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Sex-Specific Placental Responses in Fetal Development

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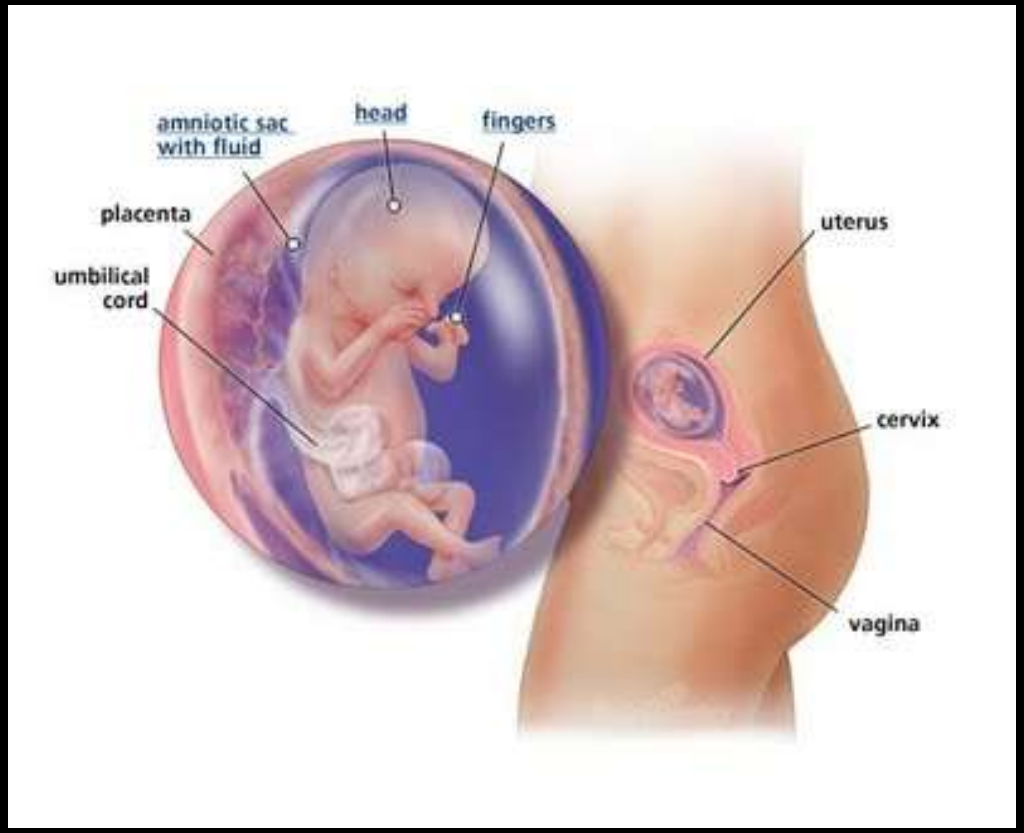
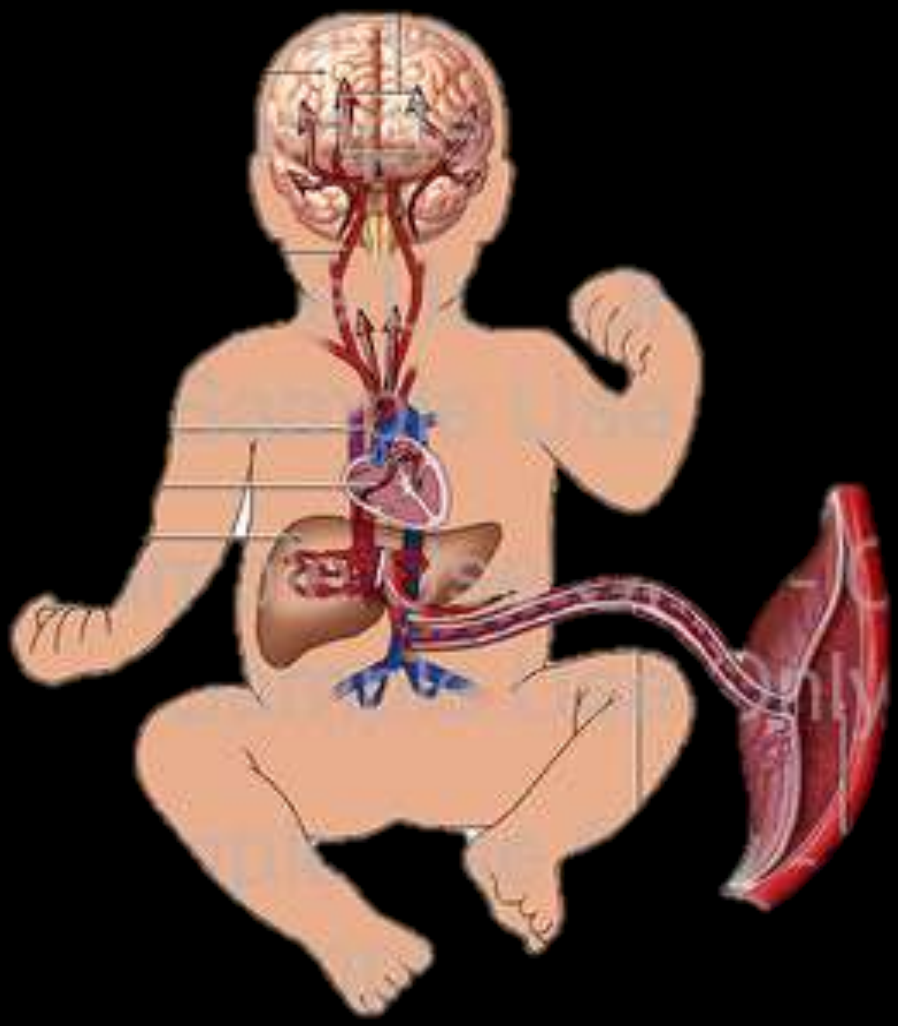
The Placenta: Guardian of the Fetus

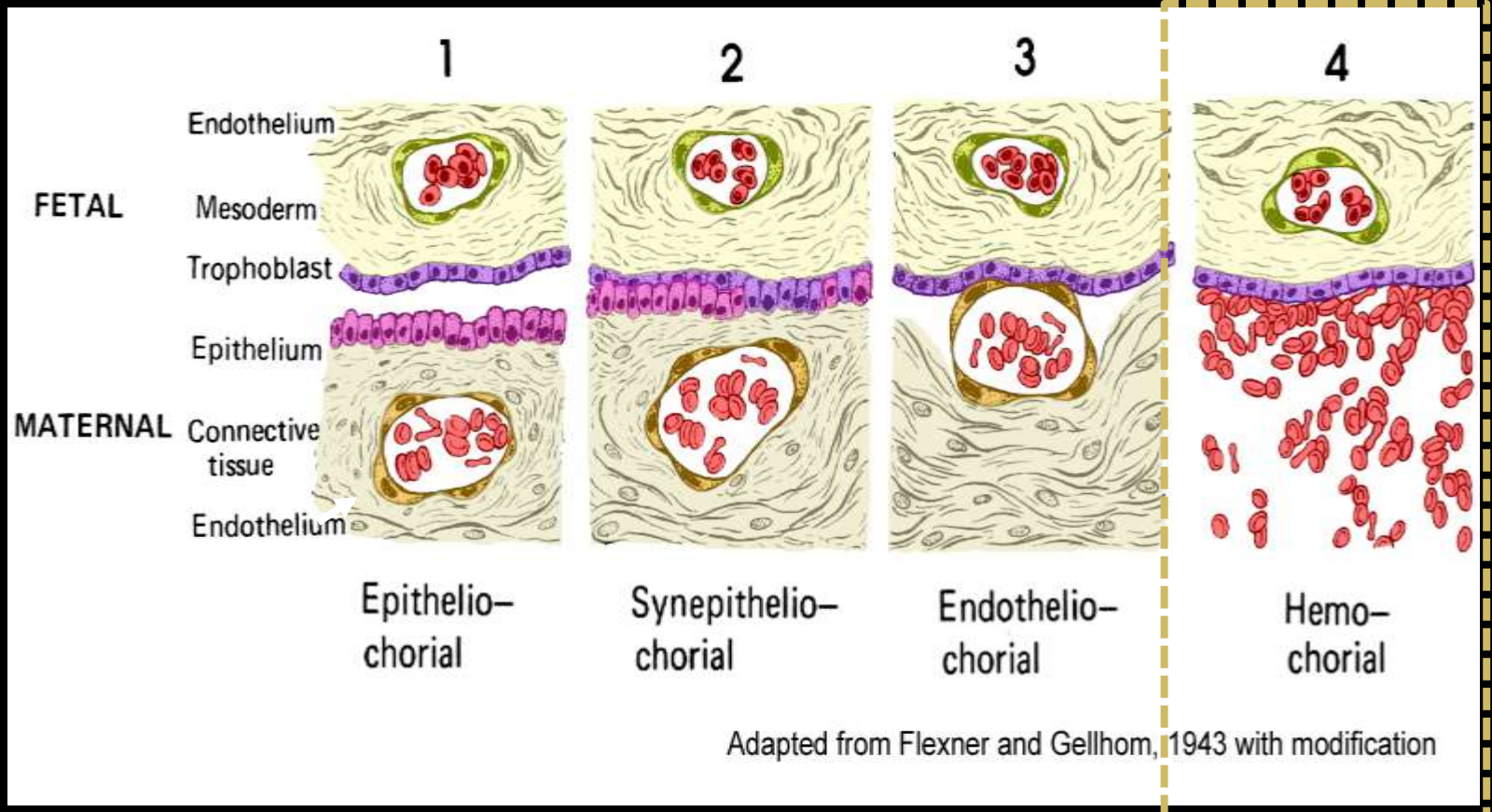


The placenta is an appropriate organ choice to begin to monitor how *in utero* environmental changes are sensed by the developing offspring.

Placental Responses Can Lead to Long-term Effects on Brain and Cardiovascular Function



Comparative Animal Placentation



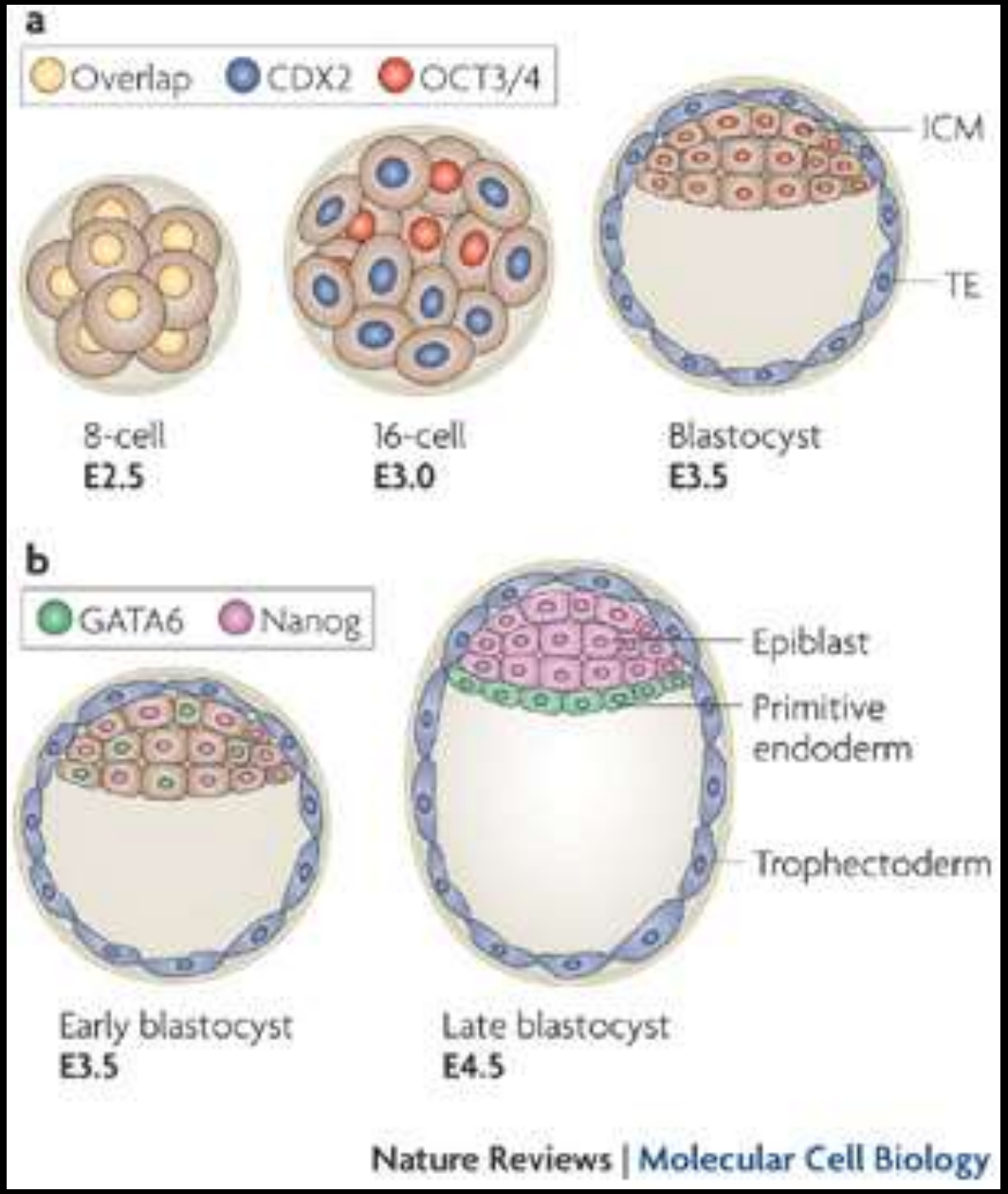
Swine
Horses
Cow

Sheep
Goats

Dogs
Cats

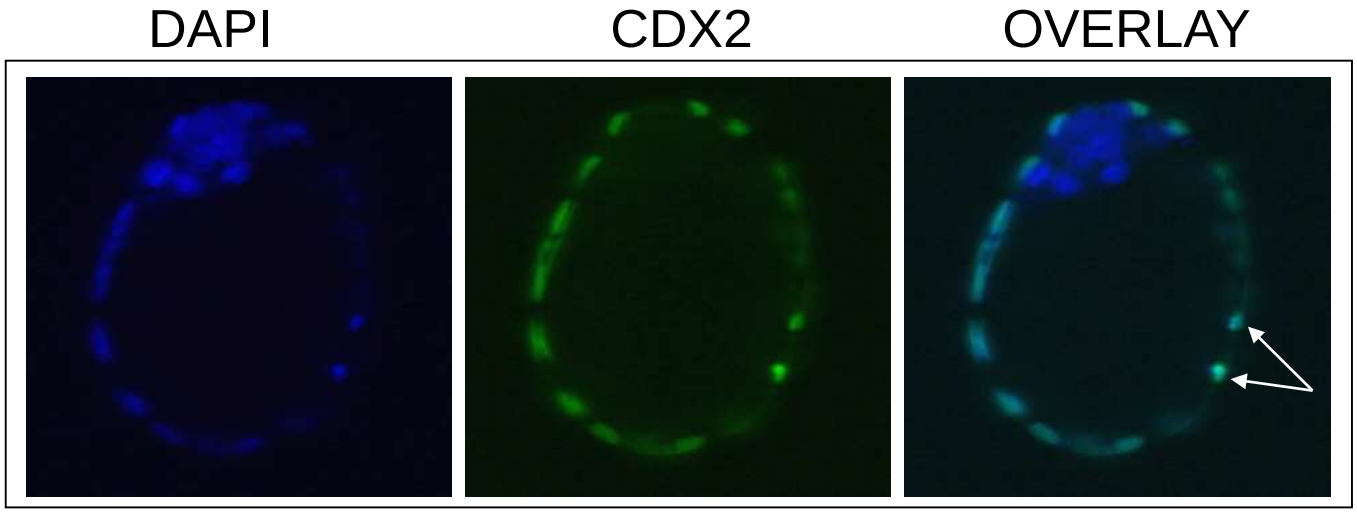
Primates
Rodents

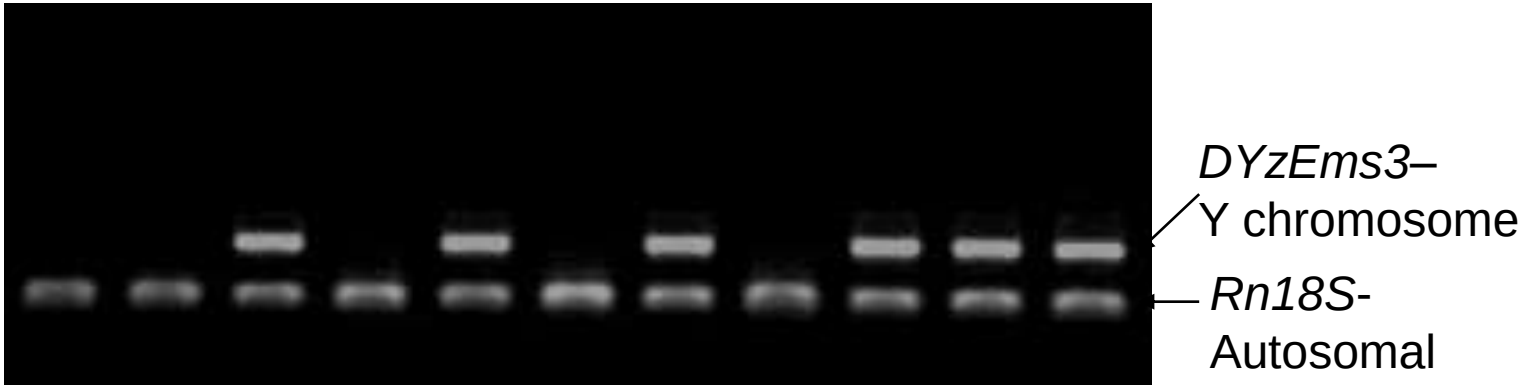
Pre-implantational Embryonic Development



Trophectoderm (TE) cells gives rise to part of the fetal placenta

Distinguishing TE Versus ICM Cells & Male Versus Female Embryos





Bermejo-Alvarez P, Roberts RM, and Rosenfeld CS. Mol Reprod Develop 2012; 79:329-336

Effect of Glucose Concentration on Embryo Cell Number According to Sex

Glucose (mM)	Sex	Total Cells Mean ± SEM	TE Cells Mean ± SEM	ICM Cells Mean ± SEM
0.2	Male	**76.3 ± 4.6^{a}**	**60.8 ± 4^{c}**	15.4 ± 1.2
0.2	Female	**76.3 ± 4^{a}**	**61.9 ± 4.8^{c}**	14.4 ± 1.4
28	Male	**61.1 ± 3.8^{b}**	**45.8 ± 3.1^{d}**	15.3 ± 1.5
28	Female	**54.8 ± 3.9^{b}**	**38.6 ± 3.6^{d}**	16.1 ± 1.3

Major Conclusions:

- No sex differences were observed in embryonic cell numbers due to *in vitro* changes in glucose concentrations.
- **Elevated *in vitro* glucose concentrations** that approximate those of diabetic maternal serum **decreases total cell and TE cell numbers in male and female blastocysts**,

Bermejo-Alvarez P, Roberts RM, and Rosenfeld CS. Mol Reprod Develop 2012; 79:329-336

Overall Goal

- We sought to examine how maternal diet might influence the full range of placental gene expression in male and female conceptuses at around mid-pregnancy (12.5 days post-coitus, dpc) in the mouse.
- This is when the morphological development of the placenta is complete but the gonads are not fully formed (i.e. minimal steroid production).

Previous Studies on Effects of Maternal Diet on Placental Gene Expression

- Prior to our study, only one published study to date examined how maternal diet governs global placental gene expression (Gheorghe et al., Placenta 2009).
- This study revealed that in mice a short withdrawal of protein for four days in mid-pregnancy has deleterious consequences on placental gene expression.
- The study, however, did not consider the possibility that male and female conceptuses might show different responses to the imposed diet.

Experimental Approach

- We employed NIH Swiss dams maintained for an extended period (35 to 40wks) on one of three diets:

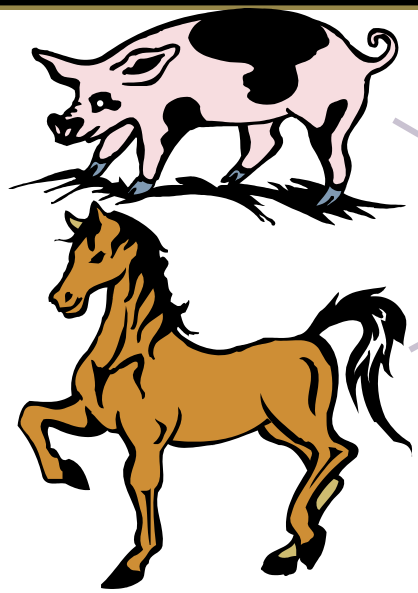
1. Low fat (LF) (Research Diets)
2. Very high fat (VHF) (Research Diets),
3. Purina 5015 chow (C) diet (Test Diets), the latter approximating standard husbandry conditions for experimental mice during pregnancy.

Experimental Approach

- Other reasons we chose to collect the mouse placental samples at 12.5 dpc:

1) The fetal discoid placenta can easily be dissected from the maternal placenta at this stage
2) This period of gestation is characterized by marked up-regulation of "*rodent specific*" placental genes that encode such products as prolactin-related proteins, carcinoembryonic antigen-related cell adhesion molecules (CEACAM), pregnancy-specific glycoproteins (PSG), and various cathepsins.

Different Types of Placentae



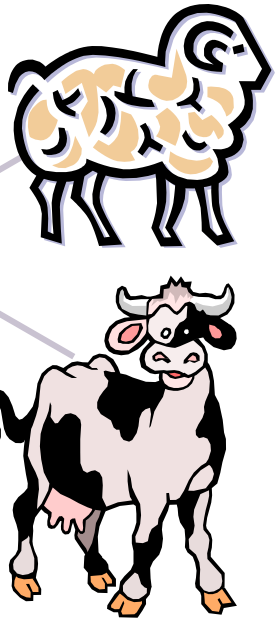
Chorion

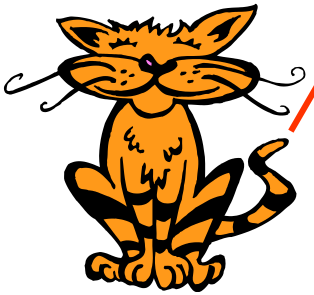
Diffuse

Necrotic tip

Cotyledonary

Cotyledon





Zonary

Haemophagous organ

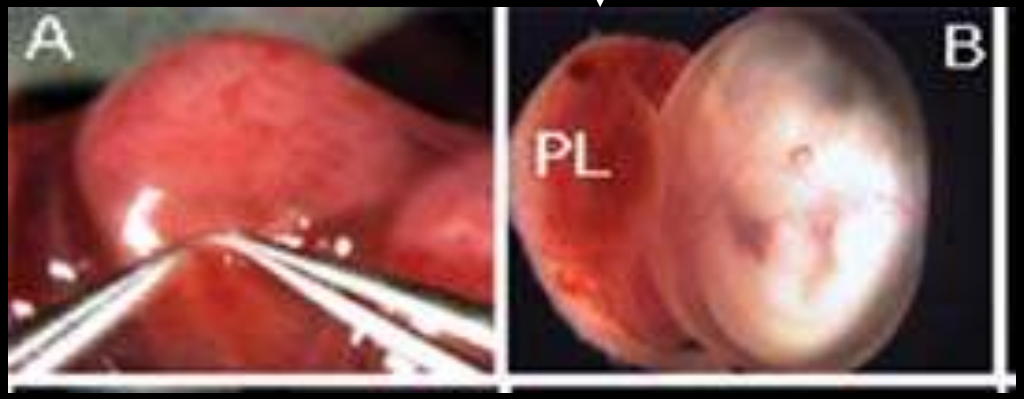
Placenta

Discoid

Amnion and chorion

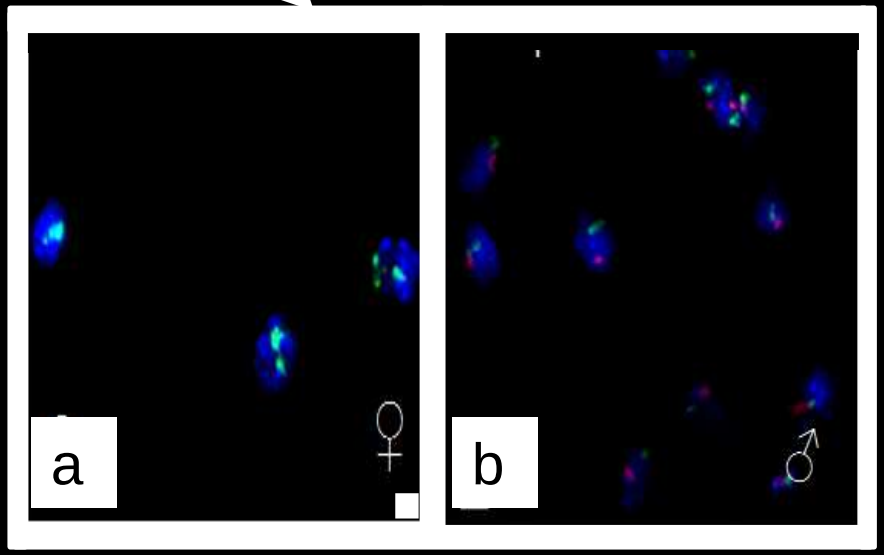
Experimental Approach

Discoid Placenta



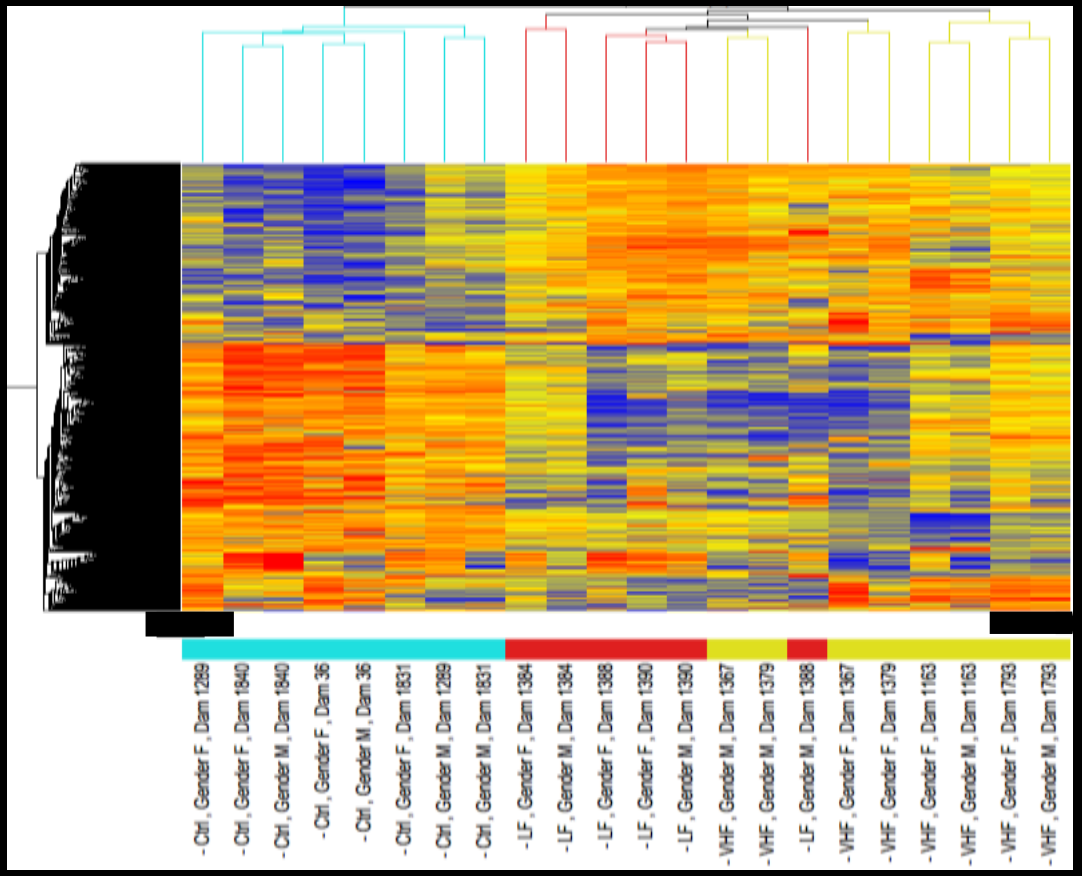
- RNA from the placenta was isolated and reverse transcribed for hybridization to Agilent Whole Murine Genome 4x44K arrays and QRT-PCR.
- Female and male placentae were pair-matched to the same mid-uterine horn region, which was on the right side for all but one VHF dam, where the pair was selected from the left mid-uterine horn.

XY FISH Analysis



Whyte et al., Theriogenology, 2007, Mao and Rosenfeld, Molecul Reprod Develop 2009.

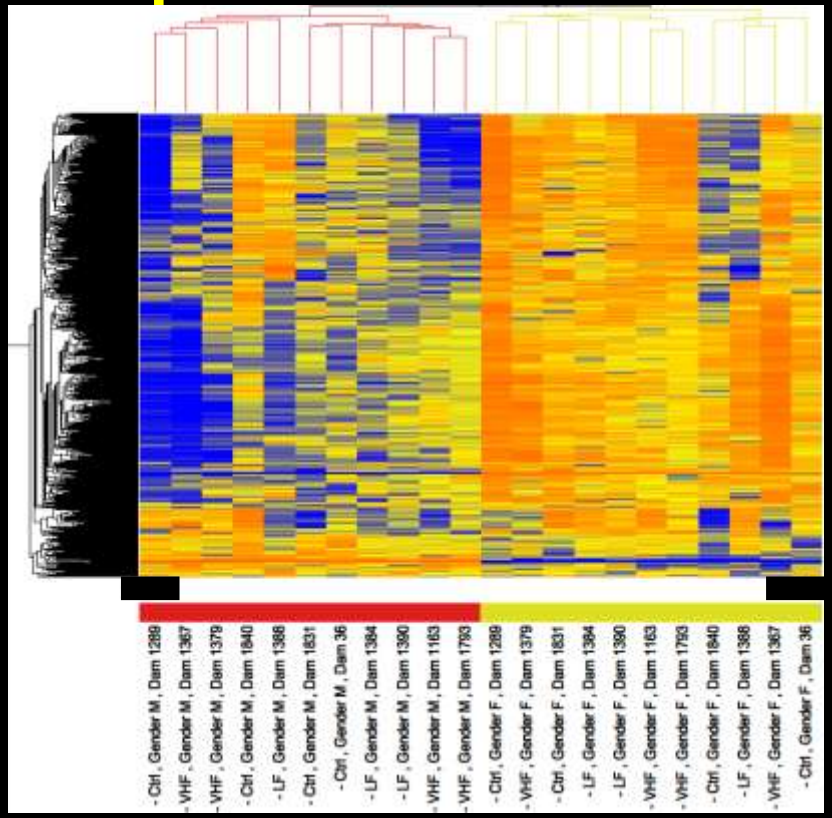
Results: Maternal Diet Alters Placental Gene Expression in Mice



Heat map based on maternal diet effects on placental gene expression. Gene tree clustering on 1,972 genes, whose expression was changed more than 2-fold with $P<0.05$.

Mao et al., PNAS, 2010; 107(12):5557-5562

Results: The Murine Placenta Displays Strikingly Sexually Dimorphic Differences in Placental Gene Expression Patterns



The placentae gene expression patterns of male conceptuses clearly clusters separately from the placentae of females, when data on the total regulated genes (with 2-fold differences) across all dietary groups are compared ($P<0.05$).

Mao et al., PNAS, 2010; 107(12):5557-62

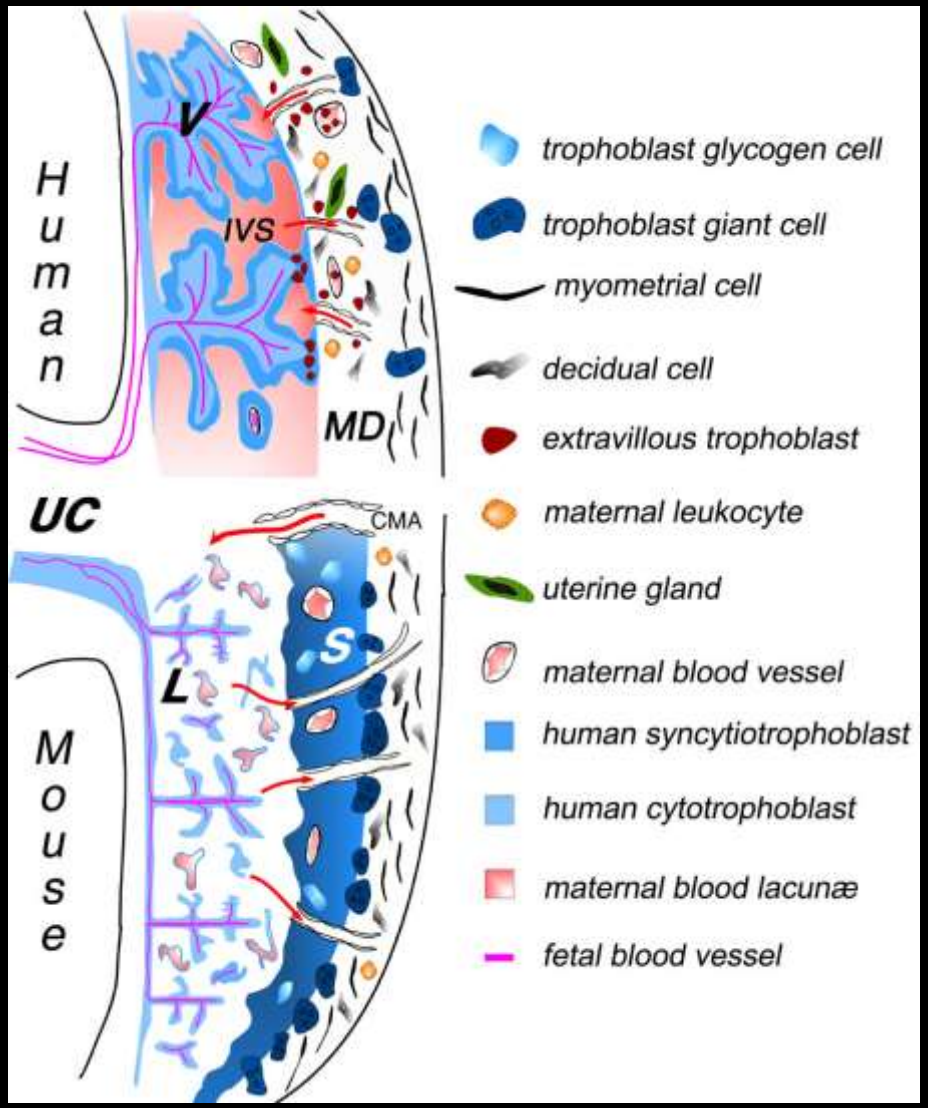
Examples of Sexually Dimorphic Expressed Placental Genes Confirmed by Quantitative Real-Time PCR Analysis

- Aquaporin 9
- Chemokine (C-C motif) receptor 3
- CEA-related cell adhesion molecule 1 (mouse placental specific gene)
- Estrogen receptor 1
- Hydroxy-delta-5-steroid dehydrogenase, 3β-and steroid delta-isomerase 5
- Olfactory receptor 1381
- Olfactory receptor 154
- Olfactory receptor 433
- Olfactory receptor 520
- Renin1
- Renin2

How do Sexually Dimorphic Differences Originate in the Placenta?

- **Sex Steroids**- Unlikely at 12.5 dpc
- **X- chromosome dosage**- Unlikely due to X-chromosome dosage, unless the paternal X chromosome is incompletely silenced in the female placentae.
- **Epimutations**- Likely mechanisms. After our study was published, it was demonstrated that fetal sex and maternal diet can alter DNA methylation patterns in the murine placenta (Gallou-Kabani et al., PLoS One. 2010; 5:e14398) and gene expression of histone demethylase paralogues (*Kdm5c* and *Kdm5d*, Gabory et al., Plos One 2012; e47988).

Need to Examine How *In Utero* Environmental Changes Affect in a Sex Dependent Manner Specific Placental Regions and Cells



In the spiny mouse (*Acomys cahirinus*):

- The female placenta has less spongy zone and more labyrinth region than males.
- There are sex-dependent and regional differences in placental gene expression.

O'Connell et al. Placenta 2013; 34: 119-126

Acknowledgements

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