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How pregnancy at early age protects against breast cancer

Fabienne Meier-Abt, MD PhD

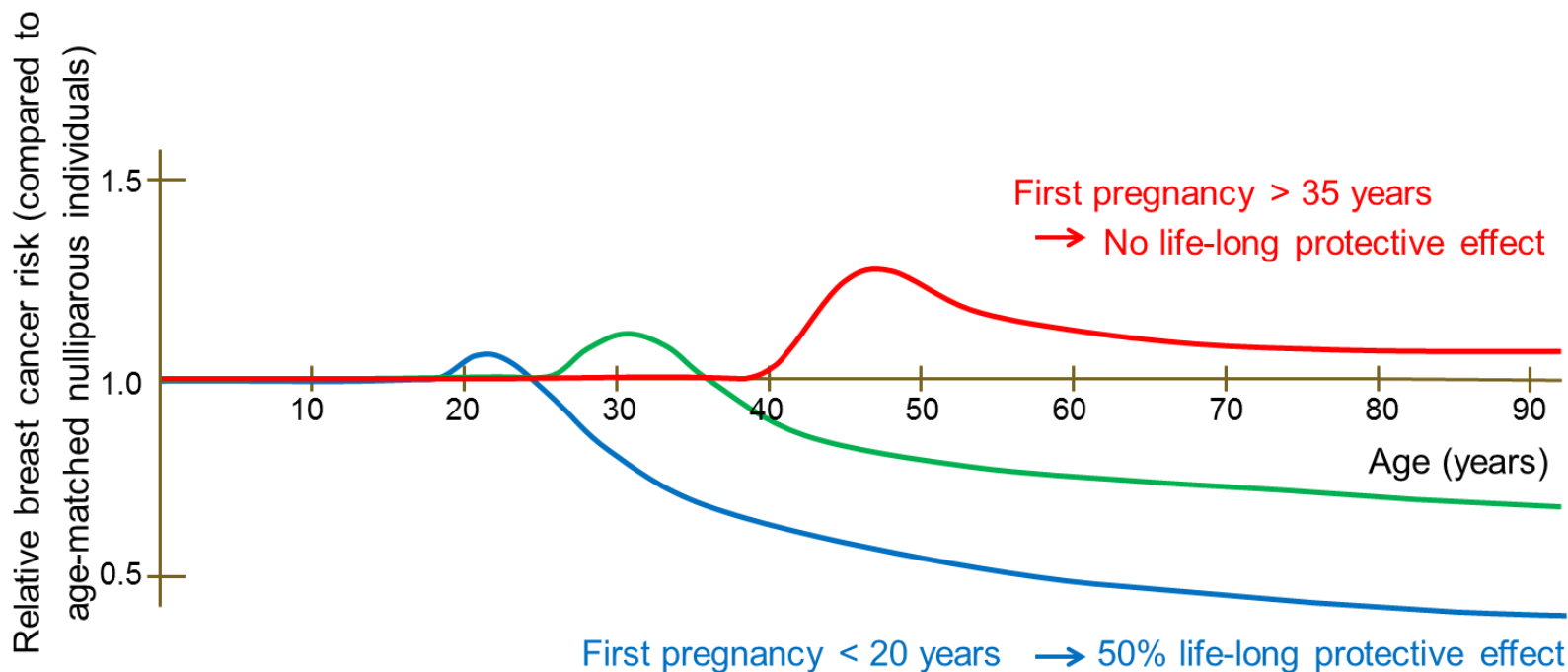
Background:

Early age pregnancy protects against breast cancer.

First pregnancy < 20 years

First pregnancy > 20, < 30 years

First pregnancy > 35 years



MacMahon, B. et al. 1970, Lambe, M. et al. 1996

Background:

Early age pregnancy protects against breast cancer.
Rodents can be used as model system.

First pregnancy < 20 years
First pregnancy > 20, < 30 years
First pregnancy > 35 years

compared to
individuals)

1.5

First pregnancy > 35 years

In rodents: early age pregnancy



>75% protective effect against
mammary tumors

Medina, D. 2005

Relative breast
age-matched

0.5

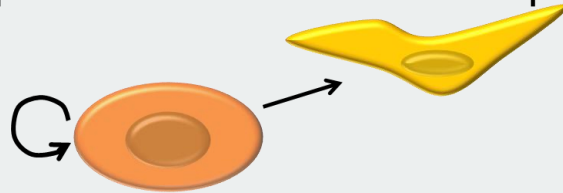
First pregnancy < 20 years → 50% life-long protective effect

MacMahon, B. et al. 1970, Lambe, M. et al. 1996

Background:

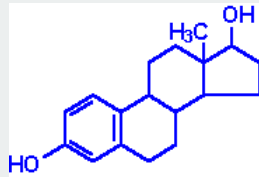
Hypothetical mechanisms:

- change in the proliferation/differentiation potential of specific mammary epithelial cells



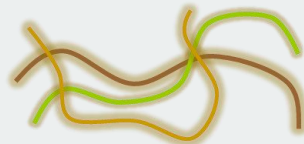
Russo, J. et al. 2005
Siwko, S.K. et al. 2008
Britt, K.L. et al. 2009

- systemic changes in circulating hormones



Thordarson, G. et al. 1995

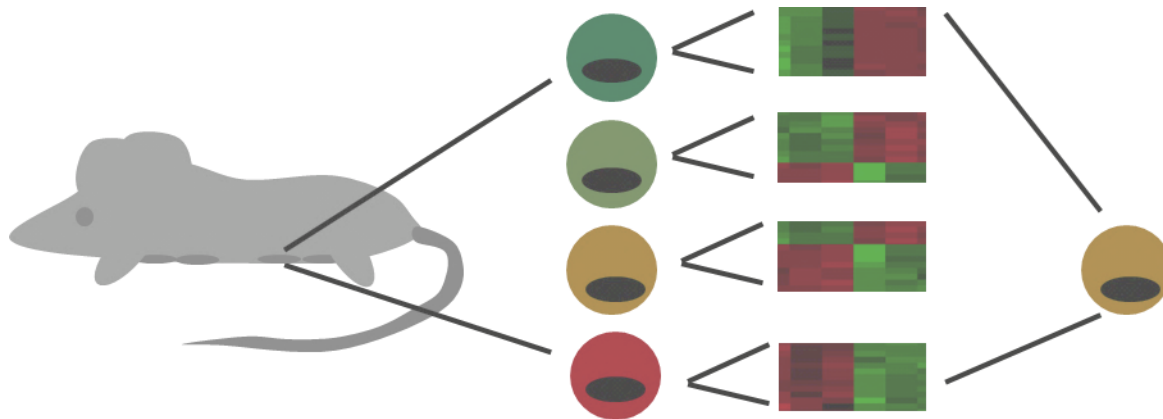
- changes in the stromal composition of the mammary gland



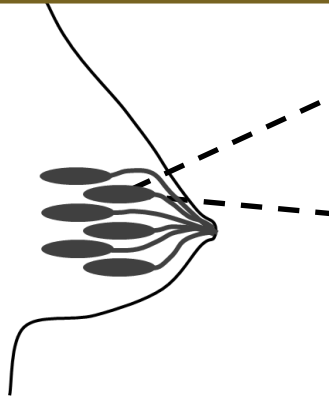
Schedin, P. et al. 2004

Research Question:

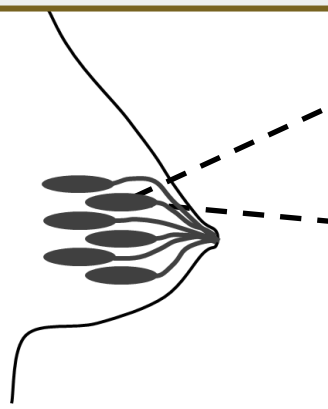
What is the effect of an early pregnancy on the gene expression profile and on the proliferation/differentiation potential of the various mouse mammary epithelial cell subpopulations?



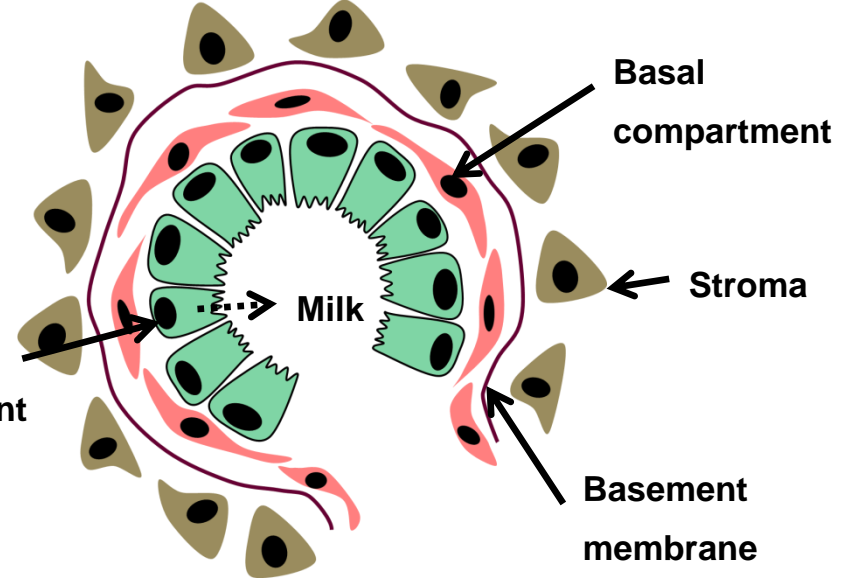
Mammary Cell Type Hierarchy:



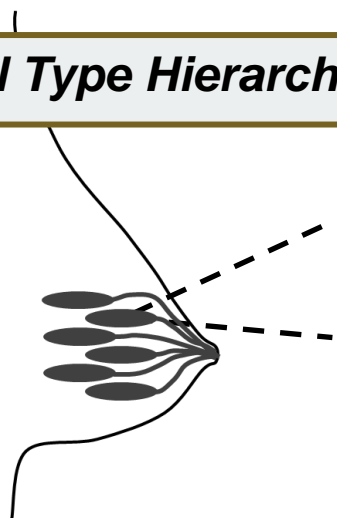
Mammary Cell Type Hierarchy:



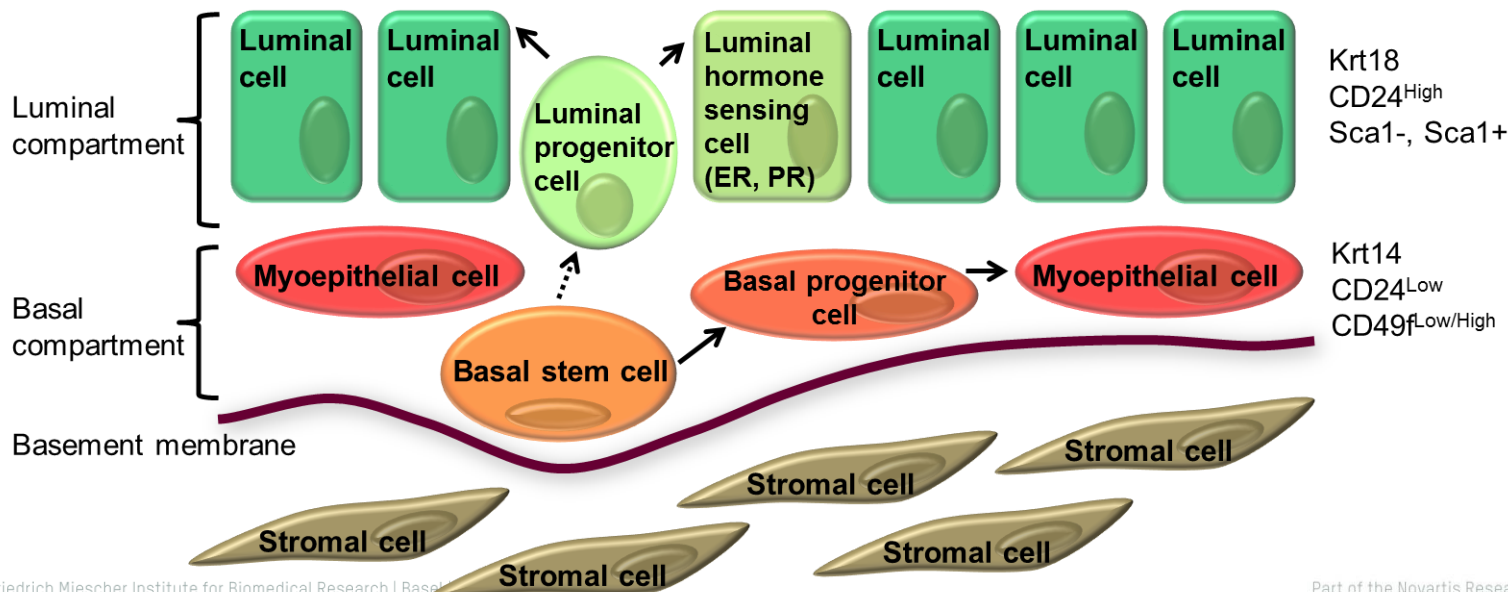
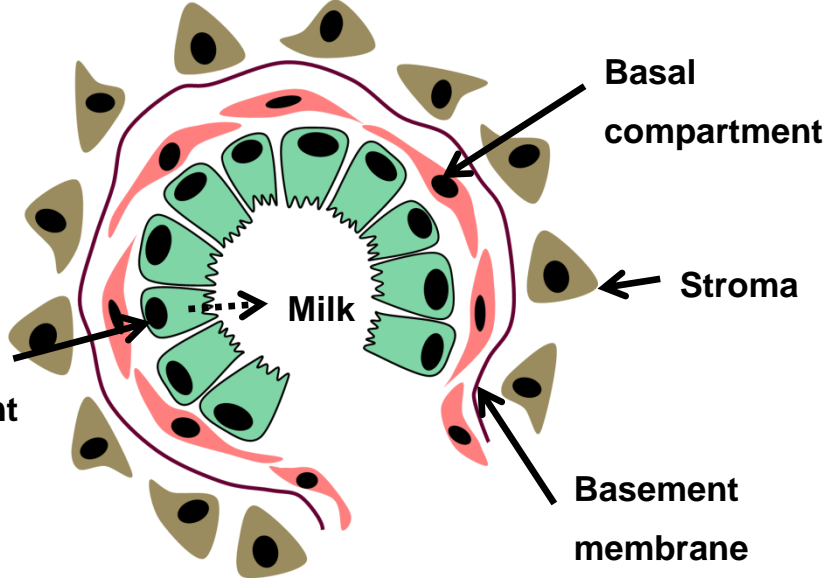
Luminal compartment



Mammary Cell Type Hierarchy:



Luminal compartment

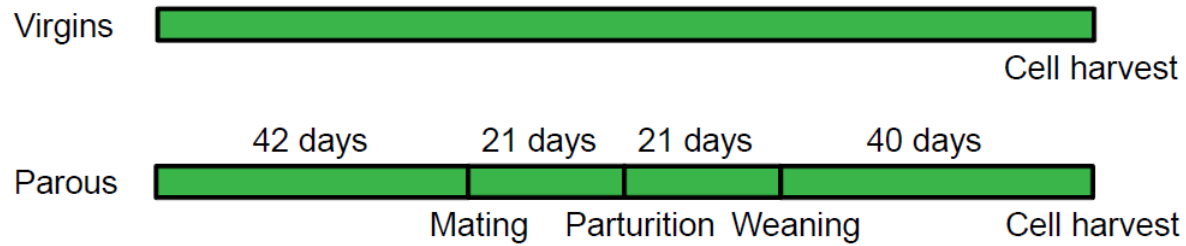


Methodology:

- 1. Isolation of mammary epithelial cell subpopulations by FACS**
- 2. Transcriptome and bioinformatic transcription factor activity analysis**
- 3. Ingenuity IPA and GSEA analysis**
- 4. Colony formation assay**
- 5. Mammary gland reconstitution assay**
- 6. Immunohistochemistry for progesterone receptor**
- 7. Rescue experiments**

Methods:

Early age parturition protocol



Results

- I – Transcriptome analysis in mammary epithelial cell subpopulations from parous and age-matched virgin control mice**

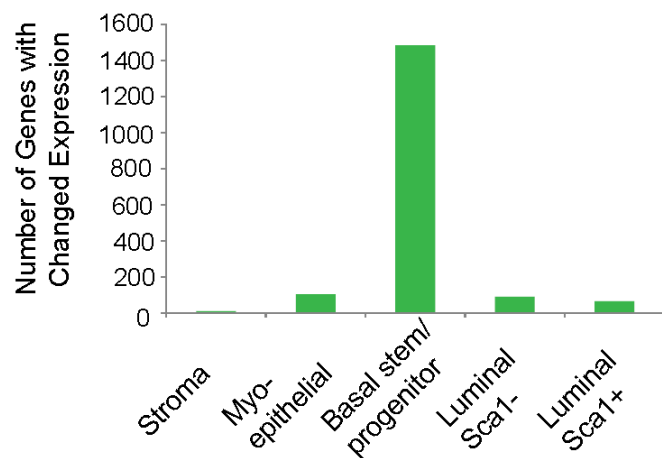
- II – Effects of early parity on the clonogenic and proliferation potential of basal stem/progenitor cells**

- III – Putative mechanism of early parity-induced biofunctional alterations in basal mammary stem/progenitor cells**

- IV – Duration and age dependency of early parity-induced changes**

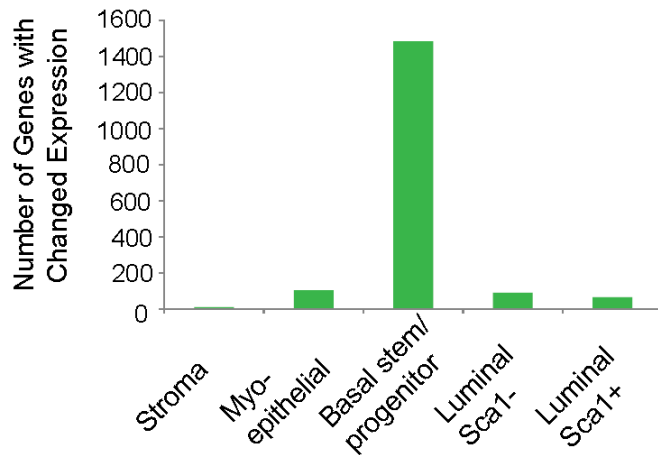
Results I:

Early age pregnancy-induced changes in gene expression in murine mammary epithelial cell subpopulations.

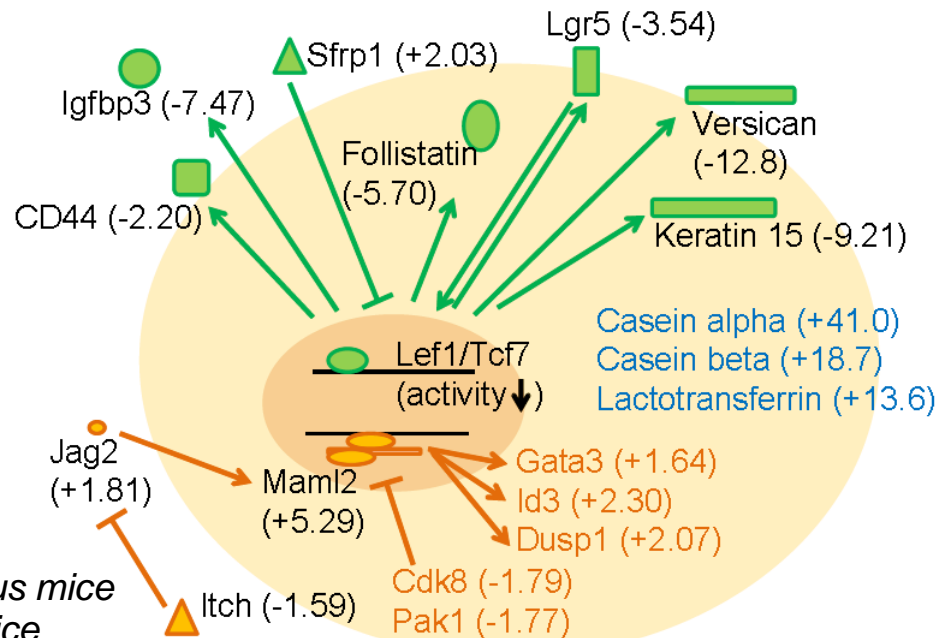


Results I:

Upregulation of differentiation genes (blue), decreased Wnt signaling (green) and increased Notch signaling (orange) in basal stem/progenitor cells from parous mice.



Gene expression in basal stem/progenitor cells from parous mice as compared to virgin control mice



Results I:

Upregulation of differentiation genes (blue), decreased Wnt signaling (green) and increased Notch signaling (orange) in basal stem/progenitor cells from parous mice.



Wnt signaling:

major cell fate determining pathway linked to cell proliferation and carcinogenesis in basal stem/progenitor cells in the mammary gland

Notch signaling:

major cell fate determining pathway linked to reduced proliferation in basal stem/progenitor cells in the mammary gland

Gene expression in basal stem/progenitor cells from parous mice as compared to virgin control mice



Results

- I – Transcriptome analysis in mammary epithelial cell subpopulations from parous and age-matched virgin control mice**

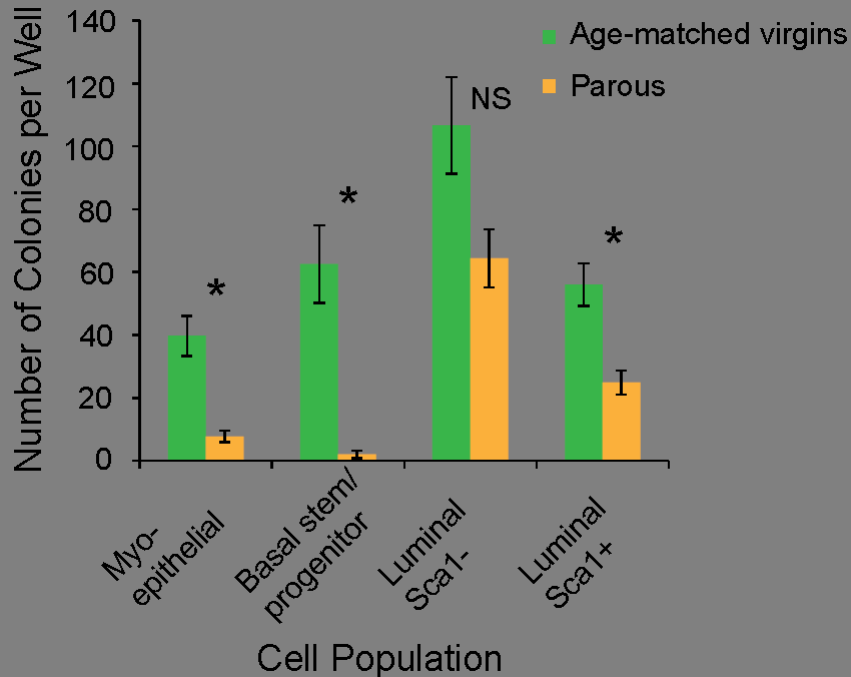
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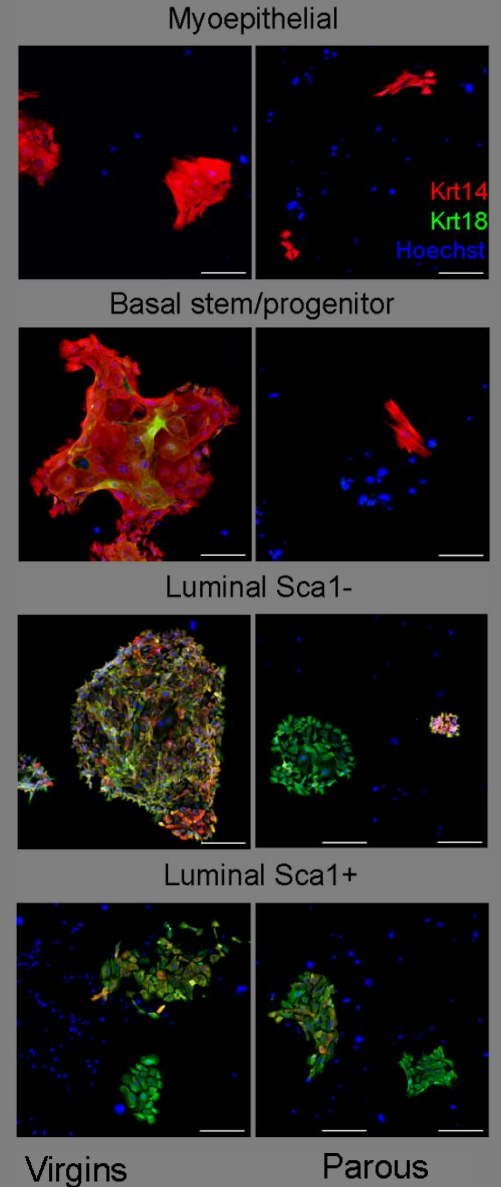
- IV – Duration and age dependency of early parity-induced changes**

Results II:

Early parity decreases the number of cells with colony formation capacity with the most prominent effect in basal stem/progenitor cells.

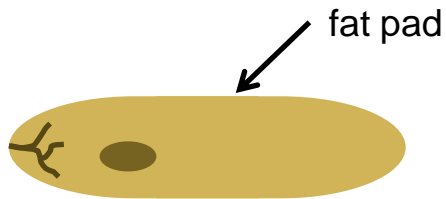


* $P < 0.015$



Results II:

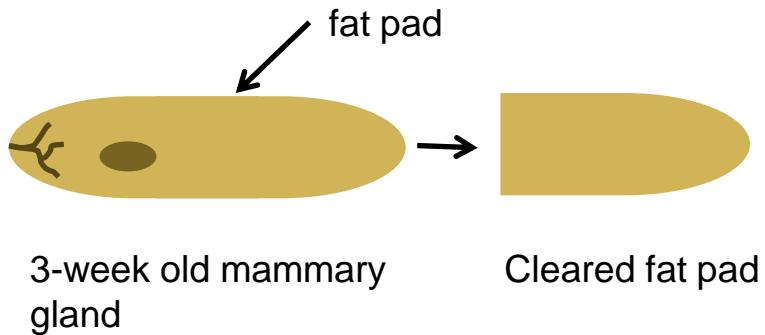
Early parity decreases the *in vivo* reconstitution efficiency but not the number of mammary repopulating units (MRUs) of basal stem/progenitor cells.



3-week old mammary
gland

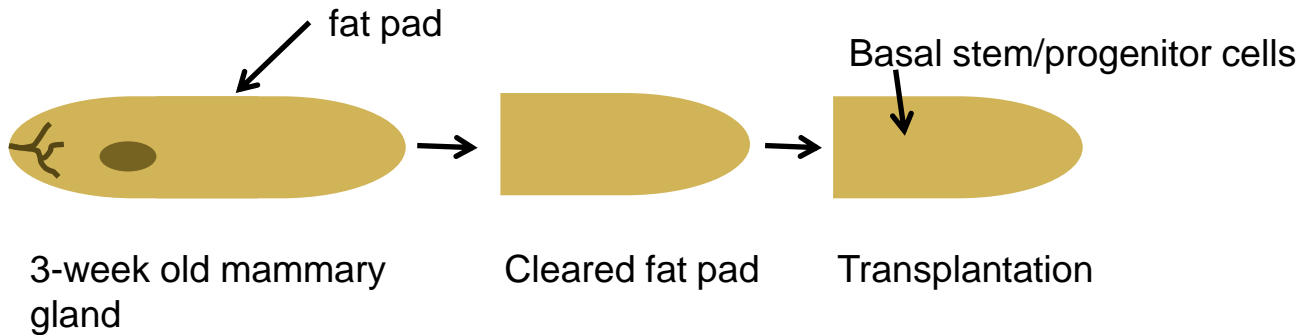
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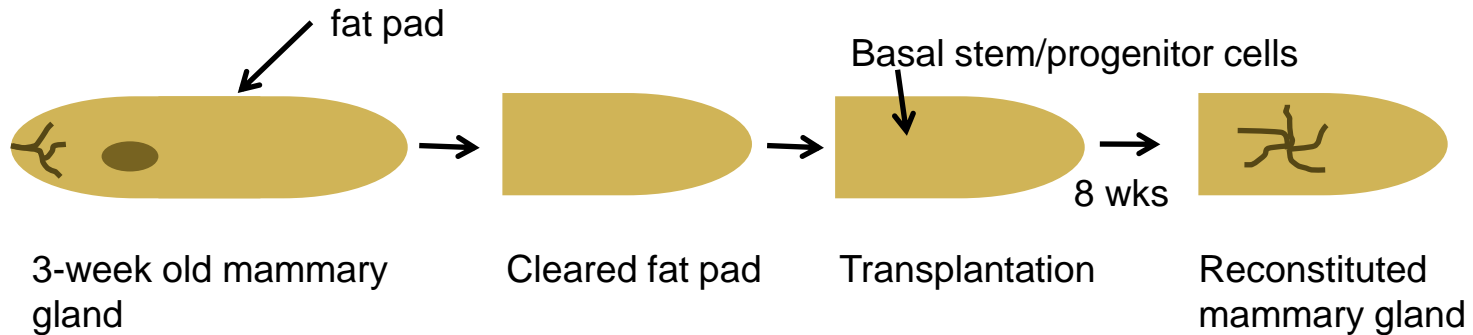
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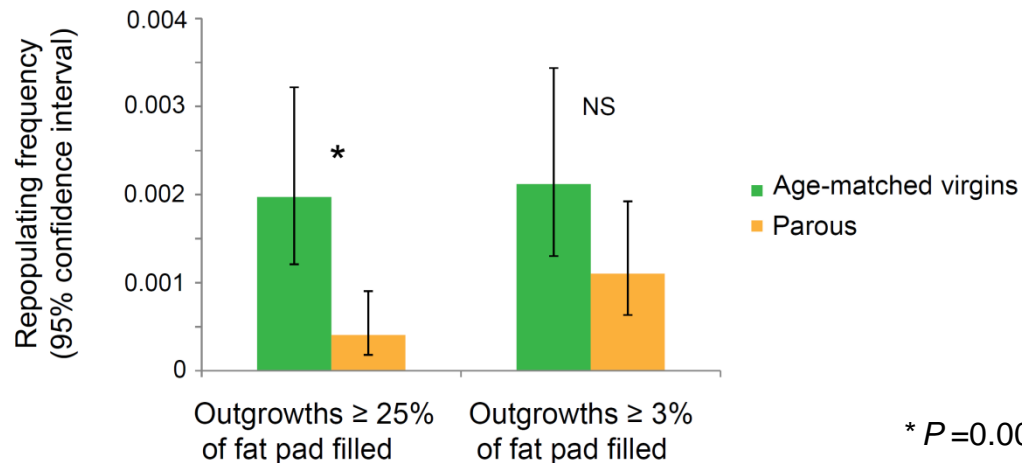
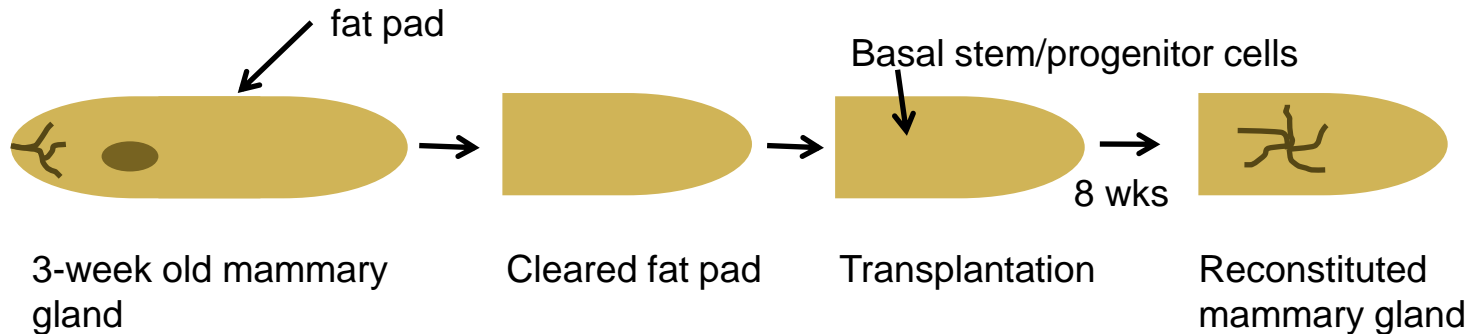
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Early parity decreases the *in vivo* reconstitution efficiency but not the number of mammary repopulating units (MRUs) of basal stem/progenitor cells.



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Early parity decreases the *in vivo* reconstitution efficiency but not the number of mammary repopulating units (MRUs) of basal stem/progenitor cells.



* $P=0.0004$

Results

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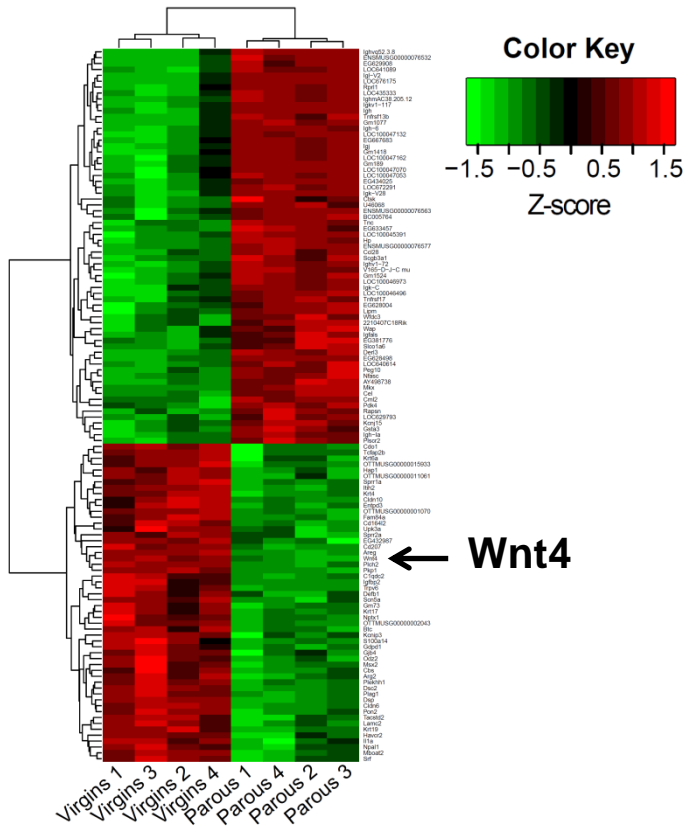
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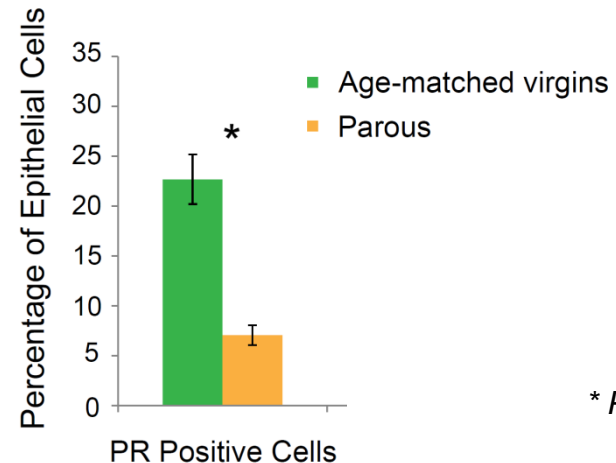
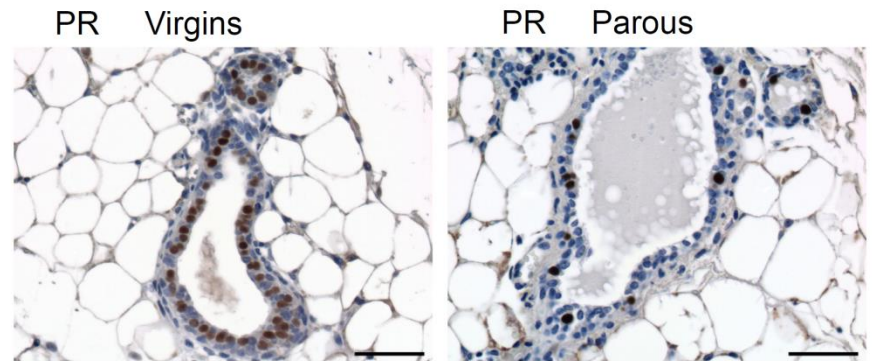
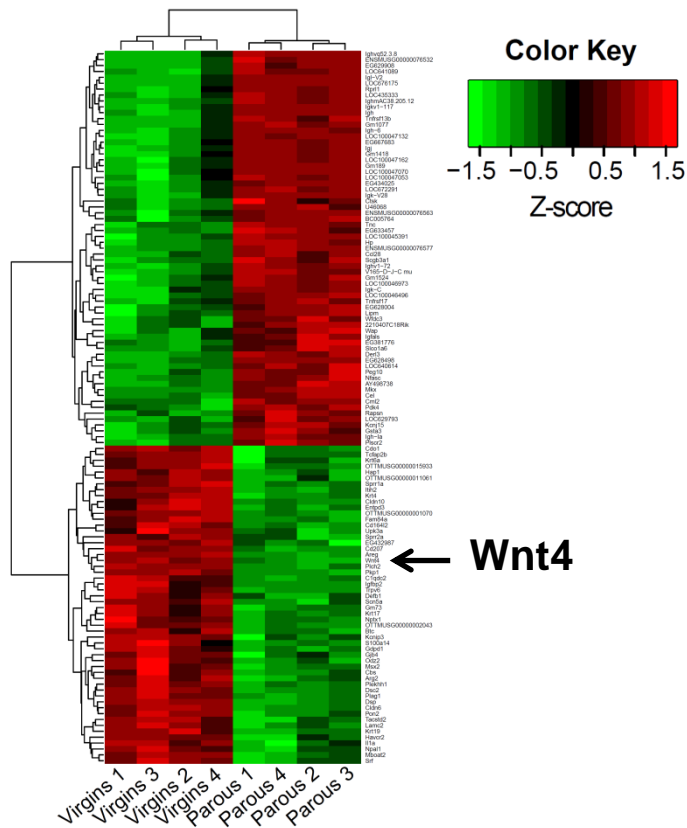
Results III:

Parity leads to a >3-fold decrease in Wnt4 expression in total cell suspensions

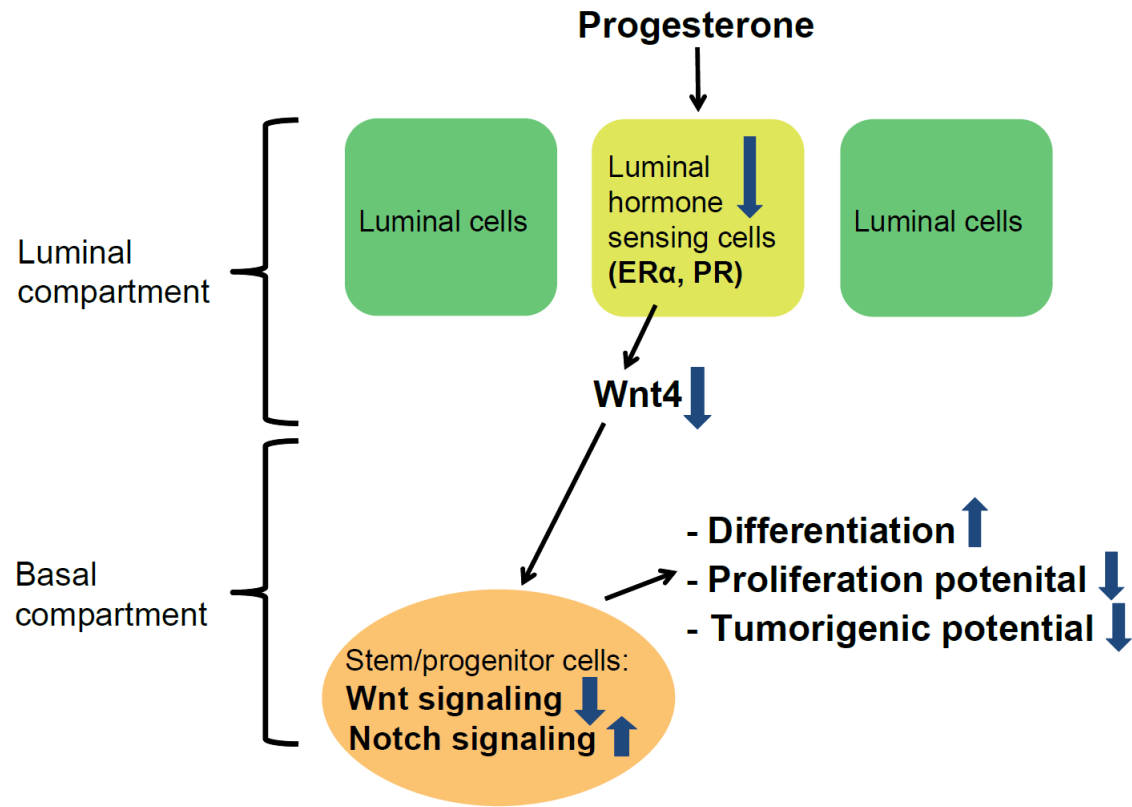


Results III:

Parity leads to a >3-fold decrease in Wnt4 expression in total cell suspensions & to a 3-fold decrease of PR positive (Wnt4-secreting) mammary epithelial cells.



Mechanistic model of the effect of an early age pregnancy



Results

- I – Transcriptome analysis in mammary epithelial cell subpopulations from parous and age-matched virgin control mice**

- II – Effects of early parity on the clonogenic and proliferation potential of basal stem/progenitor cells**

- III – Putative mechanism of early parity-induced biofunctional alterations in basal mammary stem/progenitor cells**

- IV – Duration and age dependency of early parity-induced changes**

Summary:

Early parity leads to the following changes in mammary cell subpopulations:

- 1. an induction of differentiation and a downregulation of the Wnt/Notch signaling ratio in basal stem/progenitor cells**
- 2. a downregulation of potentially tumorigenic biofunctions in the basal stem/progenitor cell subpopulation**
- 3. a decrease in the *in vitro* and *in vivo* proliferation potential of isolated basal stem/progenitor cells**
- 4. a reduction in progesterone-responsive and Wnt4-secreting luminal cells**

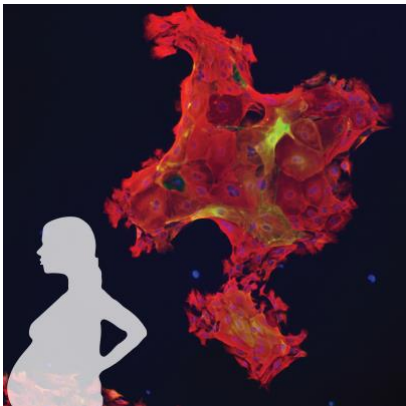
These early age pregnancy-induced changes are of life-long duration, and NOT induced by late age pregnancy. This is fully consistent with the life-long breast cancer protective effect of early but not late age pregnancy in women.

Conclusions and Perspectives:

Early age pregnancy induces life-long cellular and molecular changes in mammary glands of mice which potentially explain the breast cancer protective effect of early age pregnancy.

The decrease in the Wnt/Notch signaling ratio in basal stem/progenitor cells has now been confirmed in humans but further validations in humans are warranted.

The results provide deeper understanding of the role of parity and open the door to future studies assessing whether inhibitors of the Wnt pathway might be useful to mimic the early parity-induced protective effect against breast cancer.



Acknowledgements:

M. Bentires-Alj, S. Gasser, C. Rochlitz, D. Schübeler, M. Smalley

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MARA: Piotr Balwierz, Erik van Nimwegen

FACS: Hubertus Kohler

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Histology: Sandrine Bichet, Augustyn Bogucki



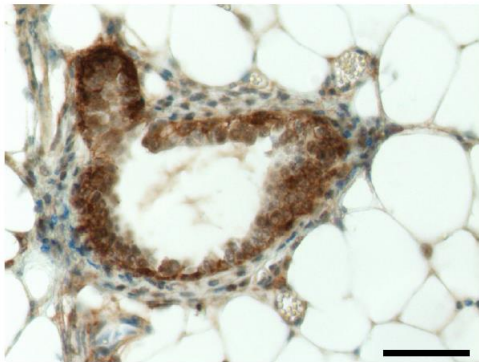
**Funding: Swiss National Science Foundation, Novartis Research Foundation,
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Thank you!

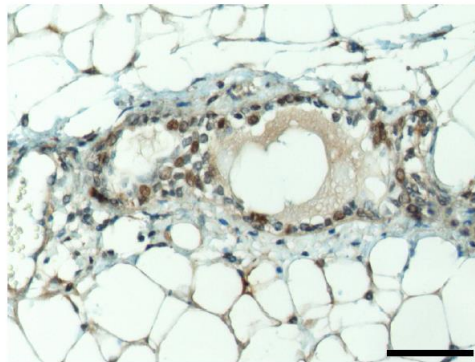
Results I:

Wnt targets are reduced on the protein level

Versican Virgins



Versican Parous



Mean number of versican (brown) pixels per image:

Virgins: $38,700 \pm 6954$

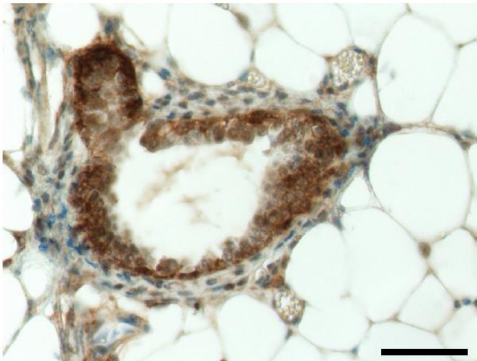
Parous: 127 ± 33

$P=5.48E-07$

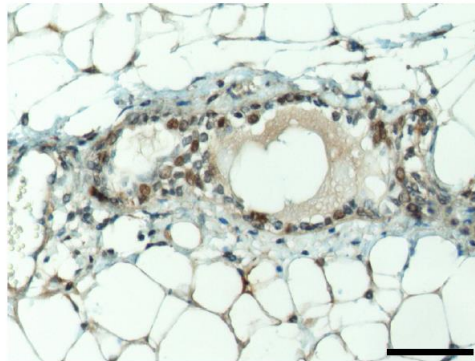
Results I:

Wnt targets are reduced on the protein level and nuclear β -catenin is decreased in basal mammary epithelial cells from parous mice.

Versican Virgins



Versican Parous



Mean number of versican (brown) pixels per image:

Virgins: $38,700 \pm 6954$

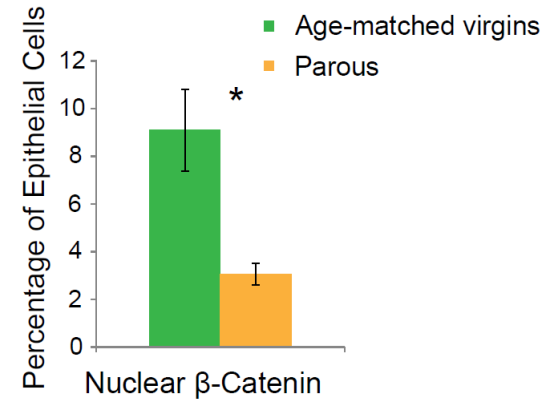
Parous: 127 ± 33

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β -Catenin Virgins

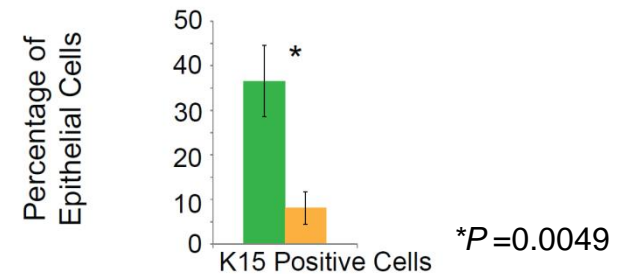
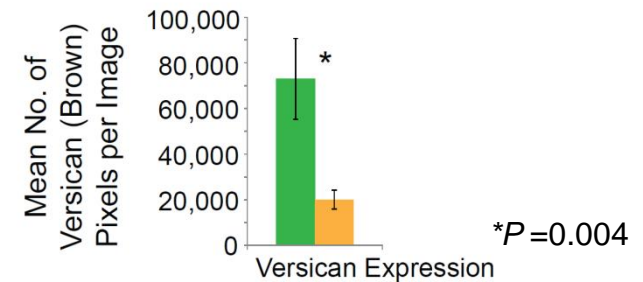
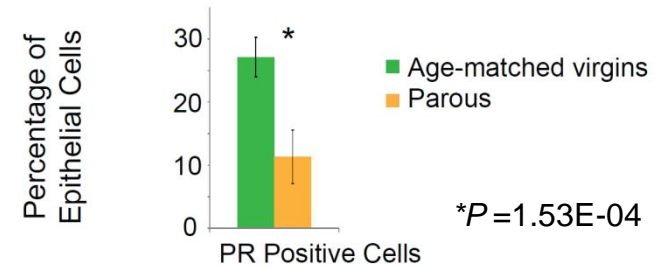
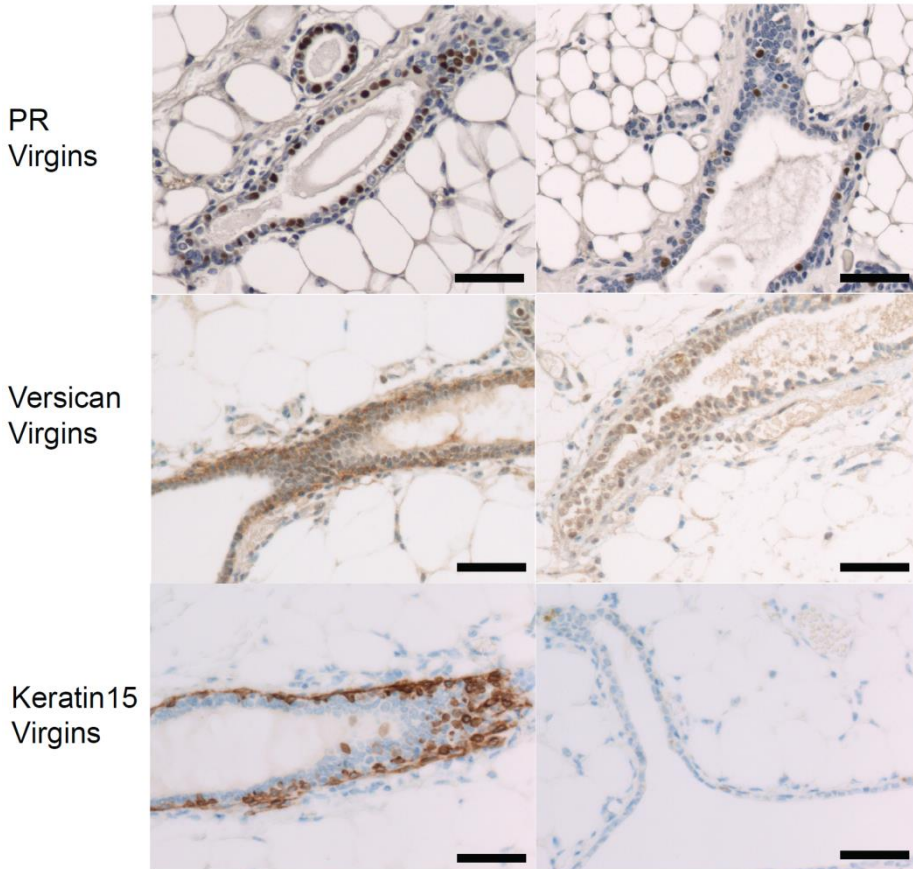


β -Catenin Parous



Results:

Early age pregnancy-induced decreases in progesterone receptor positive cells and Wnt target versican and keratin 15 expression persist in mammary glands of postmenopausal (22 months old) mice.



Results IV:

In contrast to early parity, late age pregnancy (24wks) has NO effect on PR positive cells and on Wnt target versican and keratin 15 expression.

