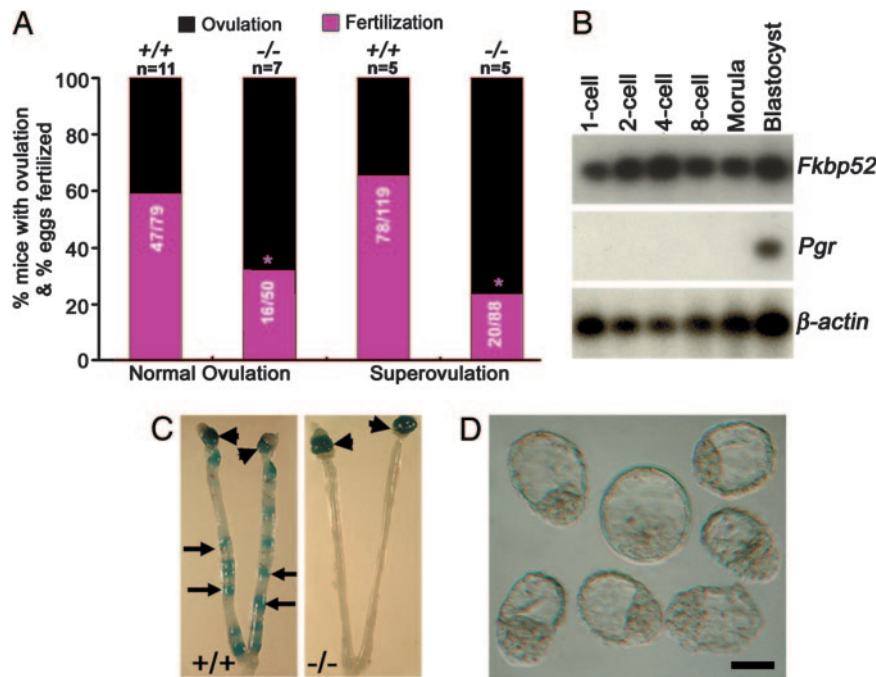
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Abbreviations:  $P_4$ , progesterone; PR,  $P_4$  receptor.

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**Fig. 1.** Female reproductive events in *Fkbp52*<sup>-/-</sup> mice. (A) Normal ovulation and fertilization were examined on day 2 of pregnancy. Superovulated eggs retrieved on day 1 were subjected to *in vitro* fertilization. Numbers within the bars indicate number of fertilized eggs/total number of ovulated eggs. Normal and superovulation rates are not significantly different between +/+ and -/- mice (unpaired *t* test); however, normal or *in vitro* fertilization rates are significantly different between +/+ and -/- mice ( $\chi^2$  analysis; \*, *P* = 0.01). (B) *Fkbp52* and *Pgr* expression in preimplantation embryos as assessed by RT-PCR. (C) Representative photographs of wild-type uteri with implantation sites (IS) and *Fkbp52*<sup>-/-</sup> uteri without IS on day 5 of pregnancy. Arrowheads and arrows indicate sites of ovary and implantation, respectively. (D) Representative photomicrograph of blastocysts recovered from *Fkbp52*<sup>-/-</sup> mice on day 5 of pregnancy. (Bar, 50  $\mu$ m.)

[<sup>3</sup>H]P<sub>4</sub> (NEN, specific activity = 102.1 Ci/mmol; 1 Ci = 37 GBq), a 100-fold molar excess of cortisol plus or minus a 100-fold molar excess of unlabeled P<sub>4</sub>. Unbound ligand was removed by incubation with dextran-coated charcoal, and bound radioactivity in the supernatant was measured by liquid scintillation counting (10).

**Transfection and PR Transcriptional Activity Assay.** Cells (8 × 10<sup>4</sup> per well) were cotransfected with 20 ng of pCMV- $\beta$ -galactosidase plasmid (Clontech), 0.3  $\mu$ g of pCR3.1-hPRB expression plasmid (provided by N. Weigel, Baylor College of Medicine, Houston), 0.3  $\mu$ g of pMTV-luc reporter plasmid (provided by J. Scammell, University of South Alabama, Mobile), and 0.3  $\mu$ g of pCI-neo vector (Promega) lacking or containing human *Fkbp51* or *Fkbp52* cDNA. Cells were incubated overnight with P<sub>4</sub> as indicated. Luciferase and  $\beta$ -galactosidase assays were performed essentially as described (10).

**In Situ Hybridization.** Sense or antisense <sup>35</sup>S-labeled probes were generated by using appropriate polymerases from cDNAs for *in situ* hybridization as described (15). Sections hybridized with sense probes served as negative controls.

**Northern Hybridization.** Total RNA (6.0  $\mu$ g) was denatured, separated by formaldehyde-agarose gel electrophoresis, and transferred onto nylon membranes. Cross-linked blots were prehybridized, hybridized, and washed as described (15). The hybrids were detected by autoradiography.

**Isolation and Culture of Embryonic Fibroblasts.** Day 13 embryos were isolated and genotyped by PCR. The head, limbs, and liver were excluded from each embryo, and the remaining tissues were minced and digested with 0.25% trypsin at 37°C for 15 min, and plated in

60-mm plastic dishes in DMEM supplemented with 10% FBS plus essential amino acids and penicillin/streptomycin. Fibroblasts were immortalized by the 3T3 protocol of Todaro and Green (16).

**Histology and Immunostaining of Ki67.** Frozen (10  $\mu$ m) or formalin-fixed paraffin embedded uterine sections (5  $\mu$ m) were subjected to hematoxylin and eosin staining or immunostaining by using a Ki67 antigen staining kit, respectively (Novacastra, Newcastle, U.K.). Nuclear brown color indicates positive Ki67 staining.

## Results and Discussion

**Ovulation Is Normal in *Fkbp52*<sup>-/-</sup> Mice.** To delineate whether FKBP51 or FKBP52 plays a critical role in ovarian and uterine functions, we generated mice with targeted deletion of either *Fkbp51* or *Fkbp52* genes. We found that, although *Fkbp51*-deficient mice show no overt reproductive failures (unpublished observations), both males and females lacking *Fkbp52* are infertile (10), indicating the importance of this immunophilin in reproduction. The infertility status of *Fkbp52*<sup>-/-</sup> females is shown (Table 3, which is published as supporting information on the PNAS web site). Whereas male infertility is due to compromised androgen receptor function (10), the site and cause of female infertility in *Fkbp52*<sup>-/-</sup> mice remains unexplored.

Because *Pgr* null mice show ovulation failure and uterine refractoriness to implantation (17, 18), and because FKBP52 associates with PR-Hsp90 complexes (5), we first examined whether ovulation is normal in *Fkbp52*<sup>-/-</sup> females. Because *Fkbp52*<sup>-/-</sup> males are infertile, normally cyclic or gonadotropin-stimulated *Fkbp52*<sup>-/-</sup> females were mated with wild-type fertile males. The number of normally ovulated or superovulated eggs in these mutant females is comparable to that of wild-type females (Fig. 1A); however, both *in vivo* and *in vitro* fertilization rates of eggs arising from mutant females are compromised (Fig. 1A). To determine whether oocyte

**Table 1. *Fkbp52*<sup>-/-</sup> female mice show implantation failure**

Day of pregnancy	Genotypes	No. of mice	No. of mice with IS (%)	No. of IS	No. of embryos recovered
5	+/+/+/-	12	9 (75)	6.5 ± 2.0	0*
5	-/-	9	0	0	37†
6	+/+/+/-	14	10 (71)	7.9 ± 2.0	2‡
6	-/-	5	0	0	5§

+/+, +/-, and -/- females were mated with wild-type fertile males and the number of implantation sites (IS) was examined on days 5 and 6 of pregnancy by the blue dye method. Uteri without IS were flushed with saline to recover any unimplanted embryos.

\*Three mice without IS yielded no embryos.

†Fifteen embryos were at the blastocyst stage, and the remaining 22 were degenerating embryos and thus whether they were fertilized or unfertilized could not be ascertained.

‡Two degenerating embryos were recovered from one mouse.

§Five blastocysts were recovered from one mouse. The results of +/+ and +/- mice were not significantly different and are presented as a pool (mean ± SD).

FKBP52 contributes to this reduction, we examined the expression of *Fkbp52* and *Pgr* in preimplantation wild-type embryos from one-cell through blastocyst stages by RT-PCR. We found that, although embryos at all stages of development express *Fkbp52*, *Pgr* is expressed only at the blastocyst stage (Fig. 1B); our observation of *Pgr* expression in preimplantation embryos is consistent with a previous report (19). These results suggest that *Fkbp52* is maternally derived in the oocyte and may have a role in fertilization and preimplantation development independent of PR. Alternatively, oocyte maturation due to follicular deficiency arising from compromised PR function in the absence of FKBP52 may contribute to the reduced fertilization rate. Indeed, there is evidence that compromised PR function impairs the expansion of cumulus-oocyte complexes (20). Further investigation is needed to address this problem. However, recovery of blastocysts from *Fkbp52*<sup>-/-</sup> uteri suggests that FKBP52 is not an absolute requirement for fertilization or preimplantation embryo development. Collectively, these results imply that complete infertility observed in mutant females is not due to failure in ovulation as in *Pgr*<sup>-/-</sup> mice, or total absence of fertilization, but is the result of defective implantation and/or pregnancy failure following implantation.

***Fkbp52*<sup>-/-</sup> Mice Show Implantation Failure.** In mice, blastocyst attachment to the uterine luminal epithelium initiates the implantation process and is accompanied by an increased endometrial vascular permeability at the sites of blastocyst apposition, which can be visualized as distinct blue bands after an i.v. injection of Chicago Blue B dye solution (15). We observed that, although wild-type and heterozygous females had an expected number of implantation sites when examined on day 5 of pregnancy, none of the mutant females showed any sign of implantation (Fig. 1C and Table 1). It is possible that blastocyst implantation is delayed but eventually occurs in *Fkbp52*<sup>-/-</sup> mice like in *cPLA2α*<sup>-/-</sup> or *LPA3*<sup>-/-</sup> mice (13, 21); however, no signs of implantation were evident even on day 6 of

pregnancy (Table 1). A small number of unimplanted blastocysts were recovered from mutant females (Fig. 1D and Table 1). Compromised fertilization in *Fkbp52*<sup>-/-</sup> mice could explain the small number of recovered blastocysts in mutant mice. Therefore, we used blastocyst transfer experiments to confirm that FKBP52 is critical to uterine receptivity for implantation. Day 4 wild-type blastocysts were transferred to wild-type, heterozygous, or homozygous mutant day 4 pseudopregnant recipients. Similar to the results of natural mating, none of the transferred wild-type blastocysts showed implantation in any of the mutant recipients when examined 24 h later, whereas wild-type or heterozygous recipients showed an expected number of implantation sites. Again, a small number of blastocysts were recovered from mutant uteri (Table 2). Reciprocal embryo transfers with homozygous mutant embryos could not be performed because *Fkbp52*<sup>-/-</sup> males are infertile (10). Collectively, these results show that, whereas P<sub>4</sub>-mediated ovulation is normal in *Fkbp52*<sup>-/-</sup> mice, the mutant uterus is completely nonreceptive to blastocyst implantation, implying an essential role for this immunophilin specifically for uterine receptivity. Our next objective was to determine whether these phenotypes resulting from *Fkbp52* deficiency are caused by impaired P<sub>4</sub> functions in the uterus.

***Fkbp52* and *Pgr* Show Overlapping Uterine Expression During Implantation.** We speculated that, if FKBP52 functions as a cochaperone to PR *in vivo*, cell specific expression of these two genes must overlap. *In situ* hybridization results show that, on day 4 of pregnancy, *Fkbp52* and *Pgr* expression overlaps in the stroma. However, *Fkbp52* is also expressed in the luminal and glandular epithelium. Both *Fkbp52* and *Pgr* become more localized in stromal cells surrounding the blastocyst on day 5 after implantation (Fig. 2A). This expression pattern coincides with an increased stromal cell proliferation on day 4 and more intensely localized stromal cell proliferation at the implantation site on day 5. Uterine *Fkbp51*,

**Table 2. Wild-type blastocysts fail to implant in *Fkbp52*<sup>-/-</sup> recipients**

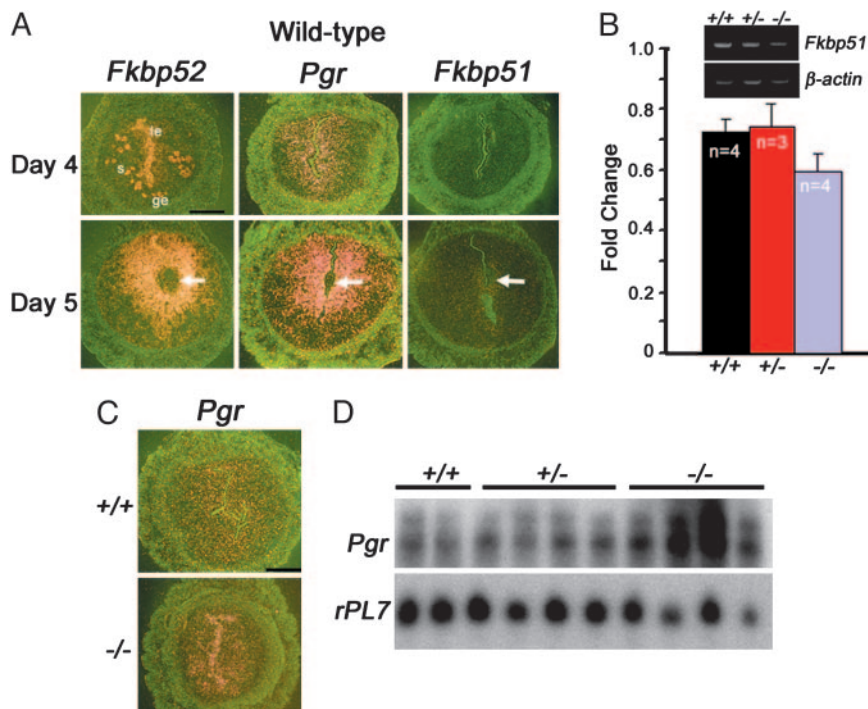
Genotype		No. of blastocysts transferred	No. of recipients	No. of mice with IS (%)	No. of IS (%)	No. of IS	No. of blastocysts recovered
Blastocysts	Recipients						
+/+	+/+/+/-	178	10	8 (80)	74 (41)	7.3 ± 4.0	2*
+/+	-/-	79	4	0 (0)	0	0	14†

Day 4 wild-type (+/+) blastocysts were transferred into uteri of +/+, +/-, or -/- recipients on day 4 of pseudopregnancy. The recipients were examined for implantation sites (IS) on day 5 by the blue dye method. Uteri without IS were flushed with saline to recover any unimplanted blastocysts.

\*Two mice without IS yielded one blastocyst each.

†Fourteen blastocysts were recovered from three recipients. The results of +/+ and +/- mice were not significantly different and are presented as a pool (mean ± SD).



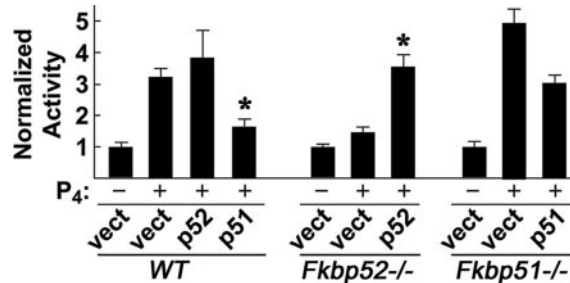


**Fig. 2.** Uterine expression of *Fkbp52*, *Pgr* and *Fkbp51*. (A) *In situ* hybridization of *Fkbp52*, *Pgr*, and *Fkbp51* in wild-type uteri on days 4 and 5 of pregnancy. (B) Comparative RT-PCR of uterine *Fkbp51* in *Fkbp52*<sup>+/+</sup> (+/+), *Fkbp52*<sup>+/-</sup> (+/-), and *Fkbp52*<sup>-/-</sup> (-/-) mice on day 4 of pregnancy. The data are presented as fold changes (mean ± SEM) of three to four independent RNA samples. Fold changes are not significantly different between +/+, +/-, or -/- mice (unpaired t test). (C) *In situ* hybridization of uterine *Pgr* in wild-type (+/+) and *Fkbp52*<sup>-/-</sup> (-/-) mice on day 4. (D) Northern blot analysis of uterine *Pgr* in +/+, +/-, and -/- mice on day 4. *rPL7* is a housekeeping gene. Arrows in A indicate the location of blastocysts. le, luminal epithelium; ge, glandular epithelium; s, stroma. (Bar, 400 μm).

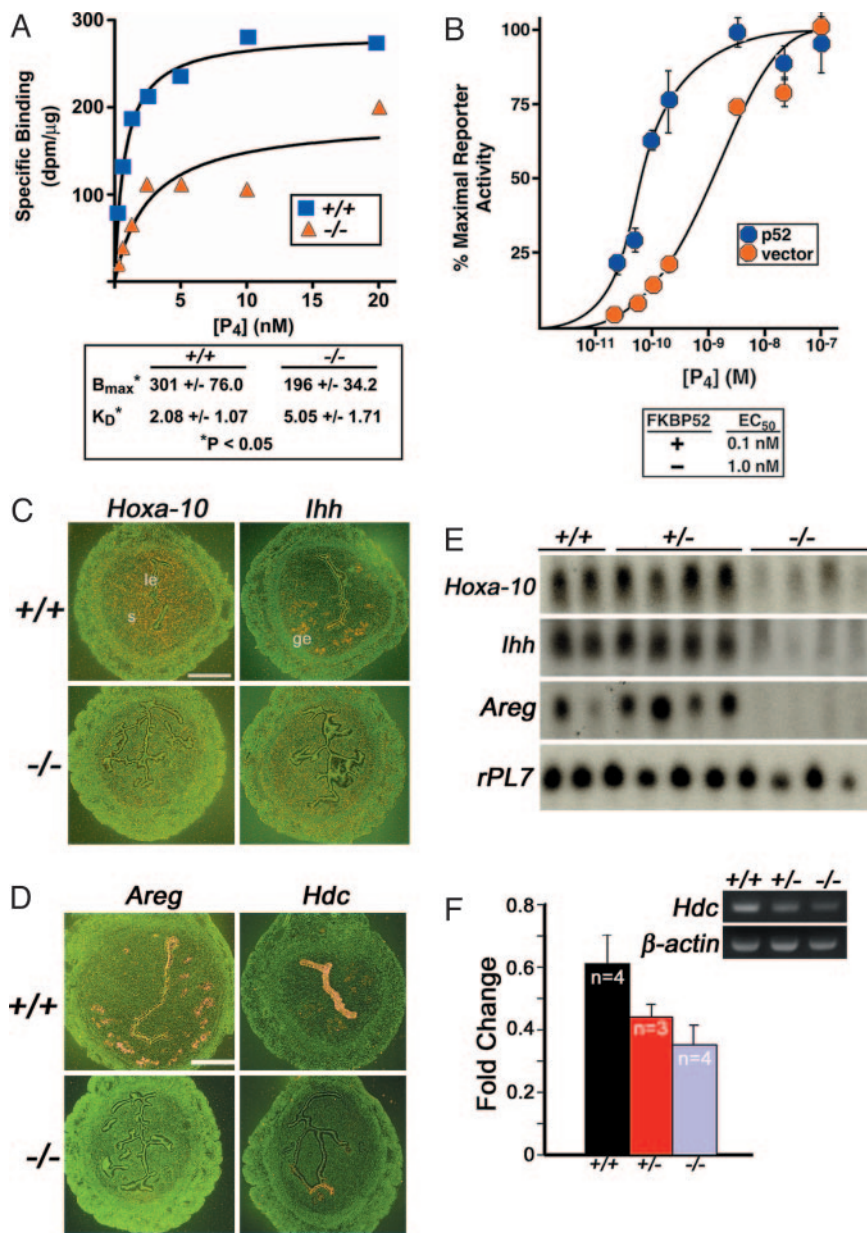
although significantly lower compared to *Fkbp52*, is also expressed on days 4 and 5. The lower expression of *Fkbp51* on days 4 and 5 implies a limited role for this immunophilin during implantation and is consistent with normal fertility of *Fkbp51*<sup>-/-</sup> mice. However, we wanted to determine whether an interaction between FKBP51 and FKBP52 modulates PR transcriptional activity. Using mouse embryonic fibroblasts in cellular assays, we found that FKBP51 is antagonistic to FKBP52 in modulating PR transcriptional activity (Fig. 3). To determine whether *Fkbp51* is overexpressed in *Fkbp52*<sup>-/-</sup> uteri producing the observed phenotypes, we used comparative RT-PCR experiments and found that *Fkbp51* expression is not exacerbated in mutant uteri on day 4 of pregnancy (Fig. 2B). Collectively, these results indicate that *Fkbp52* null phenotypes are specific to *Fkbp52* deficiency and not due to overexpression of *Fkbp51*. Given these results, we then compared *Pgr* expression in *Fkbp52* mutant uteri with wild-type uteri. *In situ* hybridization results show that *Pgr* is expressed in both the stroma and epithelium similar to that in wild-type uteri (Fig. 2C), and Northern blot results show that *Pgr* expression is not compromised in *Fkbp52*<sup>-/-</sup> uteri, but levels are rather increased (Fig. 2D). Together, these results show that uterine refractoriness to implantation in *Fkbp52*<sup>-/-</sup> mice is not due to reduced *Pgr* expression or overexpression of *Fkbp51*, but instead could be due to reduced PR function.

**Uterine PR Activity Is Compromised in *Fkbp52*<sup>-/-</sup> Mice.** If FKBP52 is required for optimal PR function, then uterine PR activity would be compromised in the absence of FKBP52. Indeed, binding assays using radiolabeled P<sub>4</sub> show that P<sub>4</sub> binding to PR is reduced ≈2-fold in *Fkbp52*<sup>-/-</sup> uterine cytosol compared with that of wild-type mice. There is also a fractional reduction in the number of P<sub>4</sub> binding sites (Fig. 4A). Reduced PR transcriptional activity in the absence of FKBP52 is also noted in a transfected cell system *in vitro*. Embryonic fibroblasts isolated from *Fkbp52*<sup>-/-</sup> mice were cotransfected with human PR-B plasmid, a luciferase reporter plasmid, and either

an empty vector or a plasmid containing FKBP52 (p52). P<sub>4</sub>-dependent reporter activity was measured over a range of P<sub>4</sub> concentrations. Cells expressing exogenous FKBP52 have enhanced P<sub>4</sub>-dependent reporter gene activation as compared to cells lacking FKBP52 (Fig. 4B). PR-B results are shown because PR-A normally displays only modest reporter transactivation in cell culture systems (22); however, loss of FKBP52 also reduced the modest activity of PR-A (data not shown). These results provide evidence that FKBP52 is required for optimal uterine PR activity;



**Fig. 3.** Differential modulation of PR transactivation by FKBP52 and FKBP51. Fibroblast cell lines prepared from wild-type (WT), *Fkbp52*<sup>-/-</sup>, or *Fkbp51*<sup>-/-</sup> mouse embryos were cotransfected with four plasmids as described in *Materials and Methods*. Cells were treated with or without 0.5 nM P<sub>4</sub> for 16 h. The bars indicate the activity (mean ± SD; n = 3) normalized to uninduced levels of luciferase activity in each cell background. In WT cells expressing endogenous FKBP52 and FKBP51, overexpression of FKBP52 show marginal increases in the reporter activity, whereas overexpression of FKBP51 decreases the reporter activity (\*, P = 0.05). In *Fkbp52*<sup>-/-</sup> cells, P<sub>4</sub>-induced reporter activity is minimal but restored by exogenous FKBP52 (\*, P = 0.05). In *Fkbp51*<sup>-/-</sup> cells retaining endogenous FKBP52, maximal reporter activity is induced; however, induced expression of FKBP51 decreases the activity. Statistical analysis was performed by using one-way ANOVA followed by paired t test.



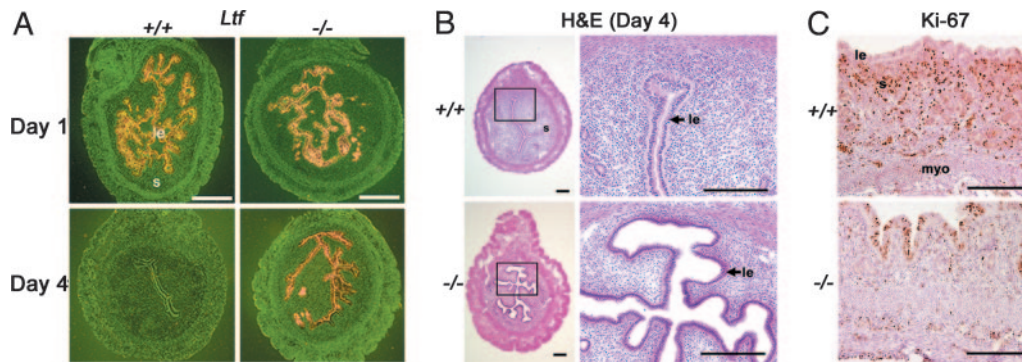
**Fig. 4.** Uterine progesterone ( $P_4$ ) binding and PR activity. (A)  $P_4$  binding in uterine cytosol of wild-type (+/+) and *Fkbp52*<sup>-/-</sup> (-/-) mice. Both +/+ and -/- female mice were ovariectomized and treated with estrogen to increase uterine PR levels. Uterine cytosolic samples from +/+ and -/- mice were used for binding assays for comparison. The data shown are representative of four independent experiments. (B) FKBP52 effects on reporter gene activation in embryonic fibroblasts isolated from *Fkbp52*<sup>-/-</sup> mice cotransfected with plasmid expressing human PR-B, a luciferase reporter plasmid, and an empty vector or plasmid expressing FKBP52 (p52). Each value represents the mean  $\pm$  SD for three replicate samples. (C and D) *In situ* hybridization of *Hoxa-10* and *Ihh* (C) and *Areg* and *Hdc* (D) in +/+ and -/- mice on day 4 of pregnancy. (E) Northern blot analysis of uterine *Hoxa-10*, *Ihh*, and *Areg* in +/+, +/-, and -/- mice on day 4. *rPL7* is a housekeeping gene. le, luminal epithelium; ge, glandular epithelium; s, stroma. (Bar, 400  $\mu$ m.) (F) Comparative RT-PCR of uterine *Hdc* in +/+, +/-, and -/- mice on day 4. The data are presented as fold changes (mean  $\pm$  SEM) of three to four independent samples. Fold changes in *Fkbp52*<sup>-/-</sup> mice are significantly different compared to combined fold changes in +/+ and +/- mice ( $P = 0.05$ , unpaired t test).

thus, defects in uterine receptivity for implantation in *Fkbp52*<sup>-/-</sup> mice are likely caused by compromised uterine  $P_4$  function.

**PR Responsive Genes and Functions Are Aberrant in Uteri of *Fkbp52*<sup>-/-</sup> Mice.** We speculated that if PR functions are compromised in *Fkbp52*<sup>-/-</sup> uteri, uterine  $P_4$ -dependent genes would be aberrantly expressed. We selected  $P_4$ -regulated genes that encode *Hoxa-10*, Indian hedgehog (*Ihh*), amphiregulin (*Areg*), and histidine decarboxylase (*Hdc*) (23–26). Wild-type and *Fkbp52*<sup>-/-</sup> females were mated with wild-type males to collect samples on day 4 when the uterus is under major  $P_4$  influence and expresses these genes.

Northern and *in situ* hybridization results show that expression of *Hoxa-10*, *Ihh*, and *Areg* are drastically down-regulated in *Fkbp52*<sup>-/-</sup> uteri, indicating compromised  $P_4$  function (Fig. 4 C–E). There are two isoforms of PR, PR-A and PR-B. Although mice lacking both PR-A and PR-B exhibit multiple defects in ovarian and uterine functions leading to female infertility, mice missing only PR-B display normal ovarian and uterine responses to  $P_4$ . These findings suggest that normal  $P_4$ -regulated ovarian and uterine functions are primarily mediated by PR-A (18). Because the mouse uterus expresses both PR-A and PR-B, we sought to examine whether FKBP52 differentially influences these isoforms. *Areg* and *Hdc* are





**Fig. 5.** Differential expression of an estrogen-responsive gene lactoferrin (*Ltf*) and cell proliferation. (A) *In situ* hybridization of *Ltf* in uteri of +/+ and -/- mice on days 1 and 4 of pregnancy. (Bar, 400  $\mu$ m.) (B) Histological examination of +/+ and -/- uteri on day 4 of pregnancy. (Bar, 200  $\mu$ m.) (C) Immunostaining of Ki67, a marker of cell proliferation, in uteri of +/+ and -/- mice on day 4. Note stromal cell proliferation in wild-type uterus versus luminal epithelial proliferation in *Fkbp52*<sup>-/-</sup> mice. Arrow points to luminal epithelial layer. le, luminal epithelium; s, stroma; myo, myometrium. (Bar, 100  $\mu$ m.)

known PR-A and PR-B regulated genes in the uterus, respectively (3). Down-regulation of both *Areg* and *Hdc* in *Fkbp52*<sup>-/-</sup> uteri (Fig. 4 D–F) provides evidence that FKBP52 influences both PR-A and PR-B functions. Because mice lacking PR-B have normal fertility (18), we believe that compromised uterine PR-A activity in *Fkbp52*<sup>-/-</sup> mice contributes to implantation failure.

To confirm that the observed changes in uterine gene expression are not due to aberrant responsiveness of the uterus to estrogen, we examined uterine expression of an estrogen-responsive gene lactoferrin (*Ltf*) on day 1 of pregnancy when the uterus is primarily under the influence of estrogen (Fig. 5A). As previously observed in the wild-type uterus (27), normal expression of *Ltf* on day 1 confirms that *Fkbp52*<sup>-/-</sup> uteri appropriately respond to estrogen. On day 4 of pregnancy, when the uterus is under major P<sub>4</sub> influence, *Ltf* expression is undetectable in wild-type uteri as expected, but persists in *Fkbp52*<sup>-/-</sup> uteri, indicating reduced P<sub>4</sub> function with exaggerated estrogenic influence in the absence of FKBP52 (Fig. 5A). This concept is consistent with a striking difference that we observed between wild-type and *Fkbp52*<sup>-/-</sup> uteri at the histological and cellular level. Normally, uterine lumens in wild-type mice on day 4 of pregnancy under P<sub>4</sub> dominance show luminal closure with a slit-like structure with nonproliferating differentiated cuboidal epithelia. In addition, stromal cell proliferation is intense on this day (2). Surprisingly, uteri of *Fkbp52*<sup>-/-</sup> mice have compromised luminal closure on day 4, displaying proliferating columnar luminal epithelia and nonproliferating stromal cells, consistent with defects in P<sub>4</sub>-regulated events (Fig. 5 B and C). However, proliferation in uterine luminal epithelia of *Fkbp52*<sup>-/-</sup> and wild-type mice on day 1 of pregnancy, when the uterus is under estrogenic influence,

remains similar (data not shown). Collectively, these results suggest that responsiveness of *Fkbp52*<sup>-/-</sup> uteri to coordinated estrogen and progesterone action is skewed to favor estrogen.

The present study identifies FKBP52 as a critical determinant of uterine P<sub>4</sub> actions in preparing the uterus receptive for blastocyst implantation. Although genetic and pharmacological evidence has previously established that P<sub>4</sub> action via nuclear PR is essential to ovulation, implantation, and pregnancy maintenance, it is remarkable to see that a particular cochaperone for PR is so critical and specific to uterine preparation for implantation. Apparently normal ovulation and a less severe fertilization phenotype as compared to uterine preparation for implantation suggest differential sensitivity of the ovary and uterus to FKBP52-mediated P<sub>4</sub> action. This tissue-specific differential sensitivity is not noted in *Pgr* mutant mice in which both severely compromised ovarian and uterine functions are responsible for female infertility. The physiological significance of PR-associated immunophilins has been underappreciated, and the dramatic infertility of mice lacking *Fkbp52* lends credence to the significance of this immunophilin cochaperone in female fertility. This study places FKBP52 as a target for improving female fertility or alternatively developing novel approaches to contraception.

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