



OVARIAN CLUB VIII

BUILDING A BRIDGE BETWEEN SCIENCE
AND CLINICAL PRACTICE

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Hormonal Control of Embryo Delivery to the Uterus

Disclosure information: Nothing to declare

<http://oc2016.cme-congresses.com/>

Epithelial Selective Roles for ER α in Female Reproductive Tract



Joy Winuthayanon



Sylvia Hewitt

Estrogens & Female Reproduction



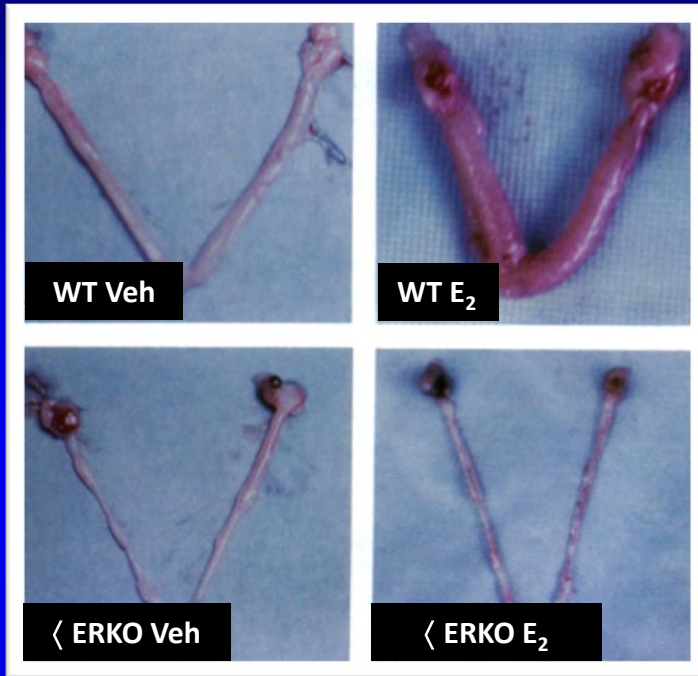
~11% of women ages 15-44 in the US have impaired fertility

**Mouse Models:
Cellular and molecular mechanisms of estrogen signals**

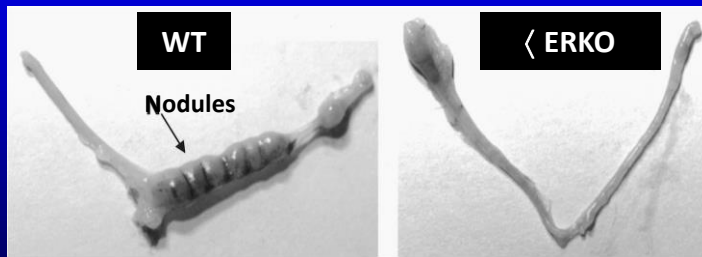


Female reproductive physiology

Role of ER α in Female Reproduction



Lubahn et al 1993 PNAS



Hewitt et al 2002 Biol Reprod

Global < ERKO (*Esr1*^{-/-}) females

- Infertile
- Hemorrhagic cystic ovaries
- Hypoplastic uteri
- Lack of E₂ responsiveness
- Implantation defect

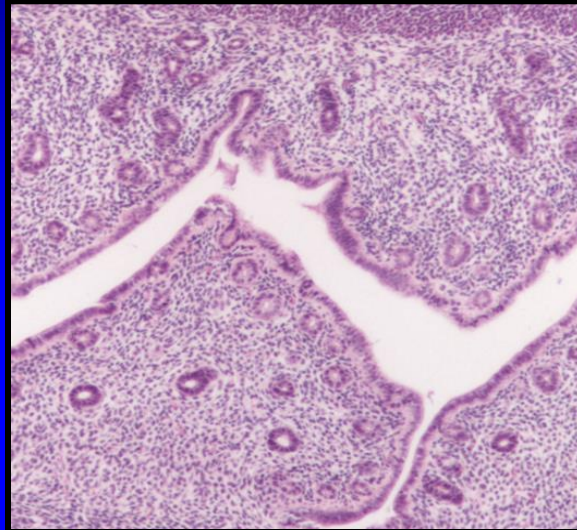
ER α is important for female fertility.



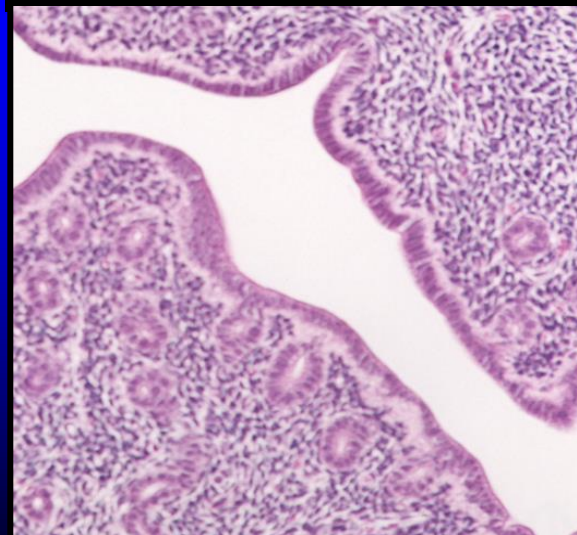
Role of tissue specific ER α in fertility?

Estrogen Stimulated Uterine Proliferation is Epithelial Cell specific

Vehicle

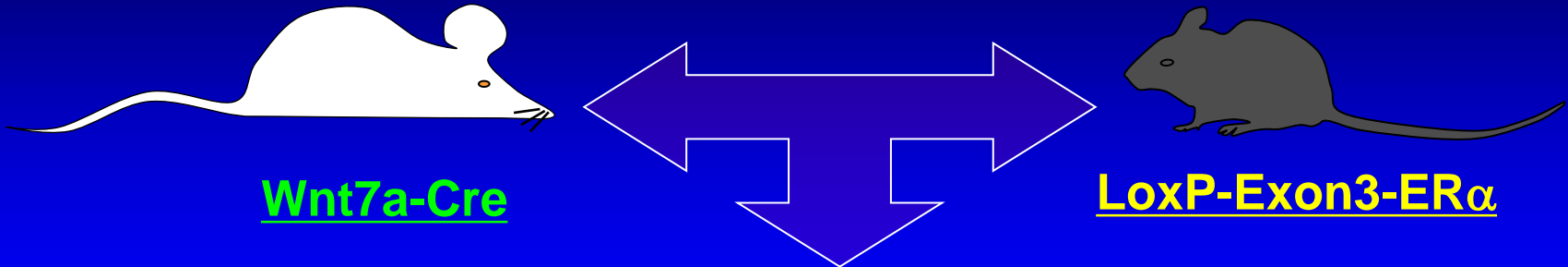


Estradiol

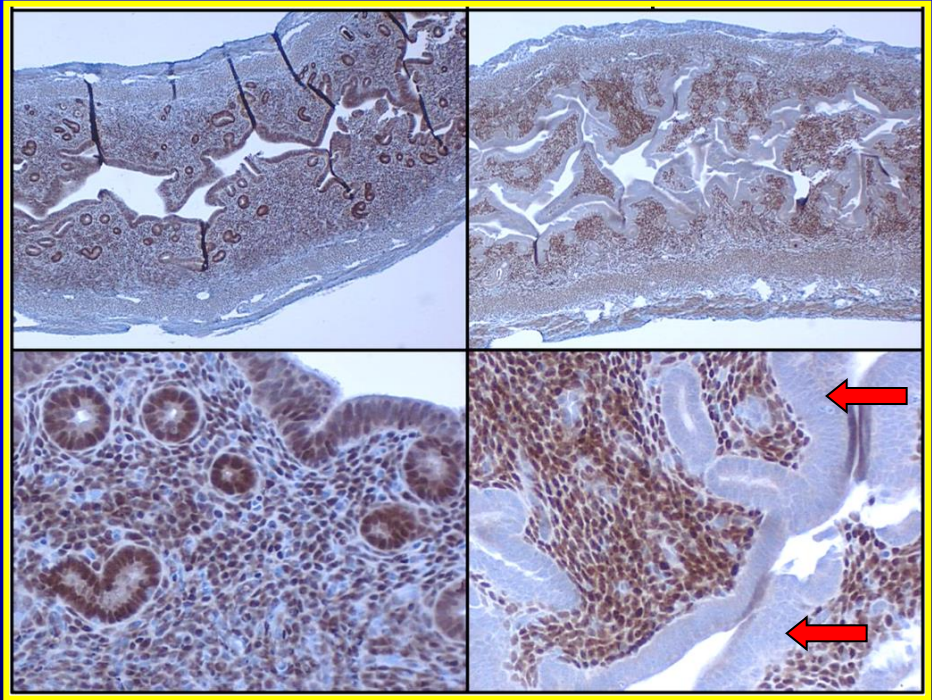


BrDu staining

Uterine Epithelial Specific Knock Out of ER α



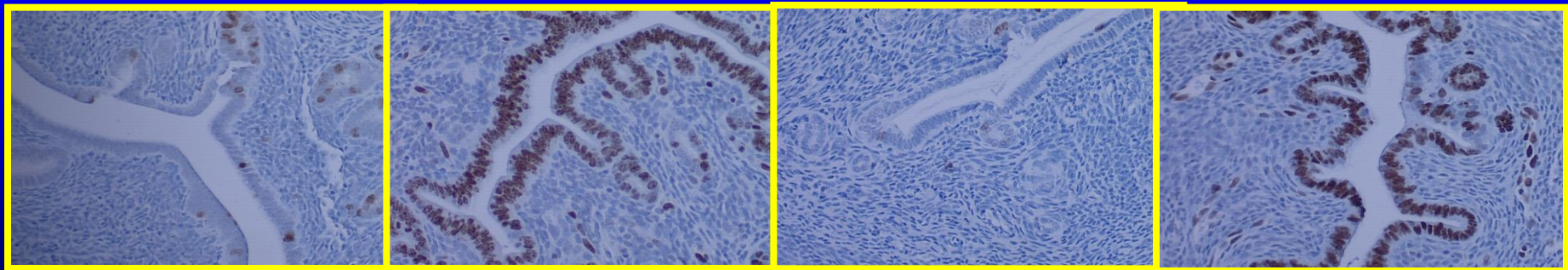
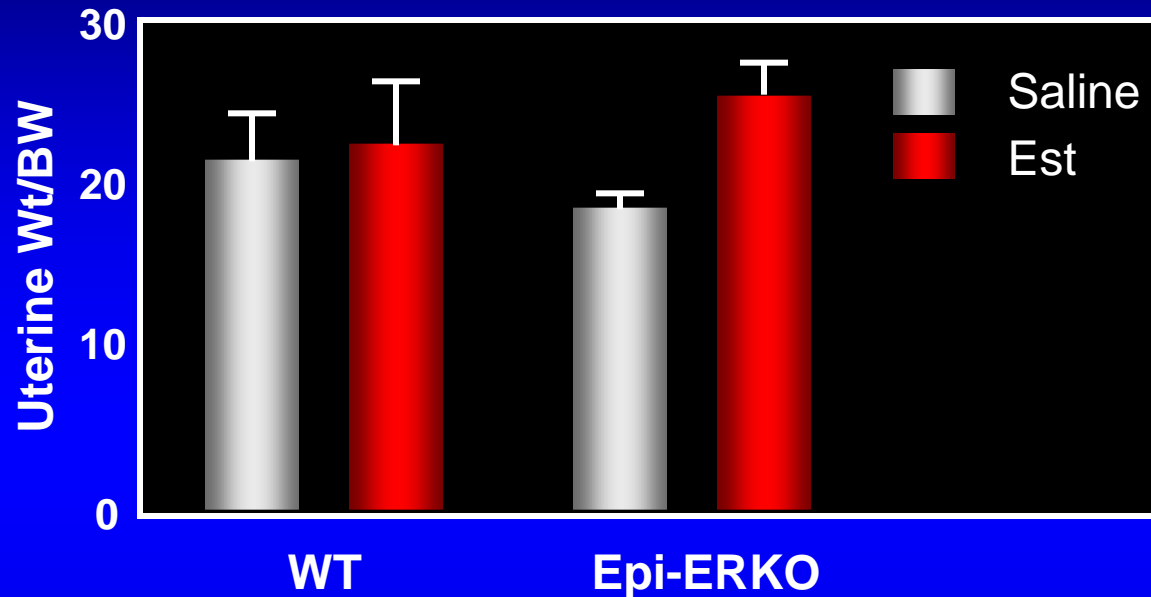
LoxP-Exon3-ER α



UtEpi- α ERKO

ER α ImmunoHistoChemistry

Estrogen Response of UtEpiERKO



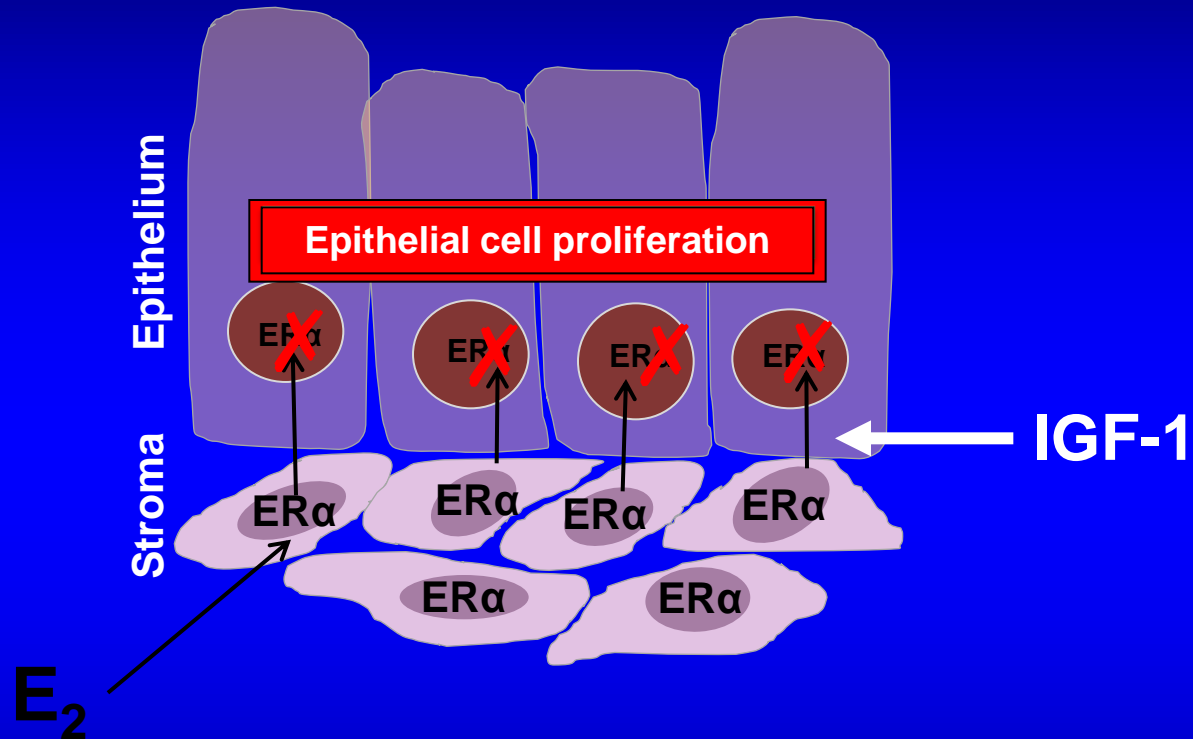
WT-Saline

WT-E2

Epi-ERKO-Saline

Epi-ERKO-E2

Stromal ER α underlying epithelial cells mediates uterine proliferation in response to E₂



Cell-specific roles of ER α during PREGNANCY?



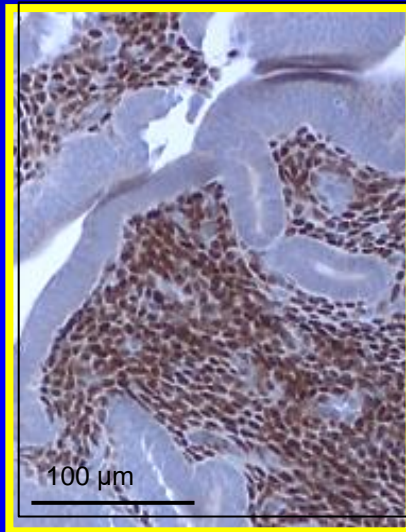
Uterine proliferation is crucial for embryo implantation

Role of ER α in Uterine Proliferative Response

- Estrogen induced uterine proliferation in adults is epithelial specific
- Stromal ER α is needed for E2 mediated proliferation through a paracrine mechanism involving IGF-1, while breast growth requires mammary epithelial ER α
- Uterine epithelial ER α is dispensable for inducing this proliferative response, but required for inhibiting epithelial apoptosis
- IGF-1 can induce the uterine epithelial proliferation independently from ER α expression
- Do uterine diseases such as adenocarcinomas, fibroids or endometriosis respond to an ER independent signal?

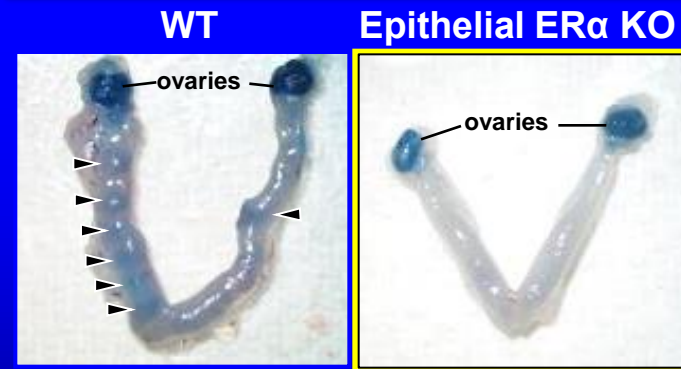
Loss of uterine epithelial ER α leads to infertility

Epithelial ER α KO



6 months breeding with WT male	# Litters/dam
WT	6.4 \pm 0.5
Epithelial ER α KO	0

1. Implantation defect in the absence of epithelial ER α

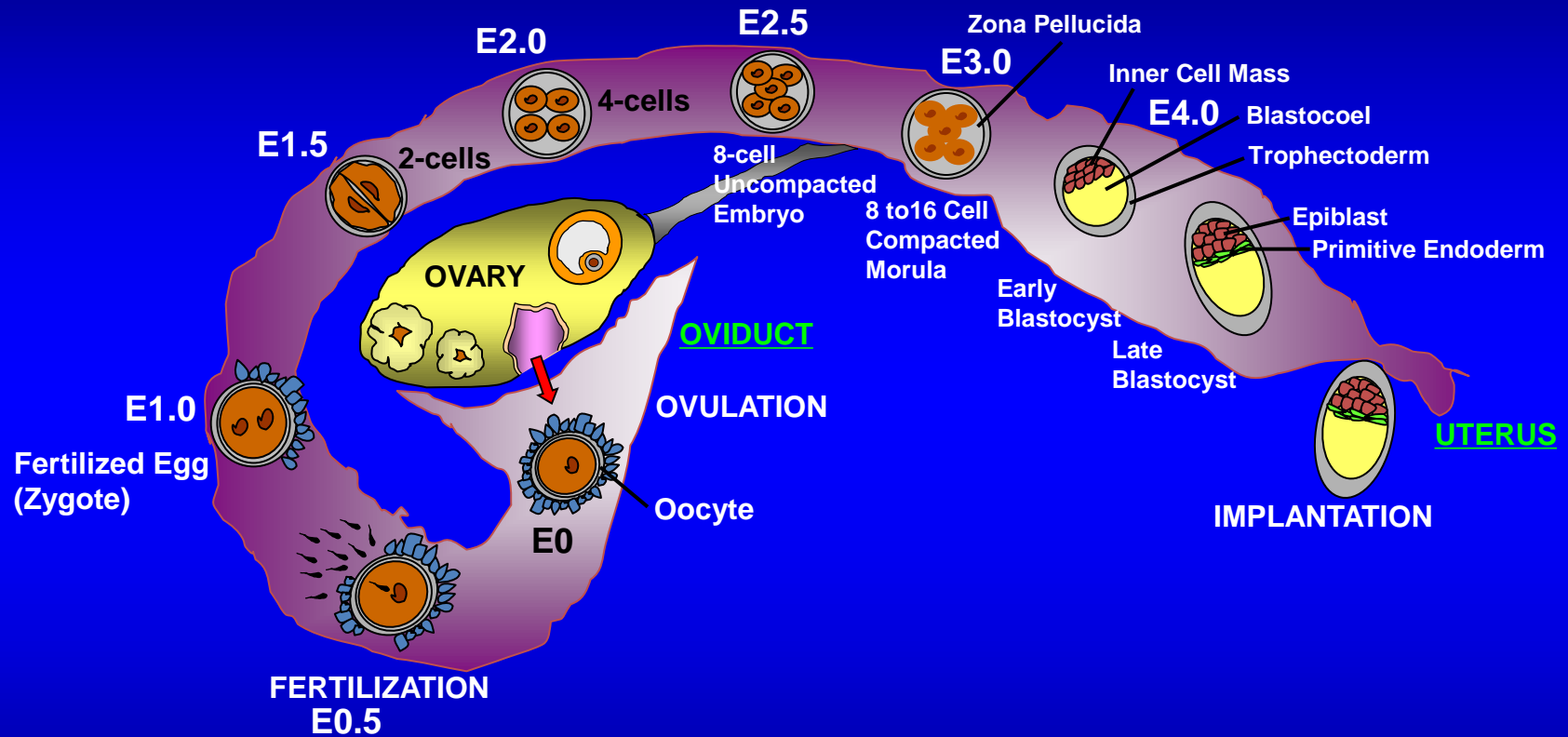


▷ Implantation sites indicated by blue dye

2. No embryos present in the epithelial ER α KO uterus

Expression of epithelial ER α is required for implantation

Embryo Development in the Female Reproductive Tract



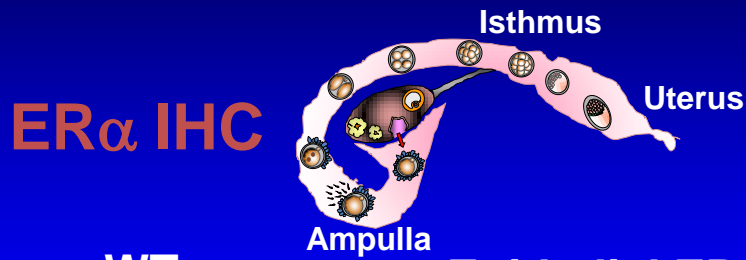
Tissue specific ER α deletion
 Uterine & oviductal epithelial ER α knockout

***Wnt7a*^{Cre/+}:*Esr1*^{fl/fl} females are Infertile**

Estrogen Receptor Functions in the Oviduct

- 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.

Cell-type specific deletion of ER α in the oviduct: epithelial vs. stromal cells

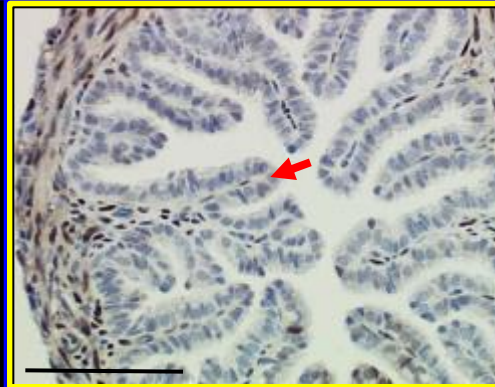
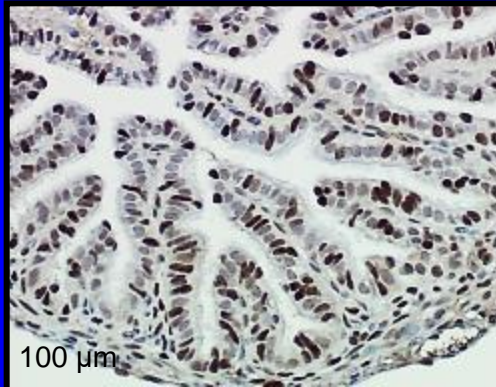


WT

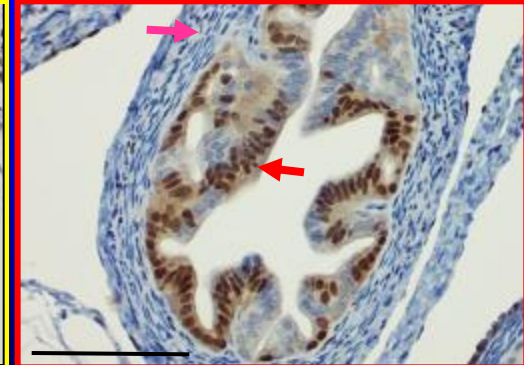
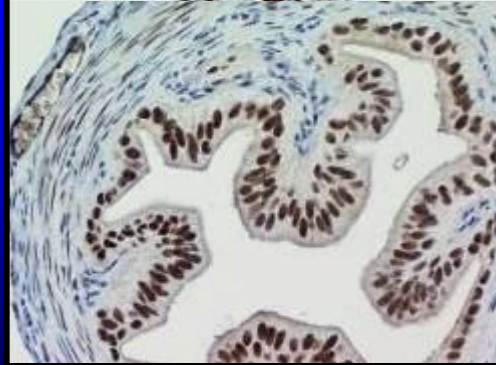
Epithelial ER α KO

Stromal ER α KO

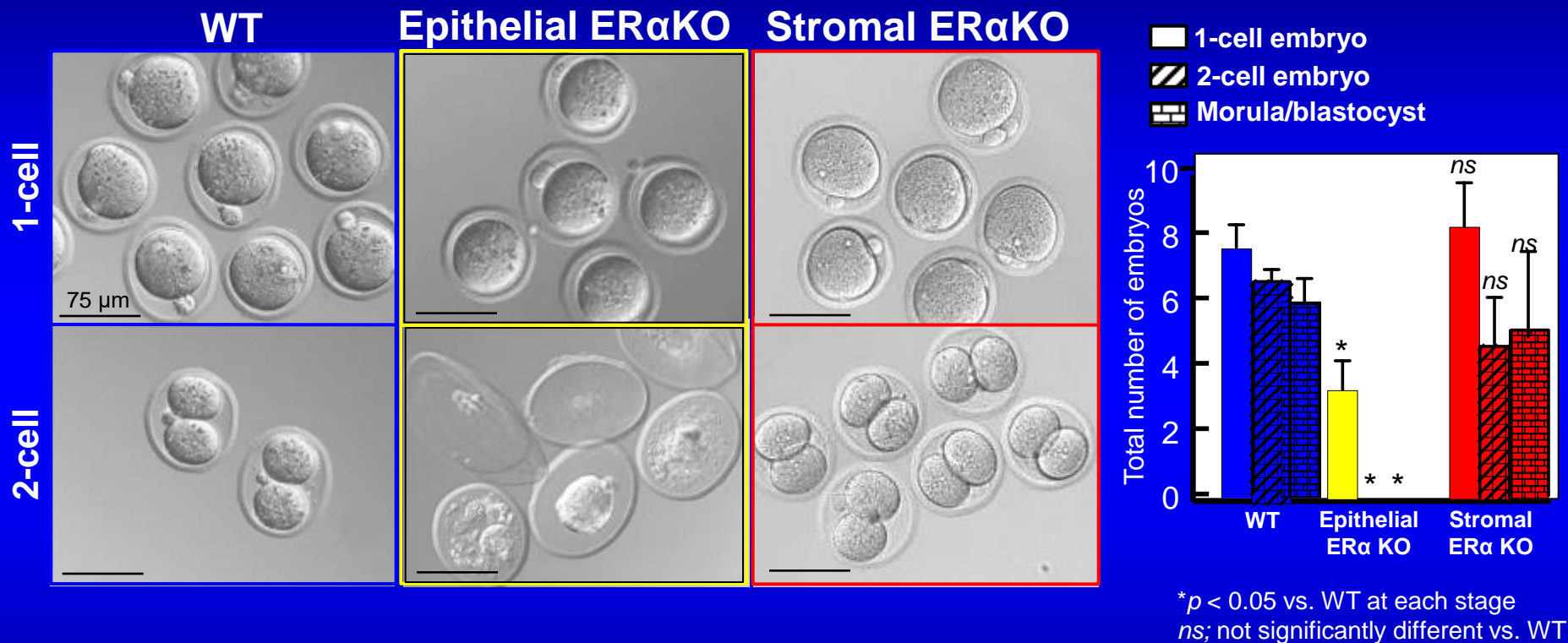
Ampulla



Isthmus

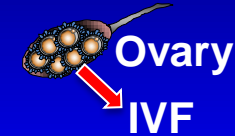
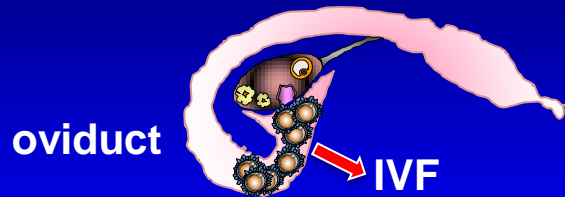


Fertilization & embryo development in mice with cell specific deletion of ER α in the oviduct

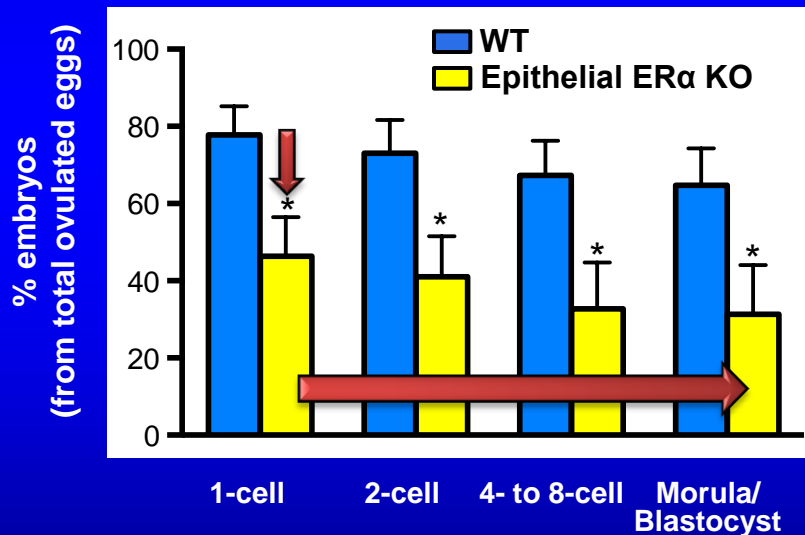


Loss of epithelial ER α in the oviduct leads to decreased fertilization and increased embryo death

Can the eggs from females with epithelial ER α KO be fertilized *in vitro*?

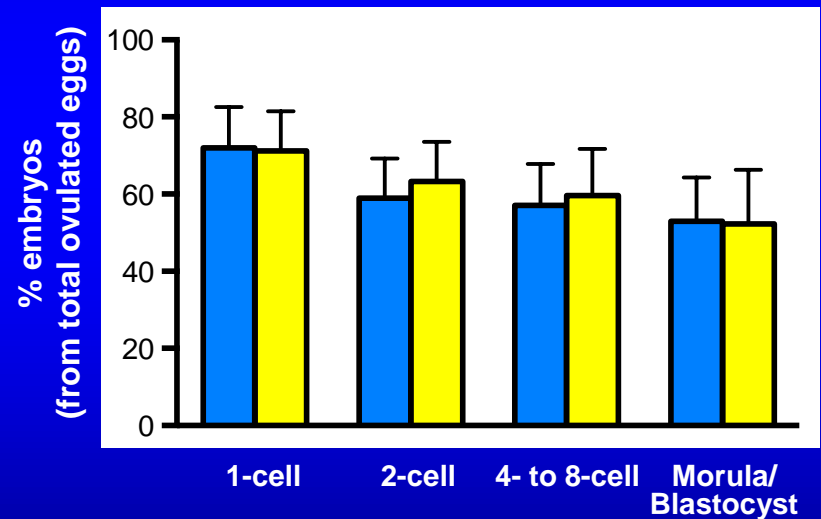


IVF: eggs collected from the oviducts



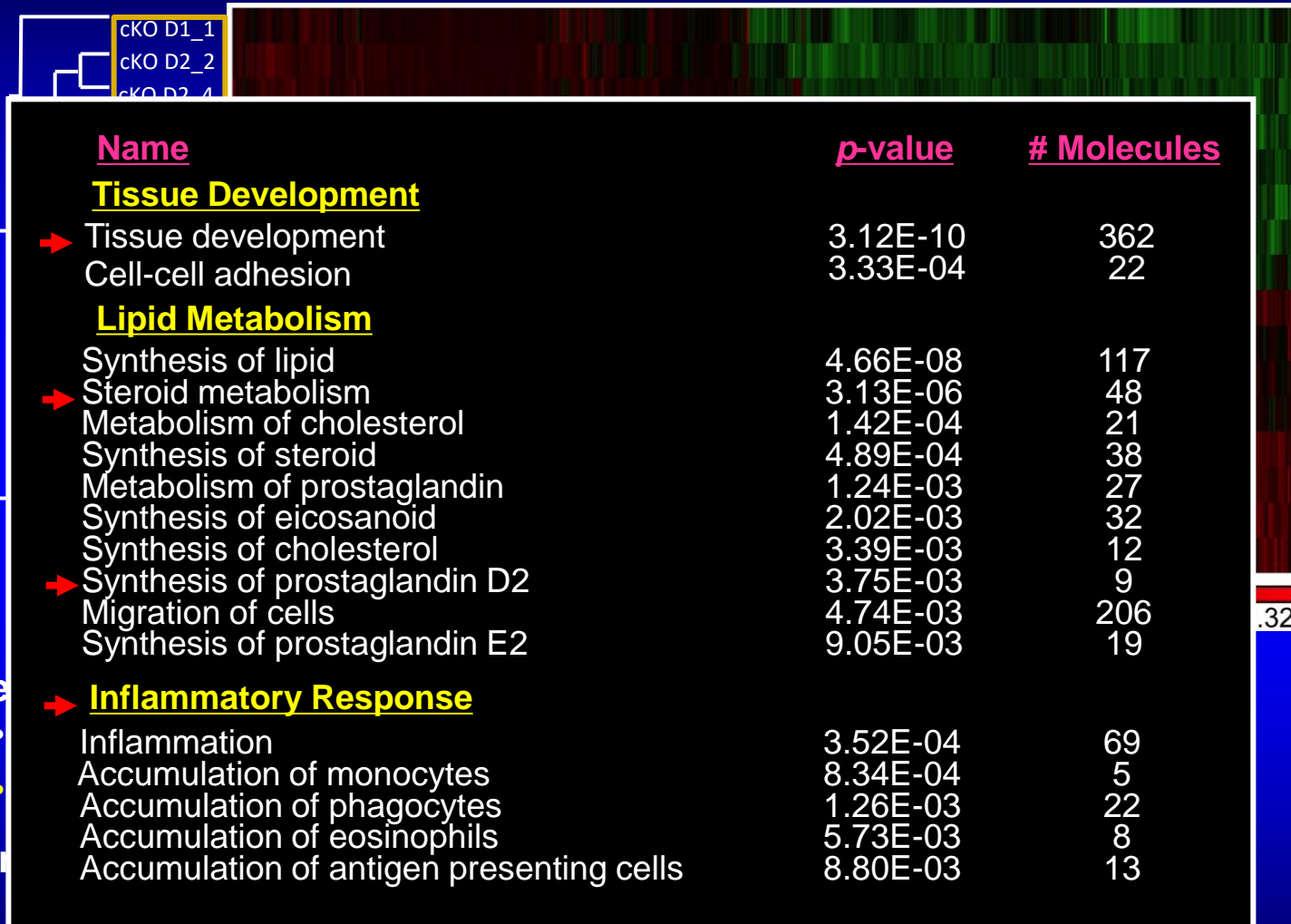
* $p < 0.05$ vs. WT at each stage

IVF: eggs collected from the ovaries



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

Altered gene expression pattern in cKO oviduct



1) Hierarchical clustering

2) Dynamic time warping

3) Group of genes over or under expressed in WT did not occur in cKO

Altered transcriptional regulation may contribute to the infertility

Altered protease/inhibitor balance in the absence of epithelial ER α in the oviduct

Microarray data

Symbol	Entrez Gene Name	Fold Epithelial ER α KO/WT	p-value
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Proteases

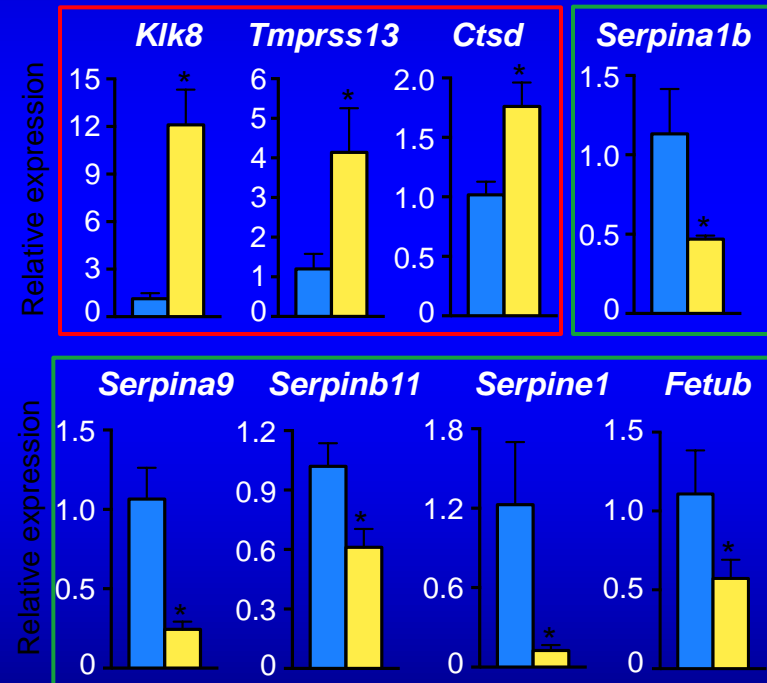
<i>Tmprss15</i>	Transmembrane protease, serine 15	16.41	2.33E-09
<i>Klk8</i>	Kallikrein related-peptidase 8	10.34	9.85E-04
<i>Prss42</i>	Protease, serine, 42	9.63	7.67E-03
<i>Prss7</i>	Protease, serine, 7 (enterokinase)	8.54	2.34E-02
<i>Klk9</i>	Kallikrein related-peptidase 9	5.62	7.06E-06
<i>Prss33</i>	Protease, serine, 33	5.35	5.07E-04
<i>Prss51</i>	Protease, serine, 51	2.96	1.65E-02
<i>Prss41</i>	Protease, serine, 41	2.69	1.36E-04
<i>Klk7</i>	Kallikrein related-peptidase 7	2.63	2.55E-05
<i>Prss32</i>	Protease, serine, 32	2.45	3.10E-02
<i>Tmprss13</i>	Transmembrane protease, serine 13	2.14	6.83E-03
<i>Cma1</i>	Chymase 1, mast cell	2.05	2.53E-02

Protease inhibitors

<i>Serpinb11</i>	Serine (or cysteine) peptidase inhibitor, clade B (ovalbumin), member 11	-2.10	2.99E-03
<i>Fetub</i>	Fetuin beta	-2.25	1.14E-02
<i>Csta</i>	Cystatin A (stafin A)	-2.65	3.80E-03
<i>Serpine1</i>	Serine (or cysteine) peptidase inhibitor, clade E, member 1	-2.69	4.02E-02
<i>Serpina3b</i>	Serine (or cysteine) peptidase inhibitor, clade A, member 3B	-3.30	2.49E-02
<i>Serpina1b</i>	Serine (or cysteine) peptidase inhibitor, clade A, member 1B	-3.49	5.80E-03
<i>Serpina1e</i>	Serine (or cysteine) peptidase inhibitor, clade A, member 1E	-4.04	4.02E-02
<i>Serpina9</i>	Serine (or cysteine) peptidase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 9	-4.22	2.52E-03
<i>Expi</i>	Extracellular proteinase inhibitor	-13.69	1.96E-06

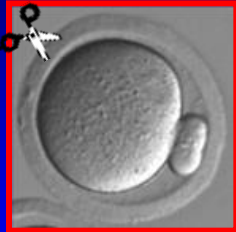
Real-time PCR analysis

■ WT
■ Epithelial ER α KO



Could altered protease activity impact fertilization?

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

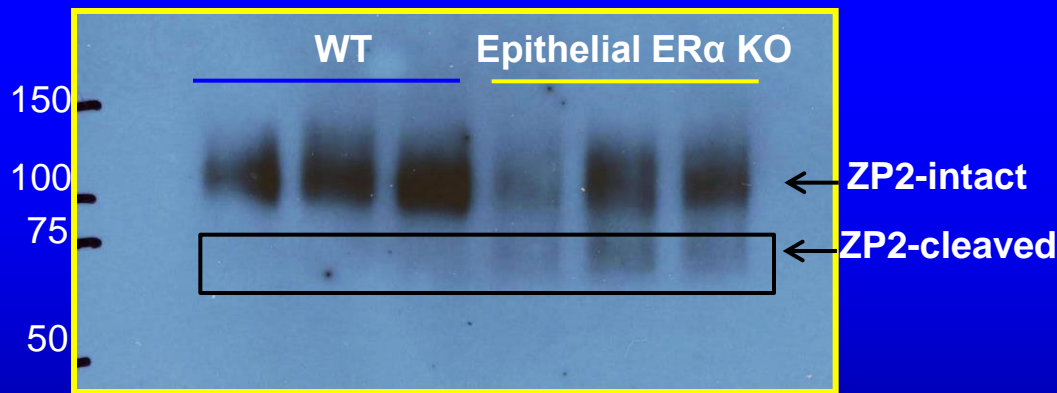


Fertilization:

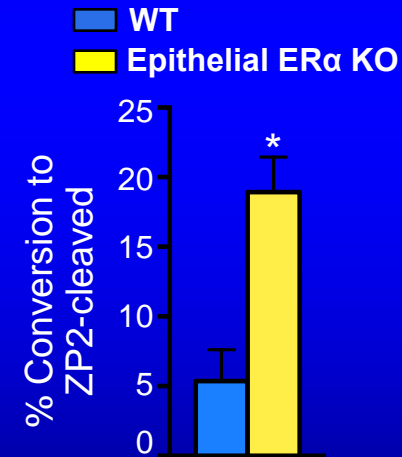
- ZP protein is cleaved due to a release of protease from eggs.
- ZP cleavage leads to a block to polyspermy.



Eggs from oviducts → WB: ZP2 protein
(4 h after ovulation)



ZP2 Ab: Jurrien Dean, NIDDK

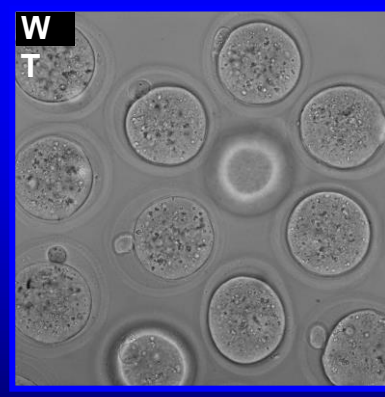
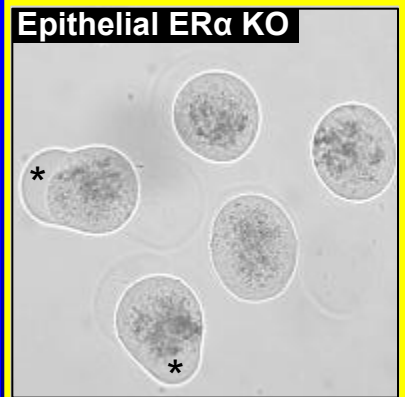
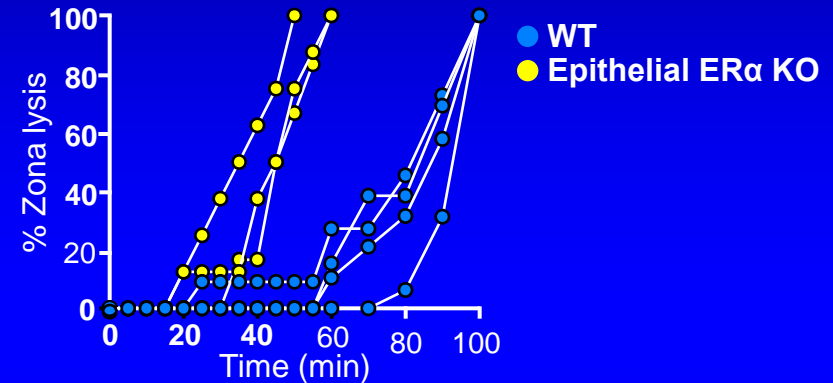
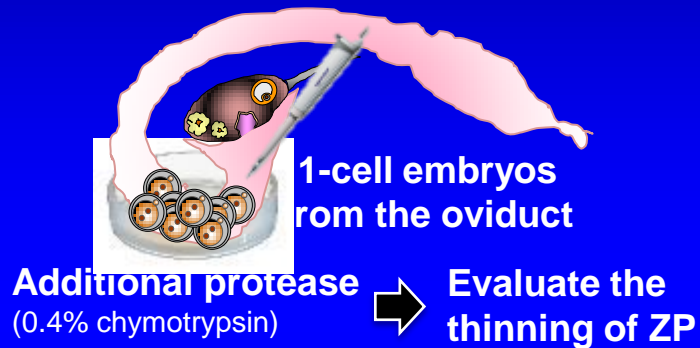


Premature cleavage of ZP2 may lead to decreased fertilization

↑ protease activity → alter ZP protein of the embryo?

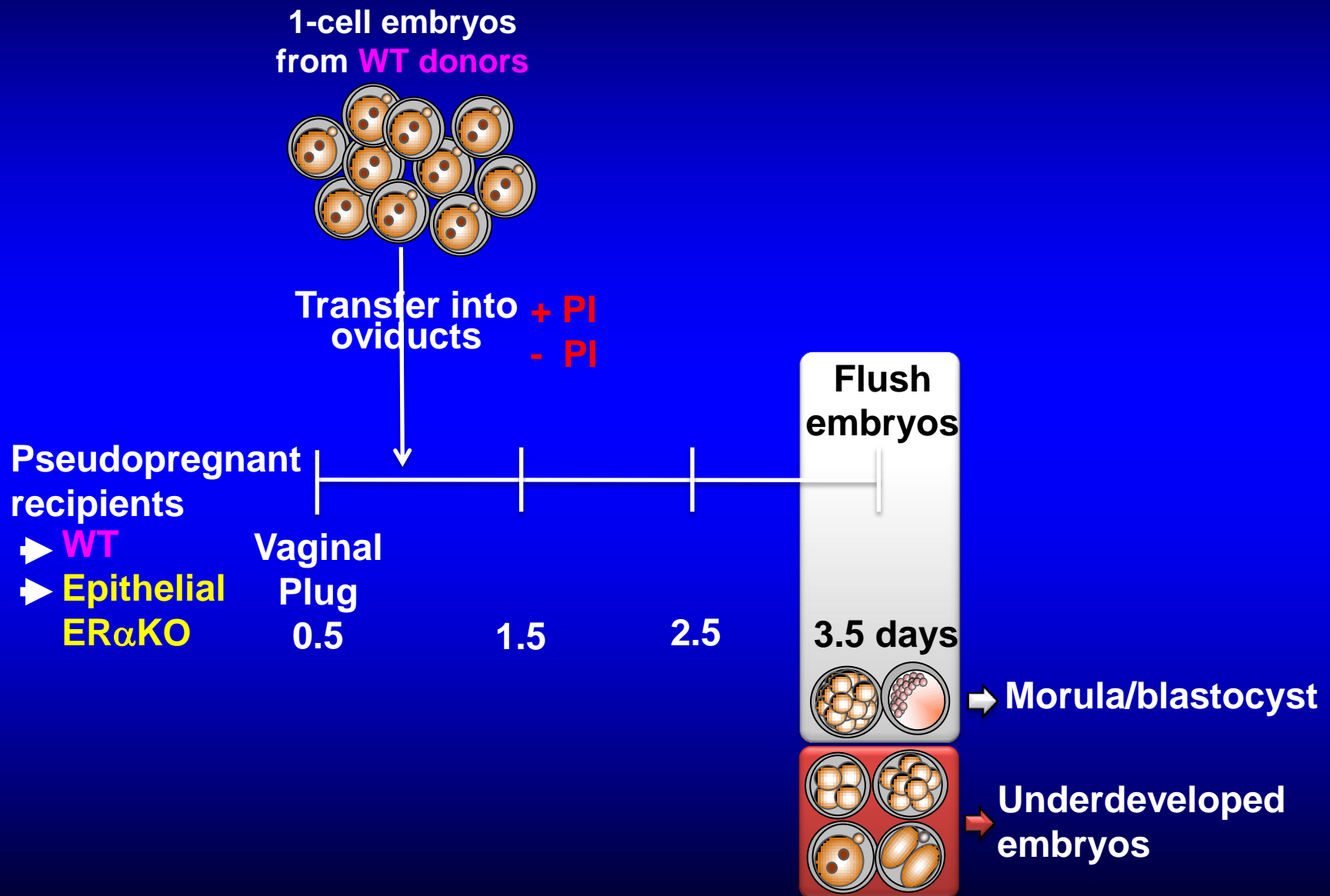
Increased protease activity leads to a thinning of zona and contributes to embryo death?

- ZP thinning ($Zp2^{-/-}$) or removal is associated with embryo death prior to 2-cell stage in the oviduct (Modlinski *et al* 1970, Rankin *et al* 1996)

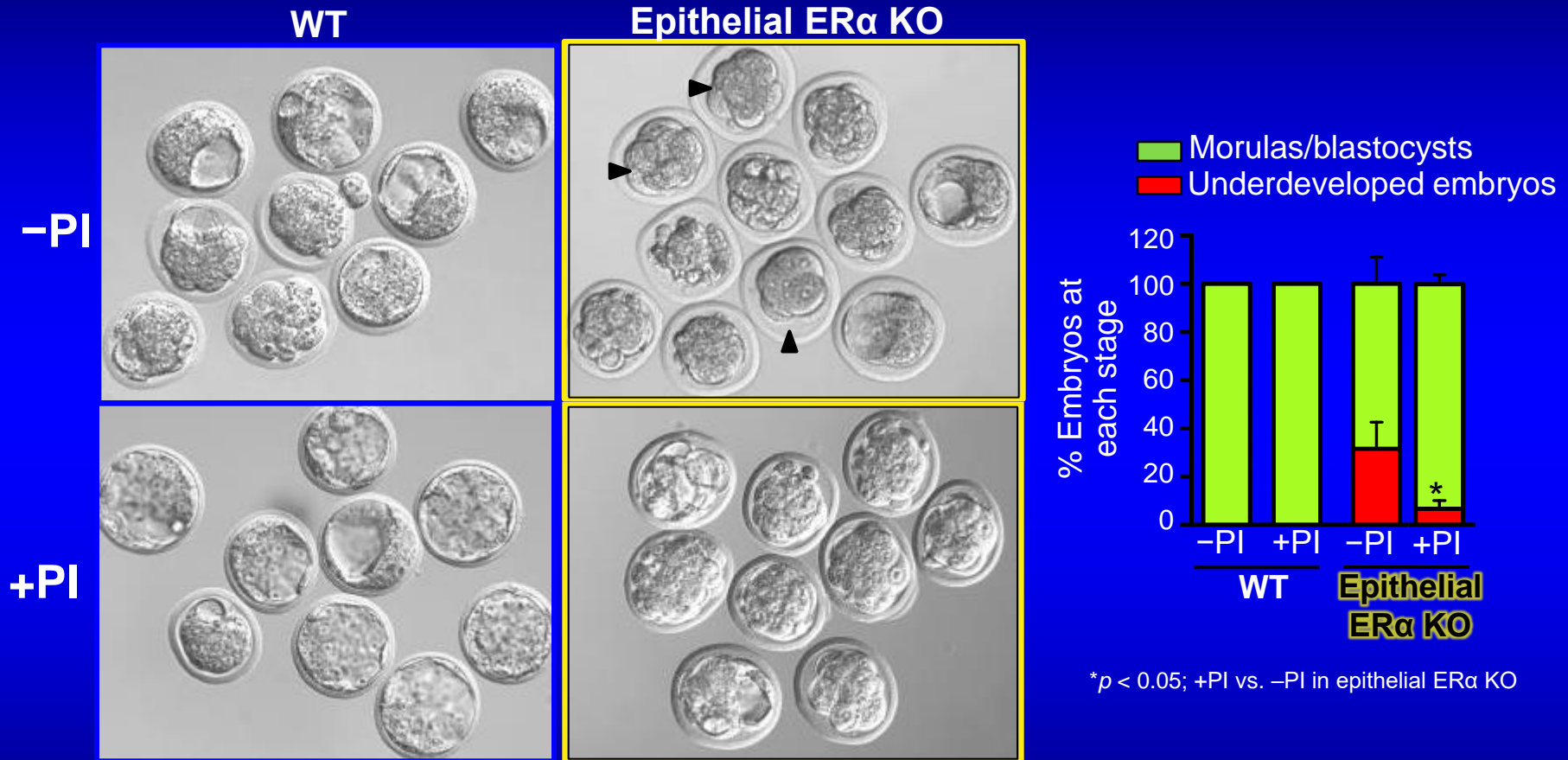


Loss of Epithelial ERα In the Oviduct → Excess protease → Embryo lysis

Effect of protease inhibitors on embryo survival

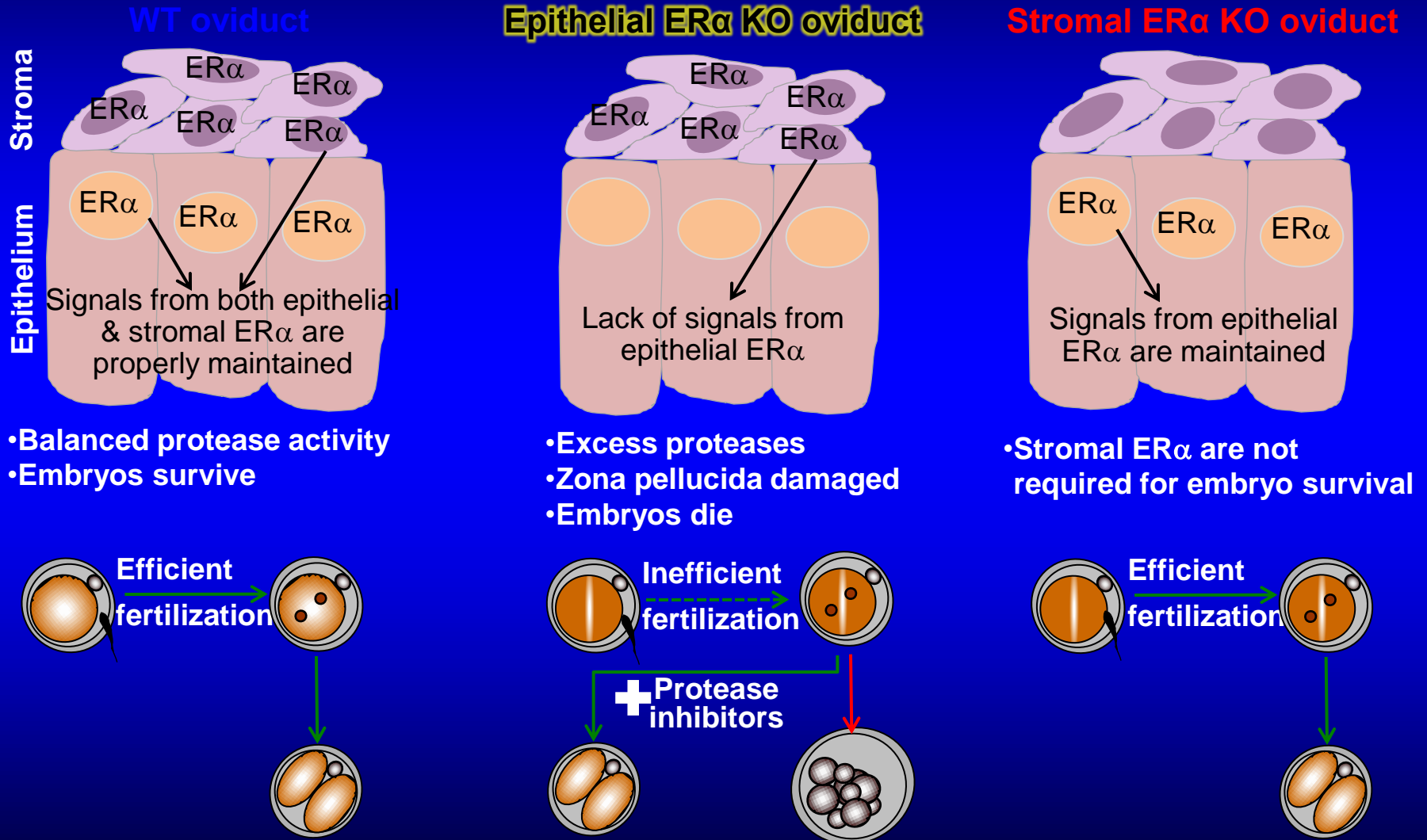


Can inhibition of the protease activity reduce embryo death in epithelial ER α KO?



Inhibition of protease activity improved embryo survival

MODEL: Requirement for epithelial ER α for fertilization & embryo development



- Balanced protease activity
- Embryos survive

- Excess proteases
- Zona pellucida damaged
- Embryos die

- Stromal ER α are not required for embryo survival

Summary & Conclusions

- **Loss of epithelial ER α 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 ER α in maintaining the proper microenvironment required for fertilization and embryo development**
- **Hydrosalpinges ? (Abnormal Innate Immune Mediators)**

- Acknowledgements -



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