Role of ER Signaling in Fertilization and Early Embryo Development
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Hormonal Control of Embryo Delivery to the Uterus

Disclosure information: Nothing to declare
Epithelial Selective Roles for ERα in Female Reproductive Tract

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Sylvia Hewitt
Infertility in the United States

~46% of women experience difficulties to have their first child after 12 months of trying.

Infertility is defined as lack of pregnancy in the 12 months prior to survey, despite unprotected intercourse with husband in each month.

Global αERKO (Esr1−/−) females
- Infertile
- Hemorrhagic cystic ovaries
- Hypoplastic uteri
- Lack of E2 responsiveness
- Implantation defect

ERα is important for female fertility.

Role of tissue specific ERα in fertility?
Early Embryo Development in the Oviduct

Mouse oviduct

- Infundibulum
- Isthmus
- Ampulla
- Uterus

Mouse oviduct

- Egg
- Ovary
- Fertilization
- Day 0.5
- Day 1
- Day 2
- Day 2.5
- Day 3
- Day 3.5
- Day 4

Human Fallopian tube

- Fimbriae
- Isthmus
- Ampulla
- Uterus

- Day 4.5
- Day 7

How is estrogen involved in these processes?

• Steroid hormone-dependent changes in oviduct cellular morphology and physiology (Hunter 2012).

• Estrogen induces biosynthesis of secretory macromolecules within the oviduct (Buhi et al. 2000).

• Requirement for estrogen-mediated regulation of oviductal physiology to support the initial event of pregnancy in vivo has never been demonstrated.

• Clinical findings: Salpingectomy augmented implantation rates in IVF with hydrosalpinx patients (Daftary et al. 2007), suggesting that aberrant oviductal functions contribute to female infertility.
Loss of uterine epithelial ERα leads to infertility

1. Implantation defect in the absence of epithelial ERα

<table>
<thead>
<tr>
<th>6 months breeding with WT male</th>
<th># Litters/dam</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT</td>
<td>6.4 ± 0.5</td>
</tr>
<tr>
<td>Epithelial ERα KO</td>
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</table>

Specific Loss of Epithelial ERα results in Infertility

Expression of epithelial ERα is required for implantation
Fertilization & embryo development in mice with cell specific deletion of ERα in the oviduct

Loss of epithelial ERα in the oviduct leads to decreased fertilization and increased embryo death
Eggs from epithelial ERαKO females can be fertilized in vitro?

Microenvironment in the oviduct of mice lacking epithelial ERα is not conducive to fertilization & embryo development

IVF = *in vitro* fertilization
Altered protease/inhibitor balance in the absence of epithelial ERα in the oviduct

### Microarray data

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Entrez Gene Name</th>
<th>Fold Epithelial ERαKO/WT</th>
<th>p-value</th>
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<tbody>
<tr>
<td><strong>Proteases</strong></td>
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<tr>
<td>Tmprss15</td>
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<td>Klk8</td>
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<td>7.67E-03</td>
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<td>6.83E-03</td>
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<td>Cma1</td>
<td>Chymase 1, mast cell</td>
<td>2.05</td>
<td>2.53E-02</td>
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<td><strong>Protease inhibitors</strong></td>
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<tr>
<td>Serpinb11</td>
<td>Serine (or cysteine) peptidase inhibitor, clade B (ovalbumin), member 11</td>
<td>-2.10</td>
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<td>Fetub</td>
<td>Fetuin beta</td>
<td>-2.25</td>
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<td>Csta</td>
<td>Cystatin A (stafin A)</td>
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<td>3.80E-03</td>
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<td>Expi</td>
<td>Extracellular proteinase inhibitor</td>
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Could altered protease activity impact fertilization?
Exposure of eggs to excess protease leads to premature cleavage of ZP protein

**Fertilization:**
- ZP protein is cleaved due to a release of protease from eggs.
- ZP cleavage leads to a block to polyspermy.

**Exposure of eggs to excess protease leads to premature cleavage of ZP protein**

Premature cleavage of ZP2 may lead to decreased fertilization

↑protease activity → alter ZP protein of the embryo?
Does increased protease activity disrupt membrane integrity of the embryo and lead to embryo death?

WT

Epithelial ERα KO

Loss of epithelial ERα in the oviduct

Excess protease

Embryo lysis

Lisa Banks, Miranda Bernhardt, Carmen Williams, NIEHS

Winuthayanon et al 2015 eLIFE. 4:e10453
Effect of protease inhibitors on embryo survival

1-cell embryos from WT donors

Transfer into oviducts + PI - PI

Flush embryos

Morula/blastocyst

Underdeveloped embryos

Pseudopregnant recipients

▶ WT

▶ Epithelial

▶ ERαKO

Vaginal Plug

0.5 1.5 2.5 3.5 days
Can inhibition of the protease activity reduce embryo death in epithelial ERαKO?

Inhibition of protease activity improved embryo survival
Fewer number of sperm at the fertilization site

Sperm in the oviduct

WT oviduct

Epithelial ERαKO oviduct

Lisa Banks, NIEHS
MODEL: Requirement for epithelial ERα for fertilization & embryo development

WT oviduct
- Balanced protease activity
- Embryos survive

Epithelial ERα KO oviduct
- Excess proteases
- Zona pellucida damaged
- Embryos die

Lack of signals from epithelial ERα

Stromal ERα KO oviduct
- Stromal ERα are not required for embryo survival

Signals from both epithelial & stromal ERα are properly maintained

Efficient fertilization

Efficient fertilization

Efficient fertilization

Inefficient fertilization

Protease inhibitors
1. Normal Liquefaction
   (low protease – Klk1, Klk1b5, KLK1B5)

2. Fertilization

3. Pre-implantation embryo development
   (high protease – Klk8, Tmprss13)

4. Embryo transport
   (ciliary function – Kif9, Kif19, Foxj1)
Summary & Conclusions

• Loss of epithelial ERα in the oviduct
  • Exposure of WT embryos to the cKO oviduct is detrimental to normal development
    • Disrupts fertilization process
    • Leads to embryonic loss at day 2 of pregnancy
  • Alters dynamic gene expression patterns during fertilization and early pregnancy
  • Reveals an important role for oviductal ERα in maintaining the proper microenvironment required for fertilization and embryo development
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