Immunophilin FKBP52 deficiency confers uterine-specific resistance to progesterone signaling during pregnancy

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Master regulators: Estrogen and Progesterone

1. Prostaglandin-nuclear receptor-angiogenic signaling axis (cPLA2α/Cox2/PPARδ/Vegf/Flik1/Ang/Tie)
2. miRNA regulation of genes during implantation
3. Cytokine-growth factor-homeobox-morphogen signaling axis (Lif/Hb-Egf/Hoxa10/Msx1/Ihh/Bmp/Wnt)
4. Ligand-receptor signaling with endocannabinoids (Anandamide-CB1)
5. Immunophilin/cochaperone signaling with PR (Fkbp52-PR)

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Events of Early Pregnancy

[Diagram]

Nat Rev Genet, 7: 185, 2006
Coordinated effects of E<sub>2</sub> & P<sub>4</sub> determine the window for uterine receptivity

Proliferation patterns on days 1 and 4 of pregnancy

- Under estrogen influence
  - Luminal epithelium proliferation
  - I<sub>e</sub>, luminal epithelium
  - S, stroma

- Under progesterone influence
  - Luminal epithelium differentiation
  - Stromal proliferation

Attachment reaction between the blastocyst and uterine luminal epithelium occurs on day 4 midnight

- Attachment site
  - Blasticid
  - Luminal epithelium
  - Struma
Localized endometrial vascular permeability occurs with the onset of the attachment reaction.

Blue dye reaction

- **Paracrine/juxtacrine interactions**
  - Trophoderm - uterine luminal epithelium interaction (Epithelial-epithelial)
  - Epithelial-mesenchymal interaction (luminal epithelium-stroma)

- **Vascular permeability and angiogenesis**

- **Regulated growth** (proliferation and differentiation):
  - Decidualization

Embryo implantation is a powerful model system to study:

1. Paracrine/juxtacrine interactions
   a. Trophoderm - uterine luminal epithelium interaction (Epithelial-epithelial)
   b. Epithelial-mesenchymal interaction (luminal epithelium-stroma)

2. Vascular permeability and angiogenesis

3. Regulated growth (proliferation and differentiation):
   - Decidualization
**Progesterone: the “pregnancy” hormone**

- Absolutely required for pregnancy success in most mammals studied
- Ovulation
- Fertilization
- Uterine receptivity
- Decidual response
- Maintenance of uterine quiescence until parturition

- Works via its nuclear steroid hormone receptor, *progesterone receptor (PR)*

**Steroid Hormone-Receptor Complex**

**Cochaperones**

- Tetra-tricopeptide repeat domain (TPR) binds Hsp90
- Two members of FK506 binding family of immunophilins: FKBP52/FKBP4 and FKBP51/FKBP5
- Cyclosporin-binding immunophilins: Cyclophilin 40 (Cyp40) and protein phosphatase PP5
- FKBP52 and FKBP51: peptidylprolyl cis/trans isomerase activity domain and catalyses conformational changes in protein substrates
- FKBP52 and FKBP51 have different functions toward steroid receptors
**Hoxa10 null mice have reduced uterine P4 responsiveness**

- Proteomic analysis of WT and Hoxa10−/− uterine stromal cells
- Identified FKBP52 as a protein downregulated in Hoxa10−/− stromal cells

FKBP52 is a cochaperone for PR

*Mol Endo* 19:683, 2005

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**PR null mice**

- Precludes using this mouse model to examine the role of progesterone (P4) in early pregnancy events

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**Fkbp52 null mice**

- Males have decreased androgen receptor responsiveness (*Cheung-Flynn et. al, Mol Endo* 19: 1654, 2005)
- Reason for female infertility remained unknown

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**Infertile Phenotype of Fkbp52/C57 null females**

<table>
<thead>
<tr>
<th>Genotypes (males x females)</th>
<th>No. of breeders</th>
<th>No. of litters</th>
<th>Average litter size</th>
</tr>
</thead>
<tbody>
<tr>
<td>+/- x ++</td>
<td>28</td>
<td>81</td>
<td>6.9 ± 3.0</td>
</tr>
<tr>
<td>+/- x +/-</td>
<td>63</td>
<td>195</td>
<td>6.7 ± 3.0</td>
</tr>
<tr>
<td>+/- x --</td>
<td>53</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Fkbp52 null (−/−) females cohabitated with wild-type (+/+;+/-) fertile males failed to produce any offspring, while the average litter sizes from Fkbp52+/+ and Fkbp52+-/ females were comparable (mean ± SD).
Modulation of PR activation by FKBP52

Examine various stages of early pregnancy in Fkbp52⁻/⁻ females
- Ovulation
- Fertilization
- Implantation

- Ovulation and fertilization are comparable to WT females
- What is the reason for infertility in Fkbp52⁻/⁻ females?
FKBP52 and PR expression overlaps in periimplantation uteri

Implantation fails in Fkbp52/C57 null mice as examined on day 5 of pregnancy

Embryo transfer experiments show that wild-type blastocysts fail to implant in Fkbp52/C57 null females

<table>
<thead>
<tr>
<th>Genotype</th>
<th>No. of blastocysts transferred</th>
<th>No. of recipients</th>
<th>No. of mice with IS</th>
<th>No. of IS (%)</th>
<th>No. of IS</th>
<th>No. of blastocysts recovered</th>
</tr>
</thead>
<tbody>
<tr>
<td>+/-</td>
<td>178</td>
<td>10</td>
<td>8 (80%)</td>
<td>4 (41%)</td>
<td>7.3 ± 4.0*</td>
<td>2a</td>
</tr>
<tr>
<td>+/-</td>
<td>79</td>
<td>4</td>
<td>0 (0%)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

*a2 mice without IS yielded one blastocyst each
*b14 blastocysts were recovered from 3 recipients
*mean ±SD
Decreased expression of PR-regulated genes in uteri on day 4 of pregnancy in Fkbp52 null females

FKBP52 is critical to uterine receptivity and implantation in mice

_C57Fkbp52/_ null mice:
- Normal ovulation
- Implantation failure
  - Reduced P4 responsiveness
  - Exaggerated estrogenic influence

_PNAS_ 102: 14326, 2005

Ovulation is not impaired suggesting uterine responsiveness to PR signaling differs from ovarian responsiveness

Locally high P4 levels in the ovary may enhance basal PR activity sufficient for ovulation

_Fkbp52-/−_ MEFs
Determine whether exogenous P4 treatment rescues the infertile phenotype of Fkbp52 null mice

- Use silastic implants placed on day 2 of pregnancy (day 1 = vaginal plug)
- Examine implantation on day 5 of pregnancy

P4 fails to rescue implantation of transferred WT blastocysts in C57 Fkbp52 null females

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Blasted Recipient</th>
<th>No. of blastocysts transferred</th>
<th>No. of Recipients</th>
<th>No. of IS (%)</th>
<th>No. of embryos recovered</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT WT</td>
<td>82</td>
<td>6</td>
<td>48/82 (59%)</td>
<td>n/a</td>
<td></td>
</tr>
<tr>
<td>WT KO + P4</td>
<td>83</td>
<td>5</td>
<td>6/83 (7%)</td>
<td>45</td>
<td></td>
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</table>

P4 supplementation fails to restore expression of P4-regulated genes (Hoxa-10, Ihh and Areg) critical for uterine receptivity.

Rescue implantation failure in Fkbp52 null uteri on a different genetic background

- CD1: outbred, more robust reproduction
- Changed background of mice to CD1 background to F10 generation

- Can P4 supplementation rescue implantation failure in Fkbp52/CD1 null mice?
  - First characterize reproductive phenotype
**Fkbp52/CD1 null females have normal ovulation and fertilization**

- **Figure A:** Graph showing the number of apposed eggs.
  - WT: 10, KO: 8
- **Figure B:** Graph showing the percentage of eggs fertilized.
  - WT: 100%, KO: 70%

**Implantation fails in CD1 Fkbp52 null females**

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Day of Pregnancy</th>
<th>No. of Mice</th>
<th>No. of Mice with IS (%)</th>
<th>No. of IS</th>
<th>No. of embryos recovered</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT</td>
<td>5</td>
<td>16</td>
<td>16 (100%)</td>
<td>12.2 ± 0.3</td>
<td>n/a</td>
</tr>
<tr>
<td>KO</td>
<td>5</td>
<td>14</td>
<td>2 (14%)</td>
<td>7.0 ± 0.3</td>
<td>66</td>
</tr>
<tr>
<td>KO+P4</td>
<td>5</td>
<td>11</td>
<td>9 (81%)</td>
<td>10.3 ± 0.5</td>
<td>17</td>
</tr>
</tbody>
</table>

**P4 implant rescues implantation failure in CD1 Fkbp52 null females**

- Day 5 implant rescues implantation failure in KO+P4 females.

**Graph:**
- **Day 5:**
  - WT: Embryos
  - KO: Embryos
  - KO+P4: Embryos
P4 supplementation restores P4-regulated gene expression in CD1 Fkbp52 null uteri on day 4

P4 supplementation restores implantation-specific gene expression in CD1 Fkbp52 null uteri on day 5

P4 supplementation rescues pregnancy through day 12 in Fkbp52/CD1 null females
P4 supplementation via implants fails to restore normal pregnancy examined on day 14 in CD1 Fkbp52/- females

P4 supplementation via implant cannot restore normal pregnancy to term

Defective placental development in P4-treated CD1 Fkbp52/- females
Does daily P4 injections rescue pregnancy to full-term in CD1 Fkbp52-/ females?

- Inject mice with P4 (2 mg/0.1ml oil/mouse) sc from day 2 of pregnancy until sacrifice (D14) or D17 to allow labor to occur.

Daily P4 injection rescues pregnancy through day 14

Daily P4 injection restores full-term pregnancy in CD1 Fkbp52-/ females
Serum P4 levels in Fkbp52 null mice treated with implant or daily injection

A

Day 14 WT: 45 ng/ml
Day 14 KO Imp: 100 ng/ml
Day 14 KO Inj: 156 ng/ml

Proposed model for pregnancy rescue in the presence of high P4 levels

Conclusions

• The major reproductive phenotype in mice missing Fkbp52 is unique to uterine deficiency in the context of implantation
• P4-P4-FKBP52 signaling is a function of genetic makeup
• More robust P4-P4 signaling is required for pregnancy maintenance than is required for uterine receptivity and implantation in Fkbp52 null mice
• Blastocyst's presence determines the ability of P4 to rescue decidualization
• FKBP52 may have a unique PR-independent role during pregnancy (placenta/embryo?)

JCI 117: 1824, 2007
Clinical Implications

* Infertility and P4 resistance
Exogenous P4 treatment results in a decrease in miscarriages in women with history of pregnancy loss

* Endometriosis and P4 resistance
P4 resistance promotes this disease process

Endometriosis model using *Fkbp52*-/- mice

CD1 female mice (7-10 wk old)
Estrous cycle: Diestrus

Donor
WT or *Fkbp52*-/-
Injection of minced donor uteri into the recipient peritoneum

Recipient
WT or *Fkbp52*-/-
Evaluation of lesions
0 1 2 (weeks)

Fkbp52 deficiency promotes endometriotic lesions
Fkbp52 deficiency promotes endometriotic stromal cell proliferation and increases recipient-derived microvessel density in ectopic lesions

**FKBP52 protein expression in human endometriosis**

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**Mouse ectopic lesions**

Hemosiderin deposition and hemorrhage

Arrow heads indicate the sites of lesions

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**Microvessel density**

WT-WT/Flk1LacZ

KO-KO/Flk1LacZ
FKBP52 deficiency promotes endometriosis
(AM J Path 173:1747-1757, 2008)

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NRSA/NIDA, NICHD, NIDA

Fkbp52 mRNA expression in human endometriosis

PP: Proliferative Phase
SP: Secretory Phase
Fkbp52 deficiency promotes endometriotic stromal cell proliferation

P4 supplementation fails to restore expression of P4-regulated genes critical for uterine receptivity

Hoxa-10, Ihh and Areg

P4 supplementation restores P4- and estrogen-target gene expression in CD1 Fkbp52 null uteri on day 4