Environmental impacts on the developing conceptus

Susanne E Ulbrich

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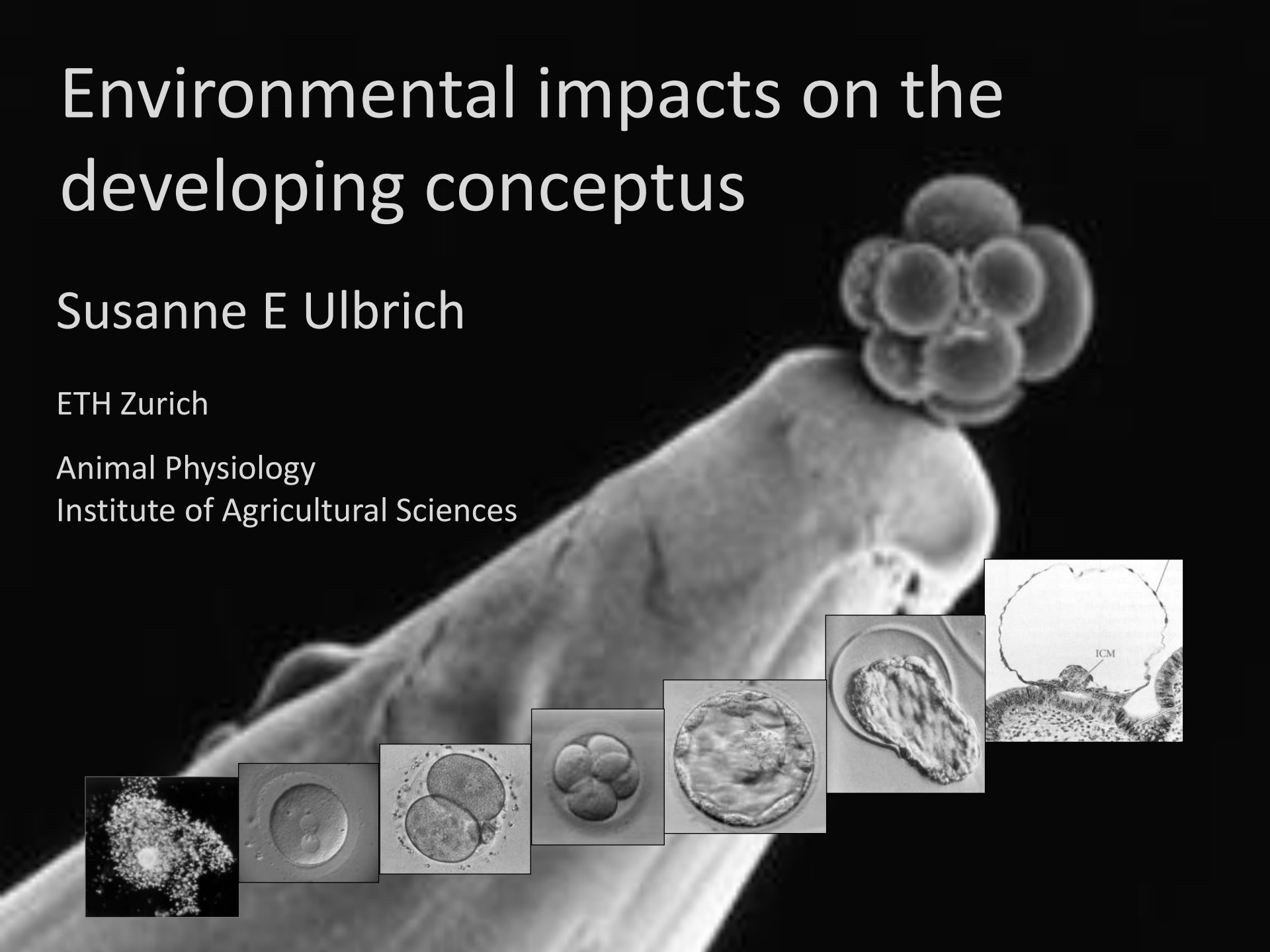
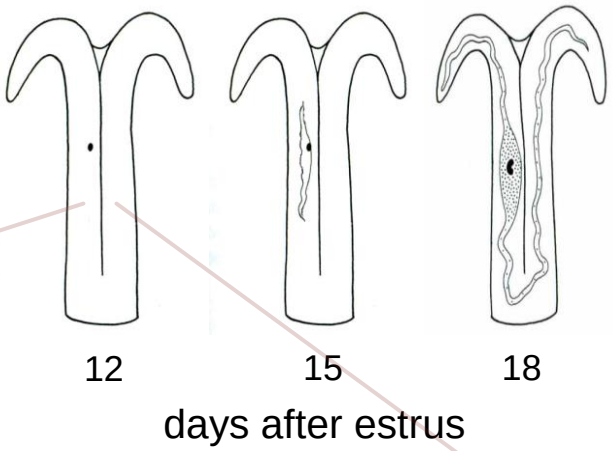
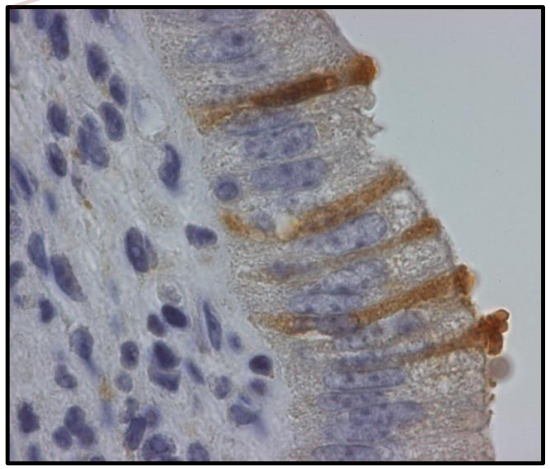
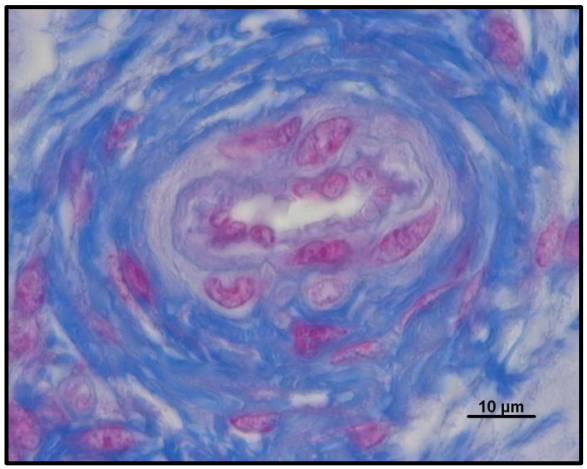


Embryo in the uterus

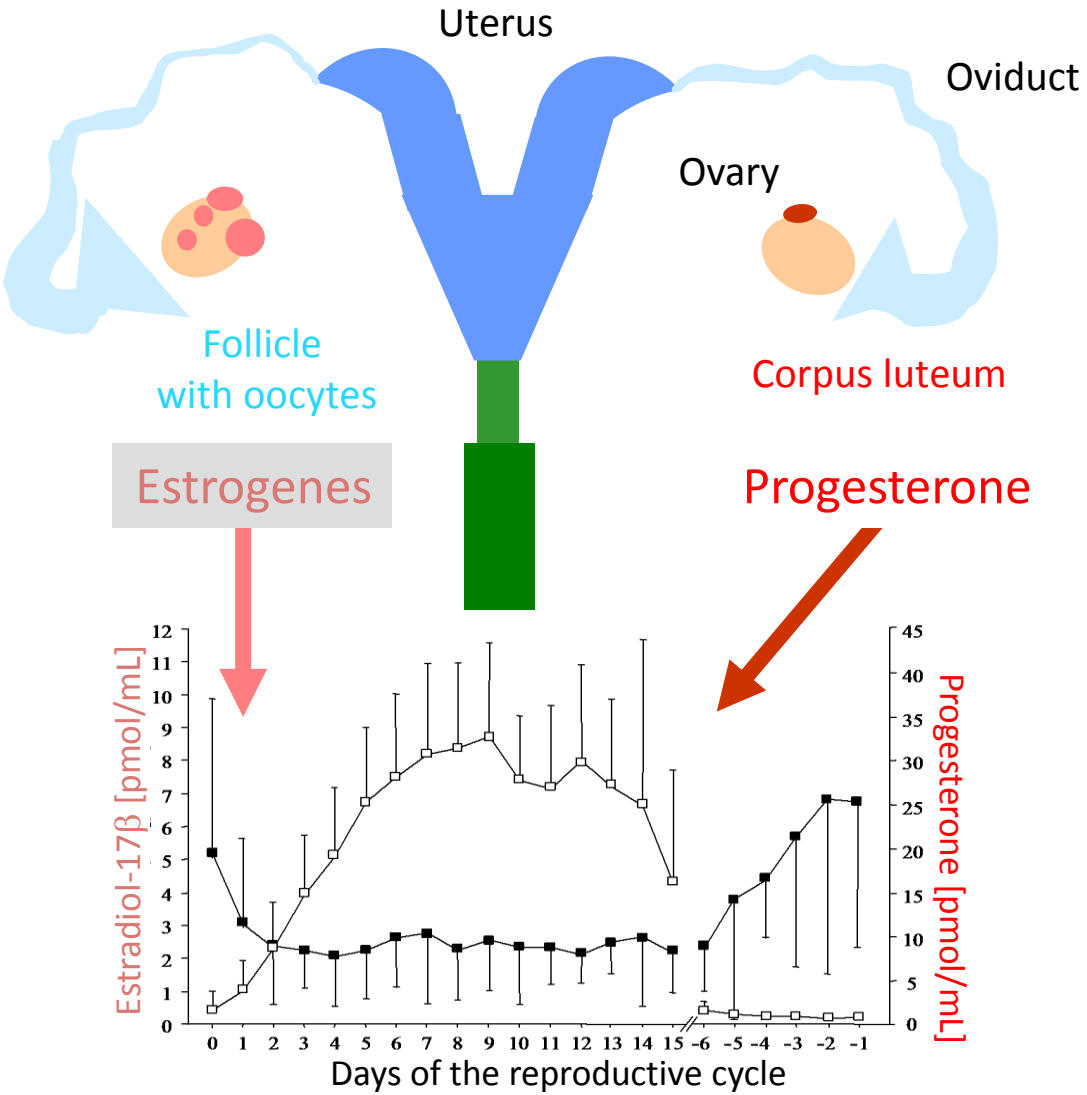






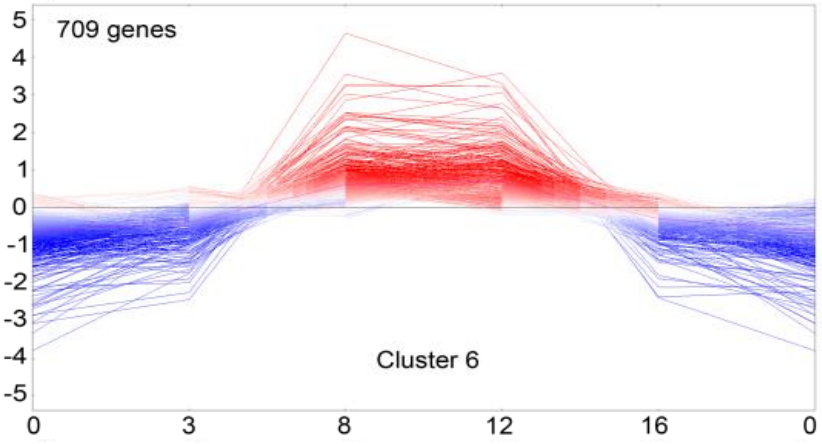
Ovarian hormones induce secretion of histotroph
to create an optimal environment for embryonic development.

Reproductive cycle

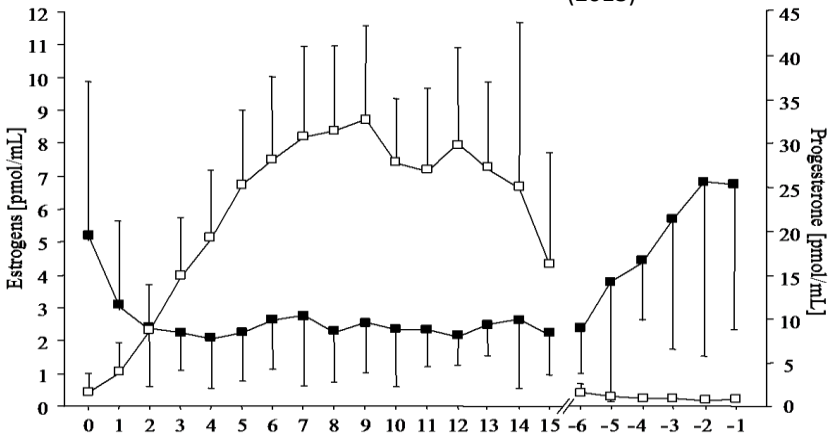


Transcriptomics of the cyclic endometrium

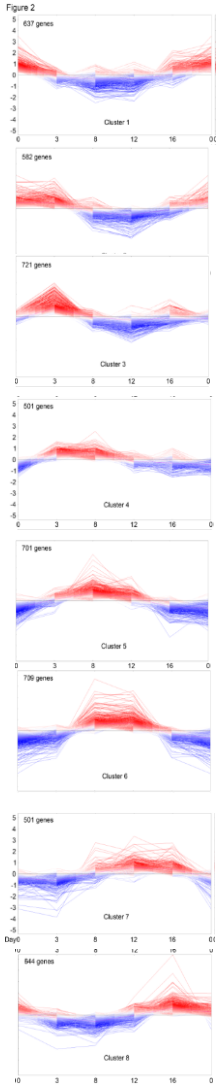
Clusters of similar expression profiles



Bauersachs et al. (2013)

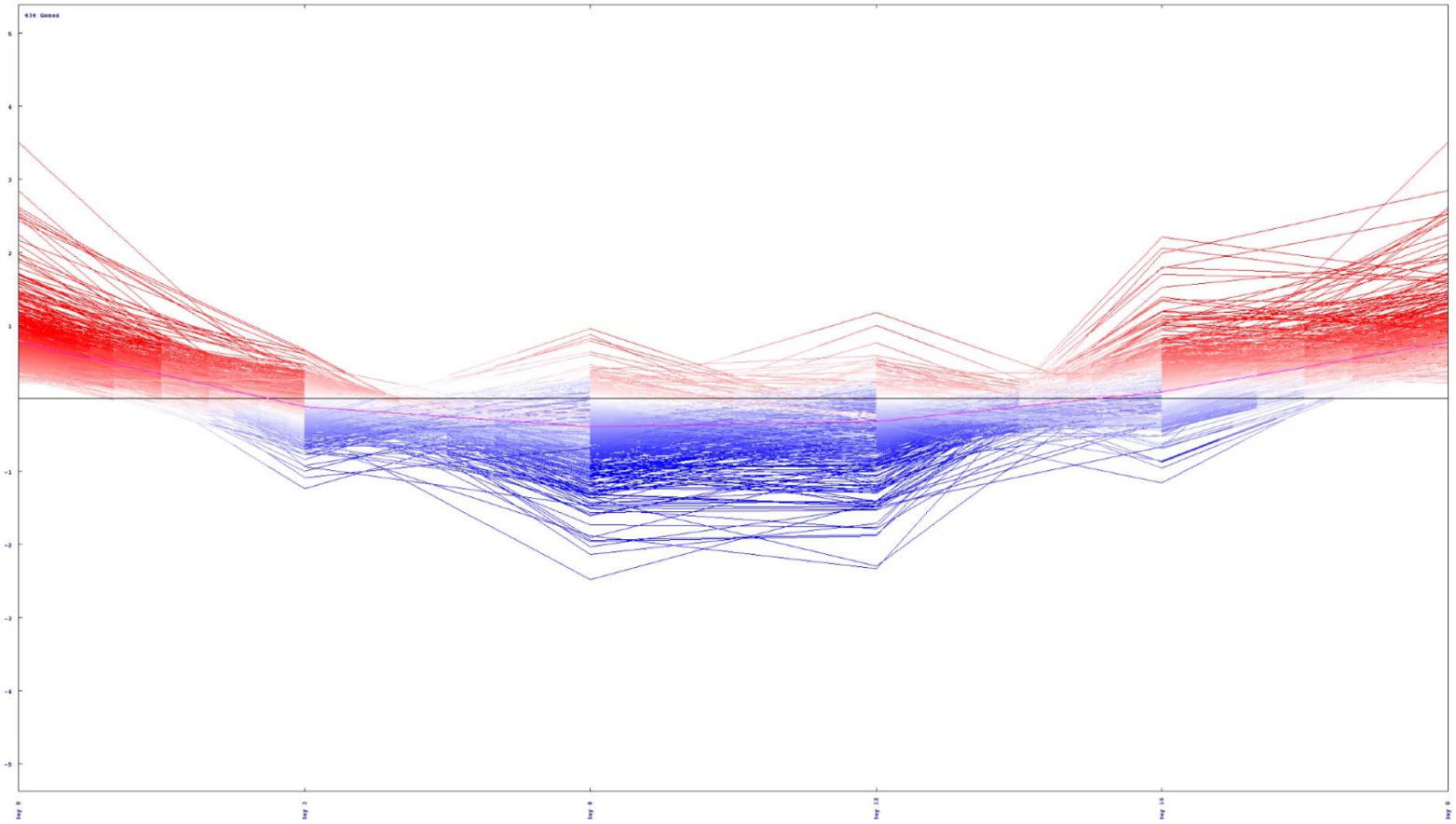


Bollwein et al. (2002)

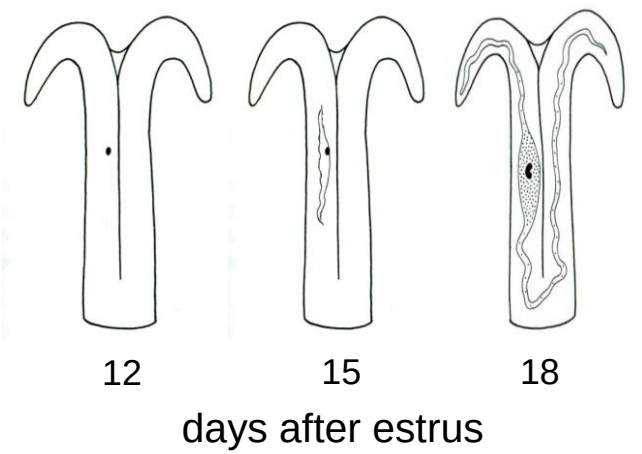


Clusters of similar expression profiles

Self-organizing tree algorithm



Maternal reaction during early pregnancy



Embryo induces…

… altered endometrial transcriptome (RNA-Seq)

Bauersachs et al (2006) *Reproduction*

… increased local prostaglandins and –metabolites (LC-MS/MS)

Ulbrich et al (2009) *Reproduction*

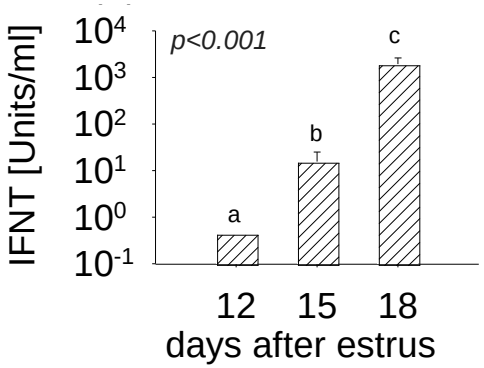
… altered endometrial proteome and histotroph composition (nano-LC-MS/MS und SRM)

Gröbner et al (2012) *Reproduction*
Forde et al (2014) *Reproduction*

... altered miRNA of extracellular vesicles of the histotroph (RNA-Seq)

Burns et al. (2014) *PLoS ONE*

Interferon-τ



Gröbner et al. (2010)
Placenta

Conclusion I

Endometrial function during preimplantation

Endometrial changes are dynamic

Functional genomics give most valuable insights to endometrial dynamics, and specified validations can substantiate functional conclusions

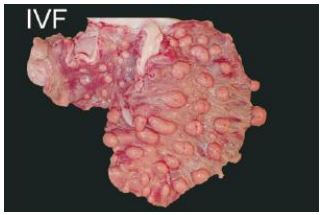
A plethora of descriptive data can not distinguish drivers from free-riders of downstream events

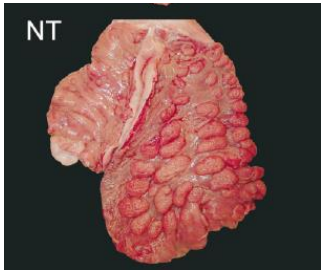
Embryo-maternal communication is intensively taking place

Maternal reaction to cloned embryos

Cloned pregnancies are often associated with the large off spring syndrome Young (1998) *Rev Reprod*

Symptoms
Dysfunction of the placenta, fewer number of enlarged placentomes….





Hiendleder et al. (2004)
Biology of Reproduction

PNAS

The endometrium responds differently to cloned versus fertilized embryos

Stefan Bauersachs[a,b], Susanne E. Ulbrich[c], Valeri Zakhartchenko[a], Megan Minten[d], Myriam Reichenbach[a], Horst-Dieter Reichenbach[e], Helmut Blum[b], Thomas E. Spencer[d], and Eckhard Wolf[a,b,1]

[a]Molecular Animal Breeding and Biotechnology, and [b]Laboratory for Functional Genome Analysis, Gene Center, Ludwig-Maximilians-Universität München, 81377 Munich, Germany; [c]Physiology Weihenstephan, Technische Universität München, 85354 Freising, Germany; [d]Department of Animal Science, Texas A&M University, College Station, TX 77843-2471; and [e]Bavarian State Institute for Agriculture, Institute for Animal Breeding, 85586 Poing, Germany

Edited by George E. Seidel, Jr., Colorado State University, Fort Collins, CO, and approved February 20, 2009 (received for review November 20, 2008)

PNAS

Endometrium as an early sensor of in vitro embryo manipulation technologies

Nadéra Mansouri-Attia[a,1], Olivier Sandra[a,1,2], Julie Aubert[b], Séverine Degrelle[a], Robin E. Everts[c,3], Corinne Giraud-Delville[a], Yvan Heyman[a], Laurent Galio[d], Isabelle Hue[a], Xiangzhong Yang[e], X. Cindy Tian[e], Harris A. Lewin[c], and Jean-Paul Renard[a]

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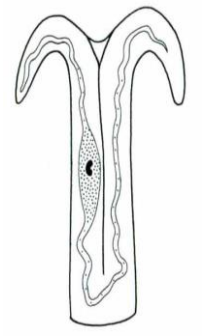
Bauersachs et al. (2011)
PNAS

Maternal reaction to cloned embryos



18
days after estrus

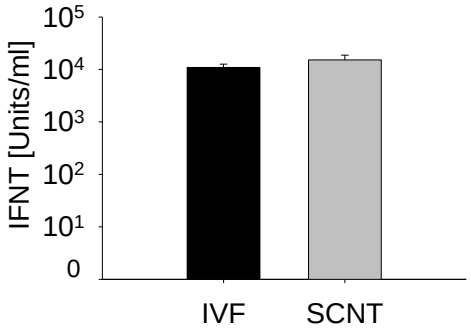
Embryo induces…

…altered endometrial transcriptome (RNA-Seq)
Bauersachs et al (2009) *PNAS*

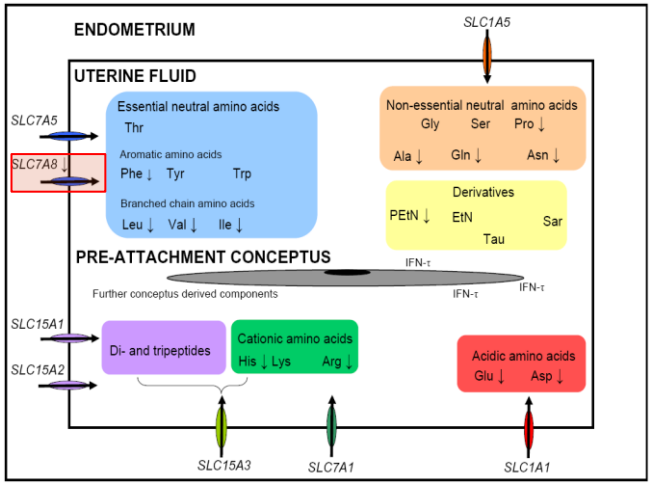
…decreased local prostaglandins and –metabolites (LC-MS/MS) Ulbrich et al (in preparation)

…altered endometrial histotroph composition (LC-MS/MS) Gröbner et al (2011) *Cell Reprogramming*

Interferon-τ



Gröbner et al. (2011)
Cellular Reprogramming



Conclusion II

Evidence of biological communication

Biological communication is necessary for reciprocal benefit.

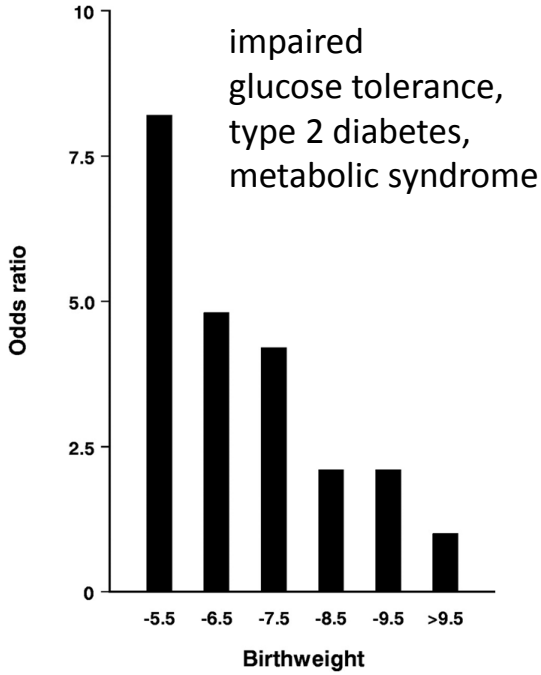
The endometrium senses the signaling of the embryo and adapts.

The embryo senses the maternal environment and adapts
by undertaking compensatory responses.

Thrifty-phenotype hypothesis

An unfavourable intrauterine supply influences the metabolic and endocrine constellation of the fetus leading to reduced birth weight which favours survival under detrimental nutritional conditions after birth.

Hales & Barker 1993



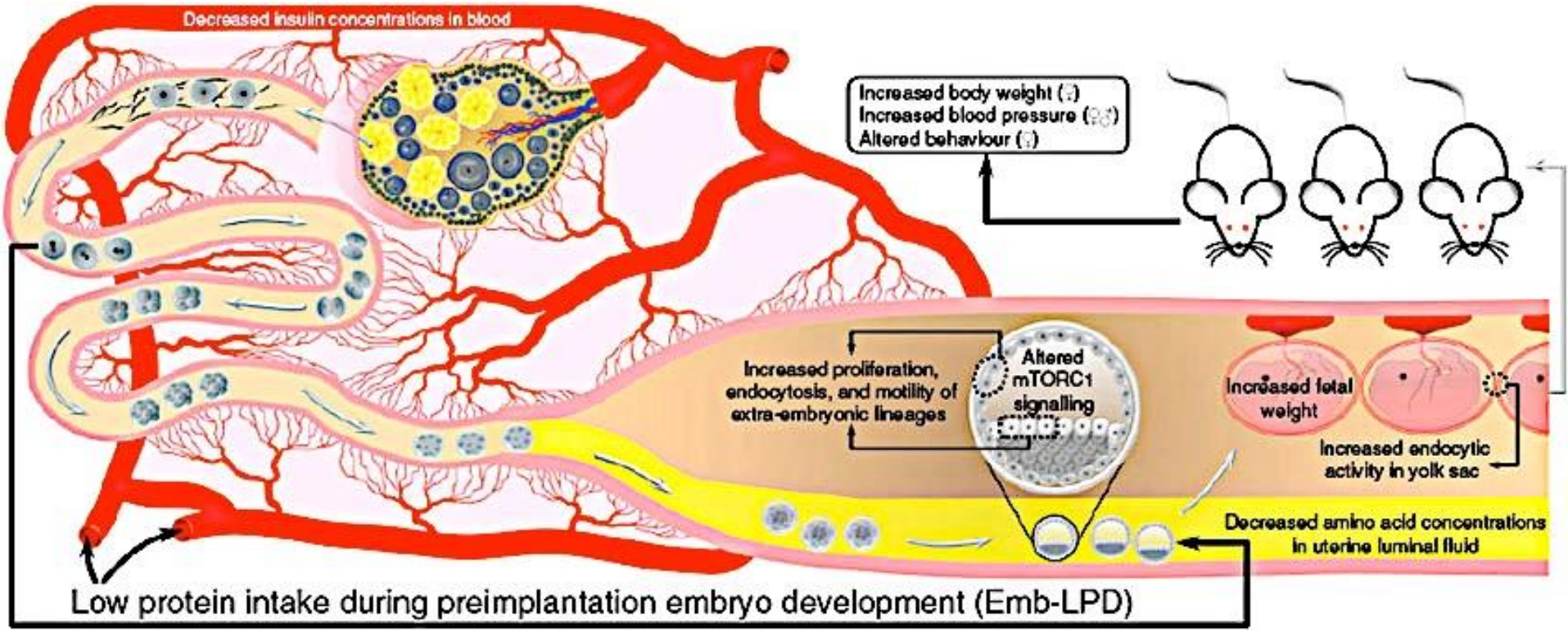
Hales & Barker, Br Med Bull 2001

Predictive adaptive response hypothesis

The degree of mismatch between the pre- and postnatal environments determines the forthcoming disposition for subsequent diseases.

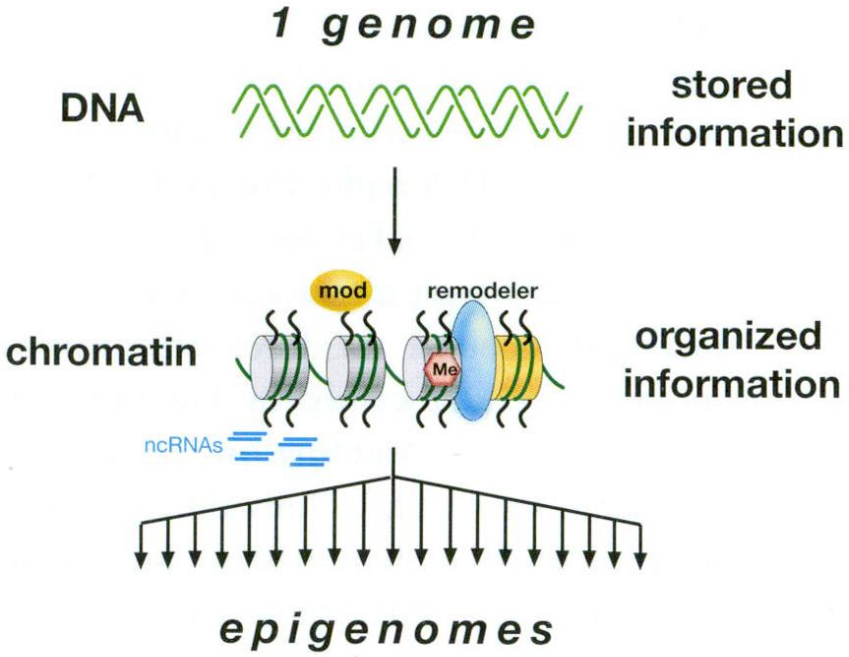
Gluckman and Hanson, 2004

Maternal dietary restriction

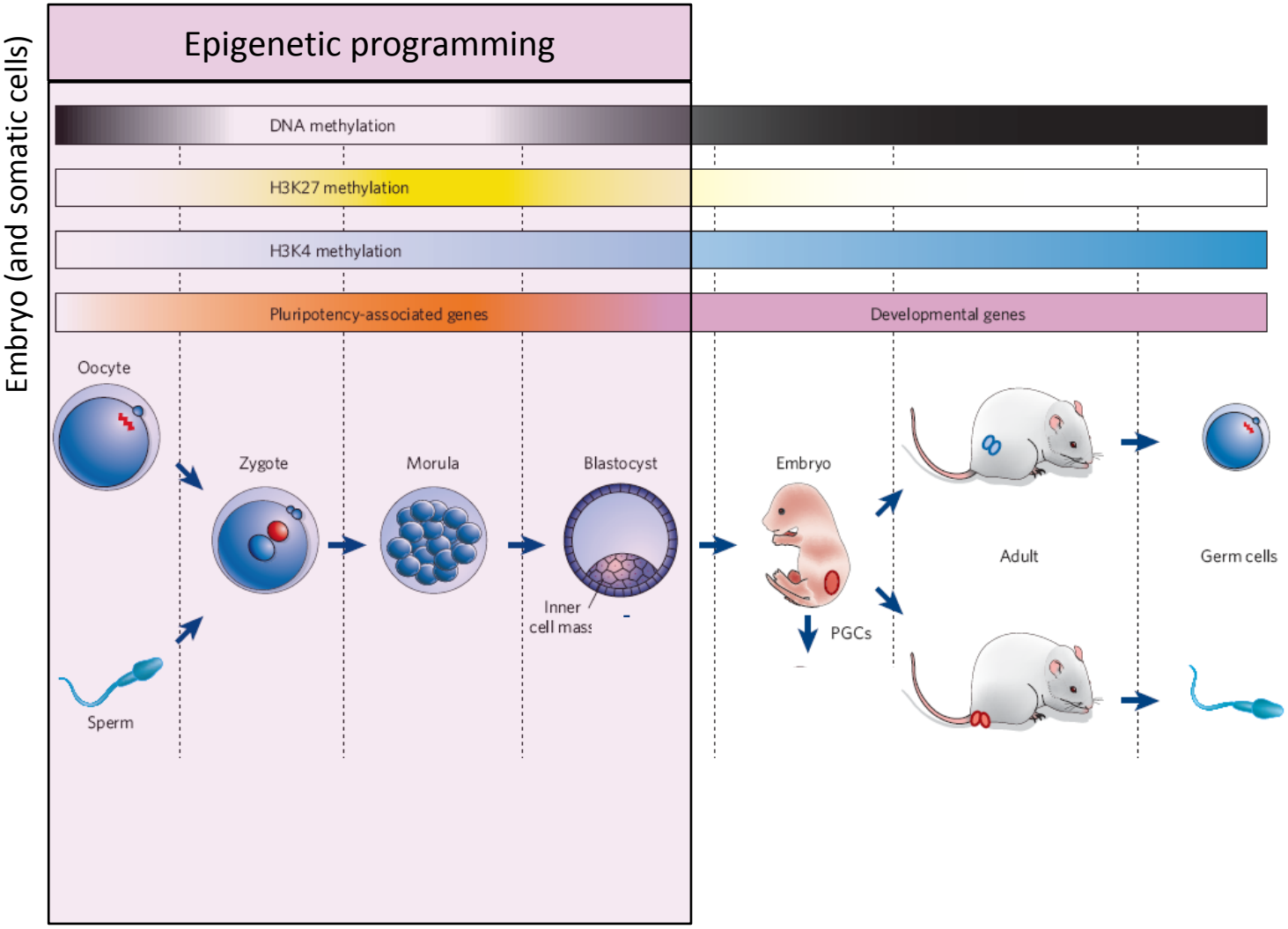


Fleming et al (2015)
Reproduction, Fertility and Development

Epigenetic code



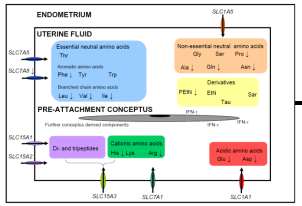
Epigenetic gene regulation

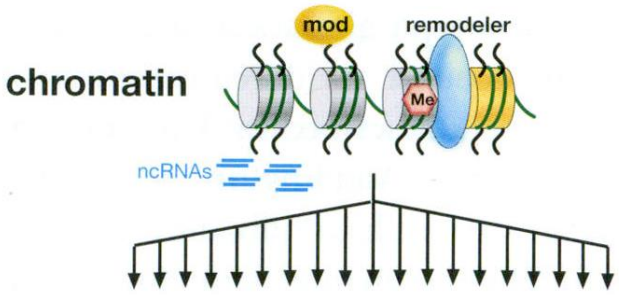


Reik *2007*

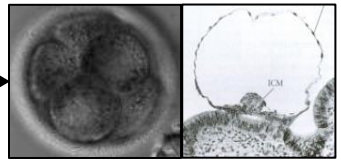
Mechanistic establishment of epigenetic modifications

Consequences of perturbed uterine environment – periconceptional programming





epigenomes



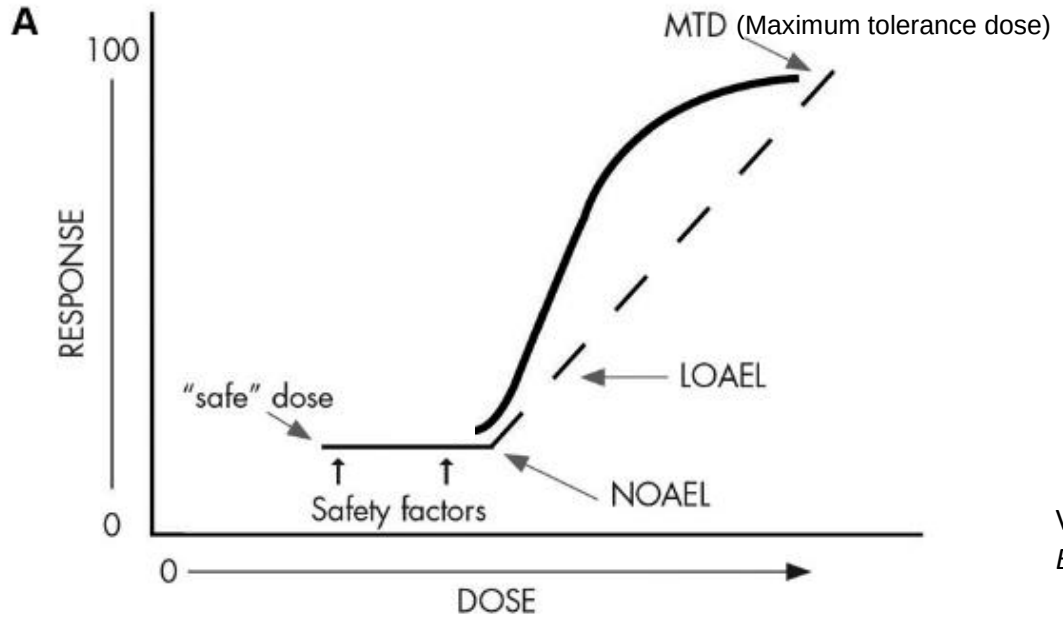
Endocrine disruptors

Endocrine disrupting chemicals interfere with endocrine signaling systems in the body, even at doses previously considered safe

Colborn et al (1993)

Endocrine disruptors

Different dose-response curves such as non-monotonic behavior ("U-shaped curve") may lead to low dose effects



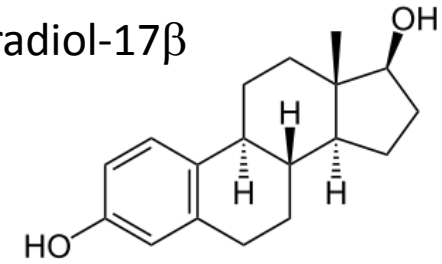
Vandenberg et al., 2012
Endocrine Reviews

Sensitive time window of exposure - *In utero*, postnatal development, puberty

Programming – Period of latency, observed effects appear later in life

Estradiol-17β - an endocrine disruptor?

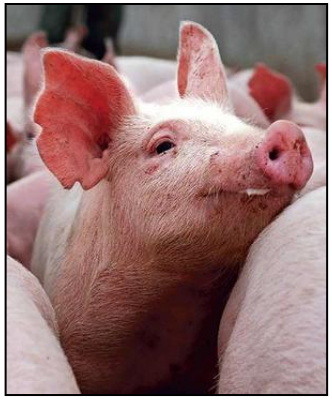
Estradiol-17β



Most potent naturally acting estrogen

Oral food contamination

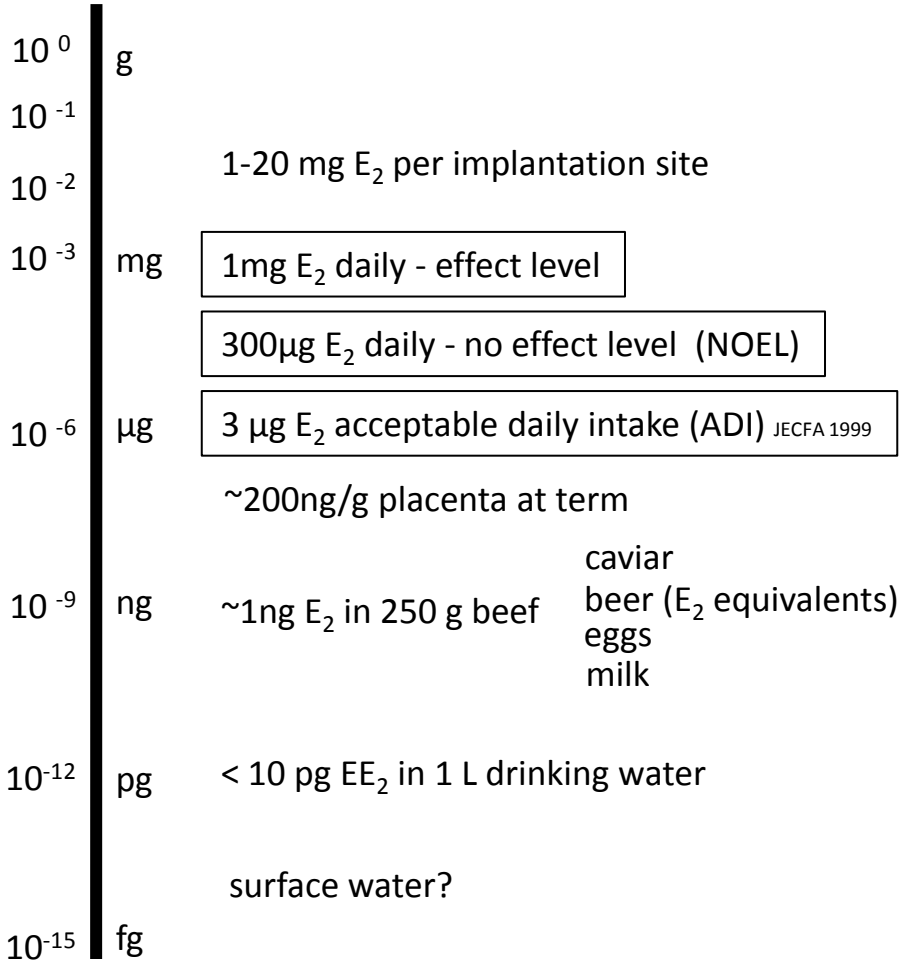
Oral contraceptives during early pregnancy



Pig as model organism

Which dose?

Safety values, occurrence and residues of Estradiol-17β



Maternal oral low-dose E_2 during pregnancy in pigs

1 mg E_2 daily: metabolic stabilization during menopause — mg E_2 daily – effect (high) dose

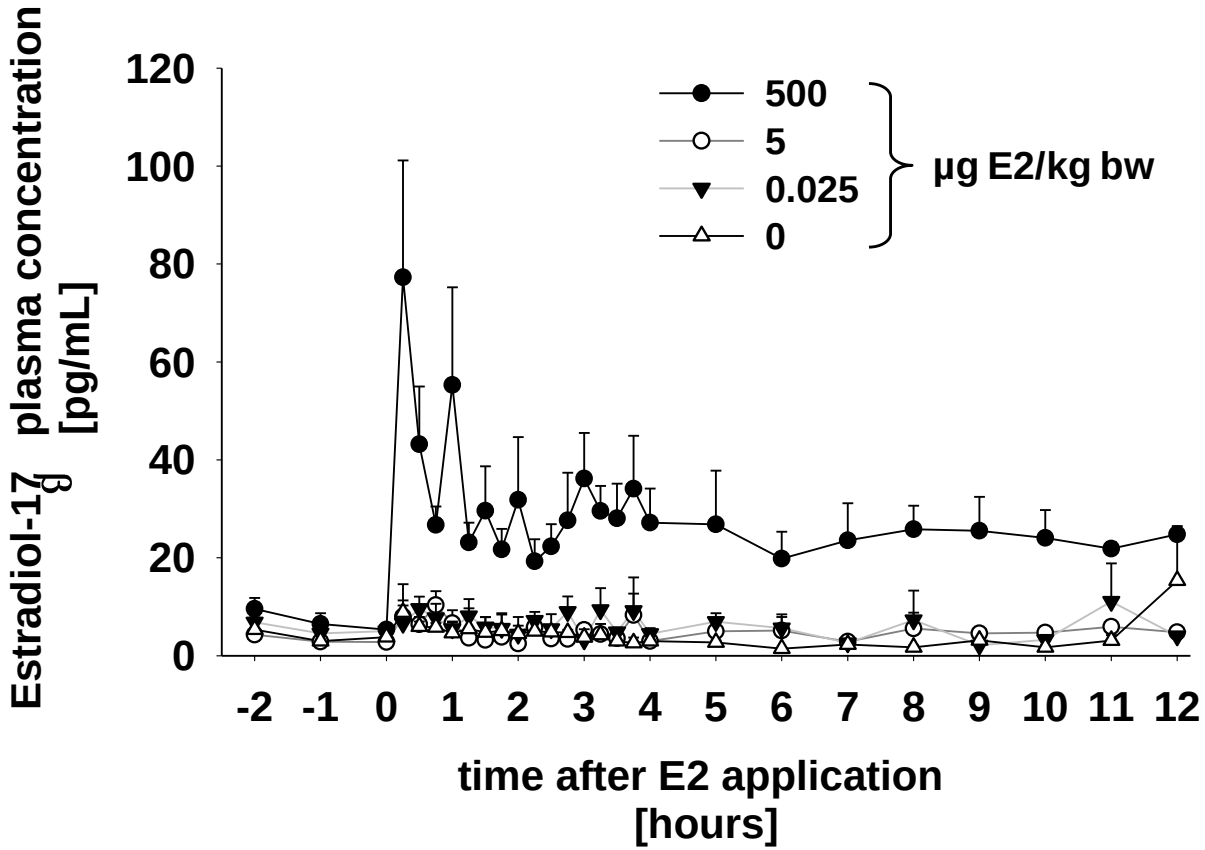
25 µg EE_2 (~0.5 mg E_2) daily: contraceptive dose — µg E_2 daily µg - NOEL dose

25-100 ng EE_2 (~1 µg E_2) daily: stimulation of growth in young Turner Syndrom females — ng E_2 daily - ADI dose

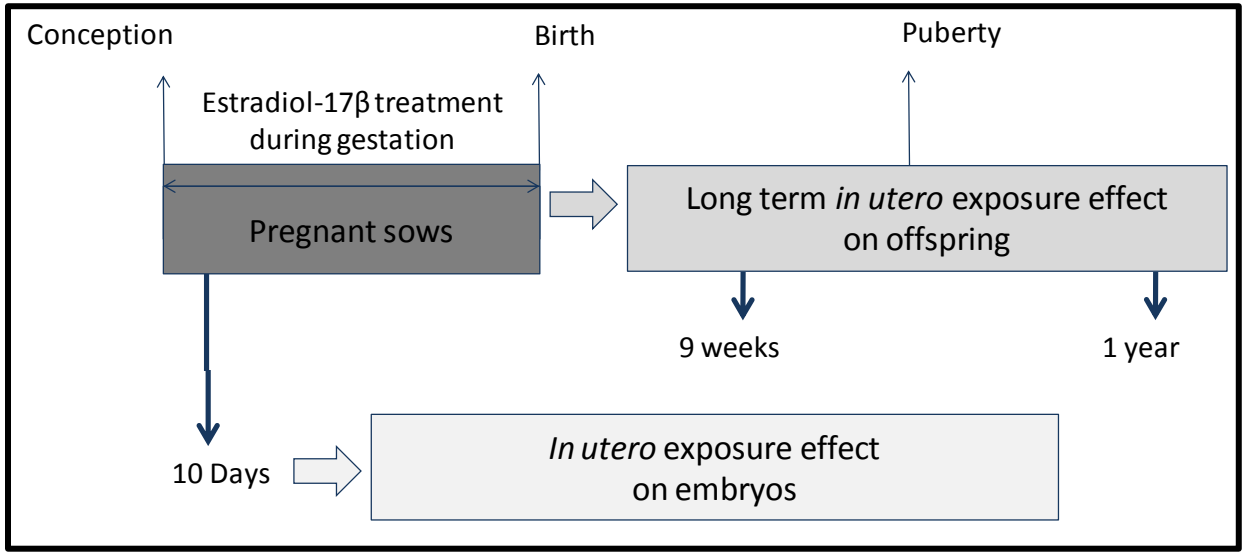
control

Direct effect of oral estrogen exposure

Elimination kinetics of estradiol-17β



Direct Effect – Programming



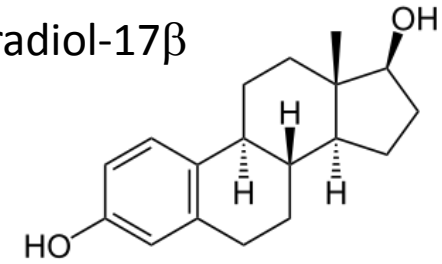
Phenotype
- Weight development,
- Body composition (DEXA)
- Bone density (qCT)

Fürst et al (2012) **Tox Appl Pharm**, 263 3 338-44
Pistek et al (2013) **JSBMB**, 138 435-44
Flöter et al (2015), ***under revision***

Hormone state (plasma, endometrium, uterine fluid)
ELISA, LC-MS/MS
Differential gene expression (endometrium/conceptus)
RNAseq

Oral Effects of estrogen exposure

Estradiol-17β



Differential endometrial gene expression (RNA-Seq)

Pistek et al.
In preparation

Dose-dependent and sex-specific differential gene expression in early embryos (RNA-Seq)

Flöter et al.
In preparation

Metabolic phenotype –
Increase in subcutaneous fat tissue in prepubertal male offspring (DXA)

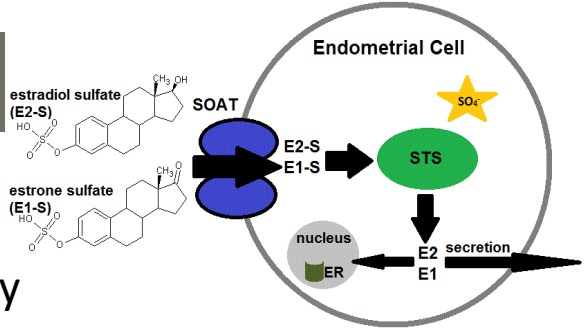
Fürst et al. (2012)
Toxicol Appl Pharmacol

Growth effects –
Altered bone density parameters in prepubertal and adult offspring (qCT)

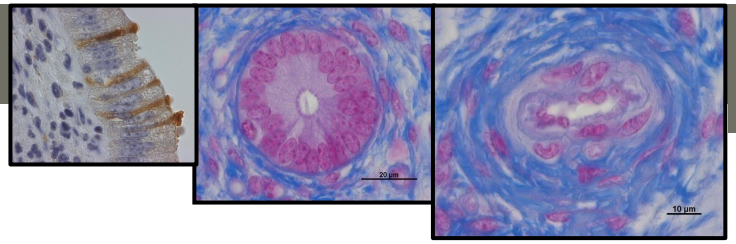
Flöter et al.
Under revision

Direct effect of oral estroges

Sex steroid hormones and metabolites at day 10 of pregnancy



	Estradiol-17β	Total Estrogens (Estradiol-17β, Estradiol-17α, Estron)	Conjugated Estradiol-17β	Conjugated Total Estrogens (Estradiol-17β, Estradiol-17α, Estron)
Plasma	[pg/mL]	[pg/mL]	[pg/mL]	[pg/mL]
Control	19.5 ± 3.2	25.3 ± 2.8	49.9 ± 5.1	95.5 ± 33.5
ADI	21.9 ± 5.4	30.4 ± 5.0	51.1 ± 1.7	109.6 ± 24.9
NOEL	25.5 ± 14.8	**71.9 ± 23.9 ***	**367.7 ± 35.7 ***	**3`503.3 ± 449.2 ***
High	**67.2 ± 7.3 ***	**419.5 ± 80.8 ***	**18`005.8 ± 4706.2 ***	**222`798.4 ± 5`1803.5 ***
Bile	[ng/mL]	[ng/mL]	[ng/mL]	[ng/mL]
Control	0.2 ± 0.08	0.7 ± 0.3	2.5 ± 0.7	20.4 ± 8.0
ADI	0.2 ± 0.1	1.3 ± 0.8	2.8 ± 0.7	17.3 ± 3.6
NOEL	2.6 ± 2.1	**34.1 ± 30.5 ***	**27.0 ± 9.9 ***	**453.0 ± 160.1 ***
High	**488.6 ± 431.9 ***	**2`188.5 ± 1400.4 ***	**1`992.8 ± 1083 ***	**8`432.0 ± 1`568.0 ***
Endometrium	[pg/mL]	[pg/mL]	[pg/mL]	[pg/mL]
Control	111.2 ± 4.2	153.5 ± 11.9	135.8 ± 16.0	90.5 ± 14.7
ADI	100.2 ± 4.0	189.8 ± 37.6	98.0 ± 14.0	73.5 ± 16.8
NOEL	194.8 ± 56.5	**881.2 ± 251.6 ***	214.4 ± 53.5	**834.6 ± 168.2 ***
High	**3`056.5 ± 830.5 ***	**24`657.2 ± 9`556.7 ***	**2`951.2 ± 1`149.2 ***	**9`497.6 ± 2`178.1 ***

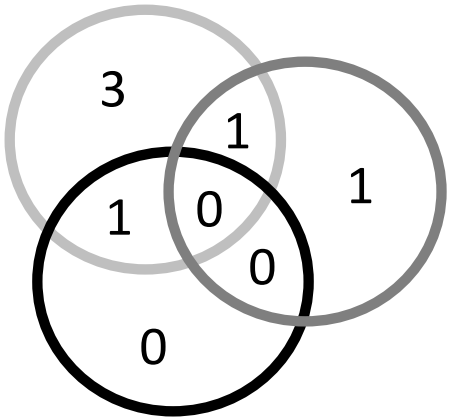


Direct effect of oral estrogens

RNA-Seq of endometrium at day 10 of pregnancy

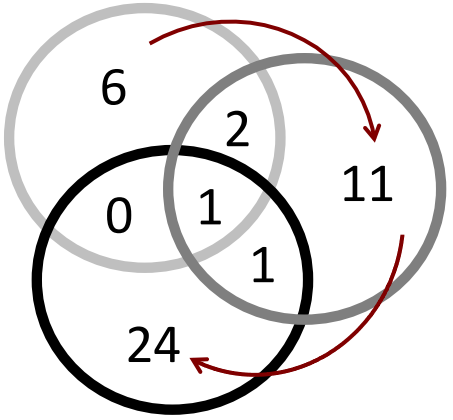
Endometrial mRNA
[# of differentially expressed genes]

Down-regulated genes



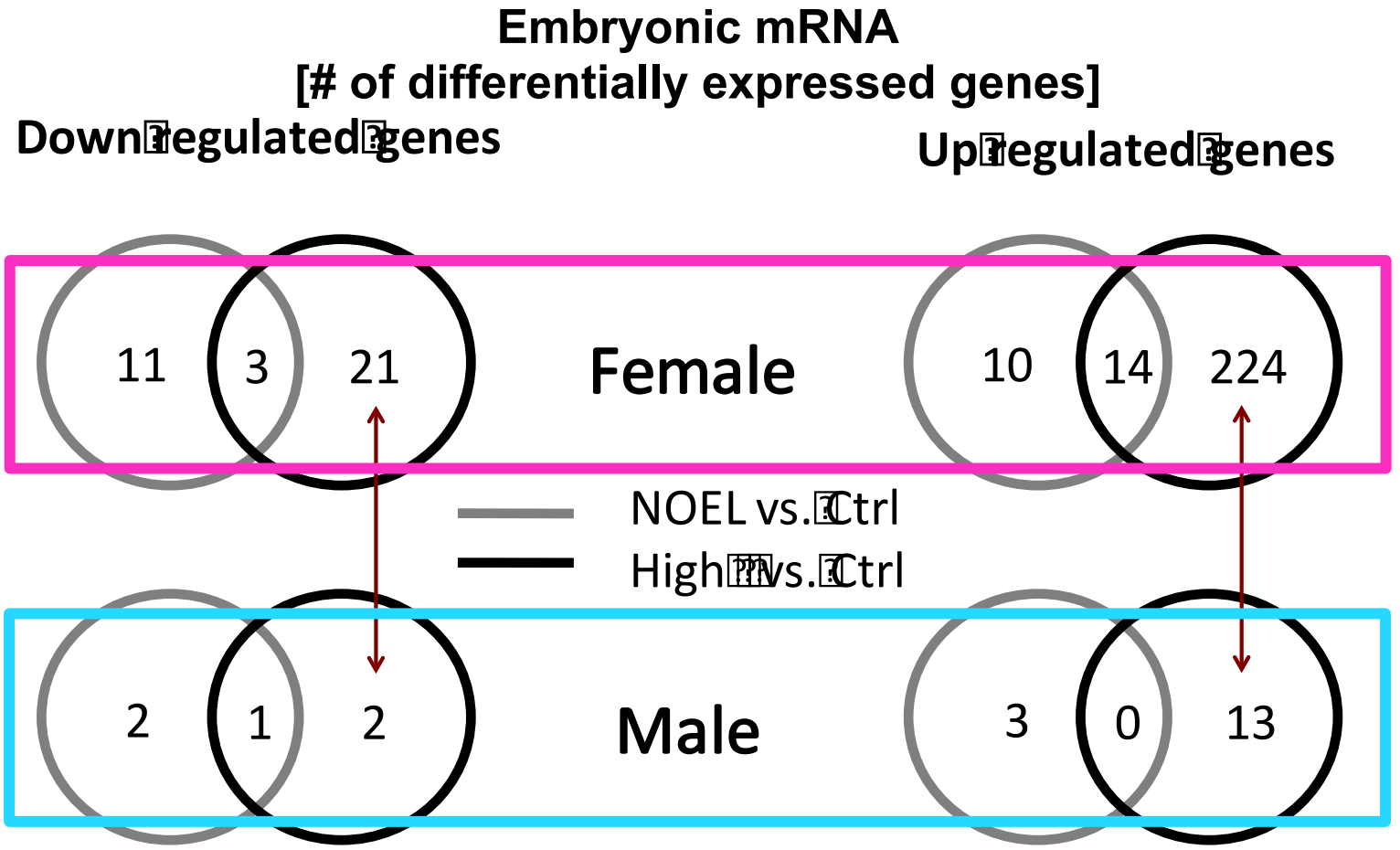
ADI vs. Ctrl
NOEL vs. Ctrl
High vs. Ctrl

Up-regulated genes



Direct effect of oral estrogens

RNA-Seq of single embryos at day 10 of pregnancy

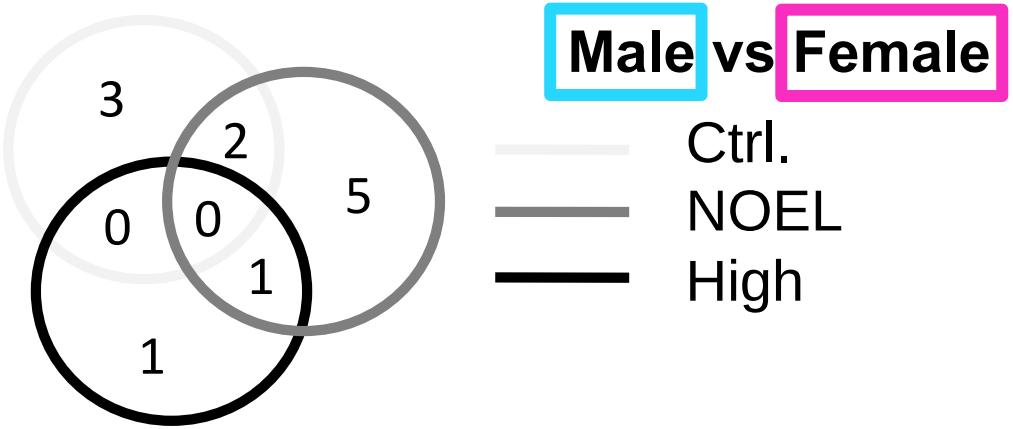


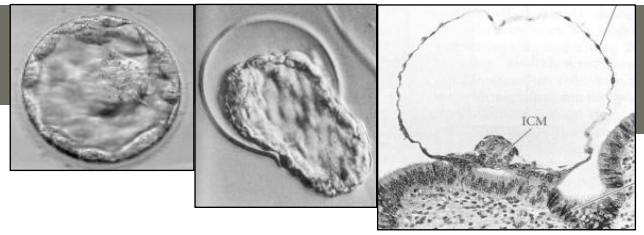
Direct effect of oral estrogens

RNA-Seq of single embryos at day 10 of pregnancy

Embryonic mRNA
[# of differentially expressed genes]

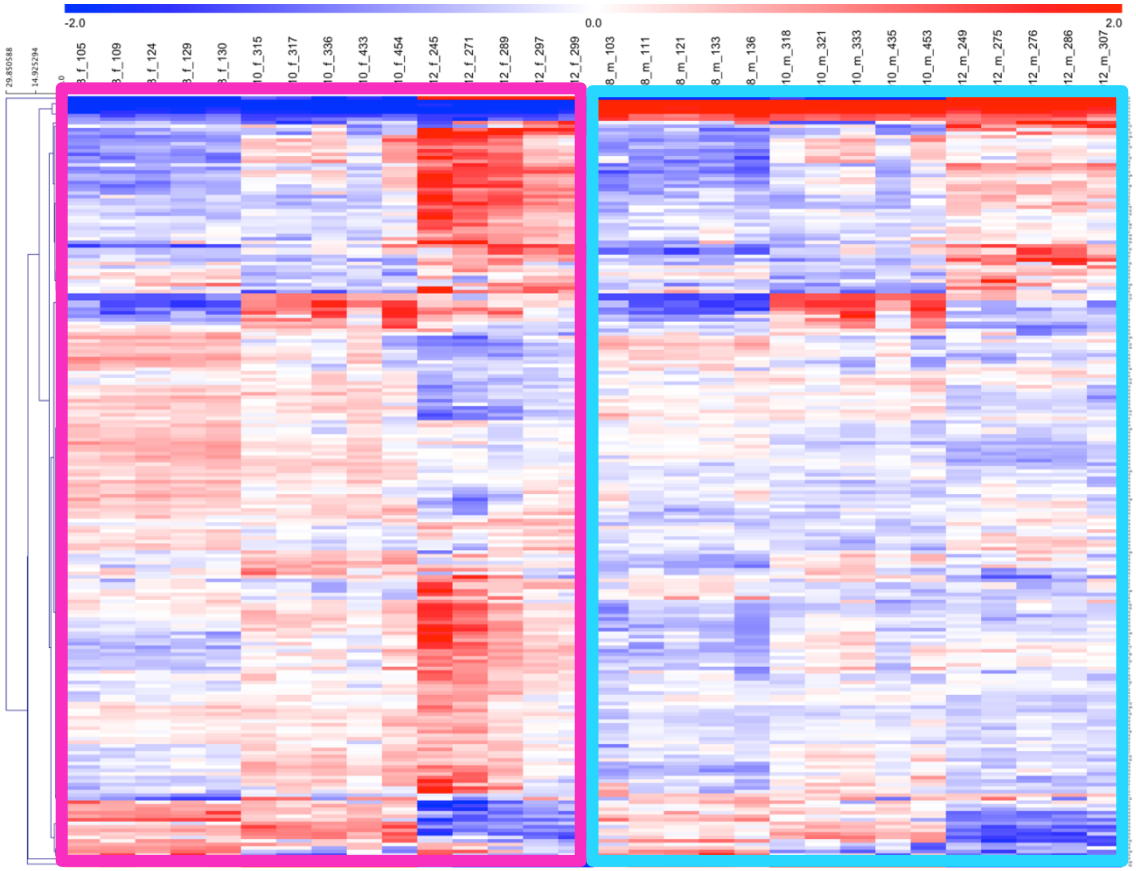
Down regulated genes | **Up regulated genes**





Effect of developmental stage

RNA-Seq of single sexed embryos at day 8, 10, 12 of pregnancy



Conclusion III

Consequence of adaptive signaling and responding

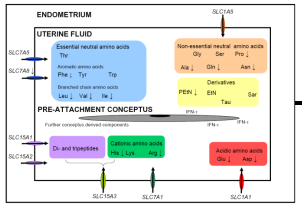
Genetic, epigenetic, or environmental cues may alter biological communication

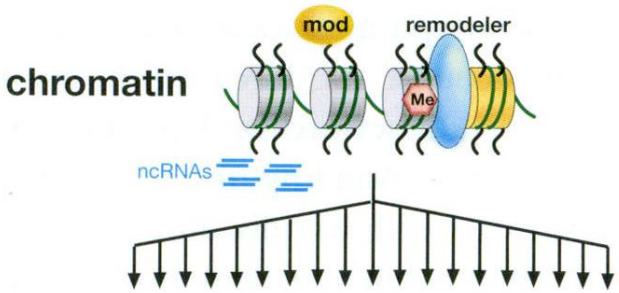
Developing embryos are highly susceptible to the impact of exogenous stressors

Flexibility of communication may determine maternal effects on postnatal development

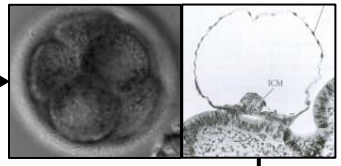
Mechanistic establishment of epigenetic modifications

Consequences of programmed uterine environment





epigenomes



Conclusion

Endometrial function is dynamic.

Endometrial fingerprint resembles embryo characteristics.

Uterine environment may critically impact on embryonic development.

Low dose matters - Reconsideration of effect levels based on an epigenetic perspective is required.

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