

Preliminary Transcriptomic Analysis of Porcine Maternal- Embryonic Interactions

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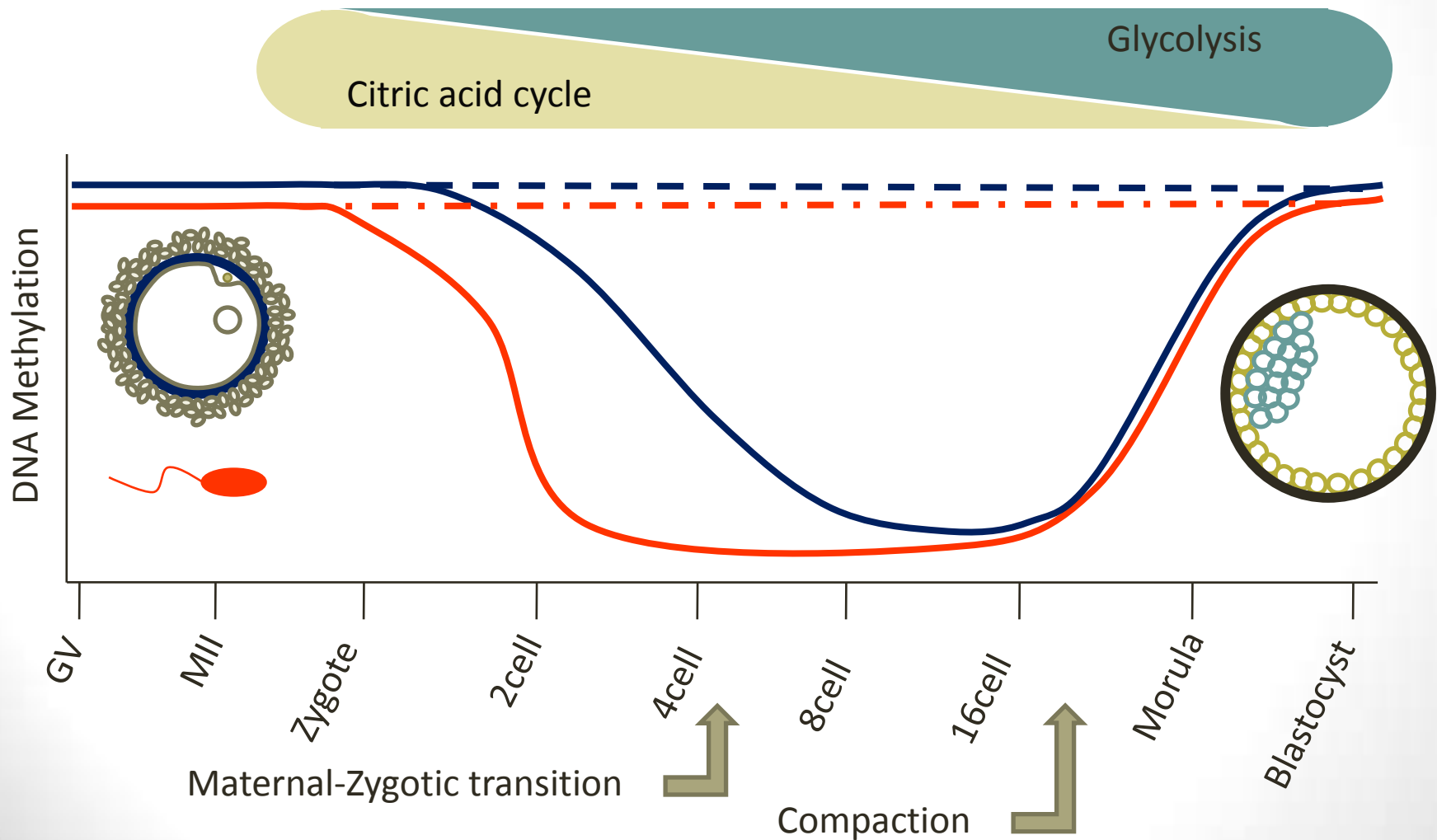
Outline

- Experimental background
- Preliminary methodology for analysis of embryo-endometrial interactions
- Selective preliminary results

Effects of Embryo Culture

- Significant alteration to the transcriptome as a result of in vitro culture - Miles et al. (2008)
- Embryo culture results in aberrant methylation of a number of imprinted genes - Mann et al. (2004) and Khosla et al. (2001)
- Poor development of in vitro embryos results from inappropriate methylation of a few genomic regions - Bonk et al. (2008)

Period of Embryo manipulation

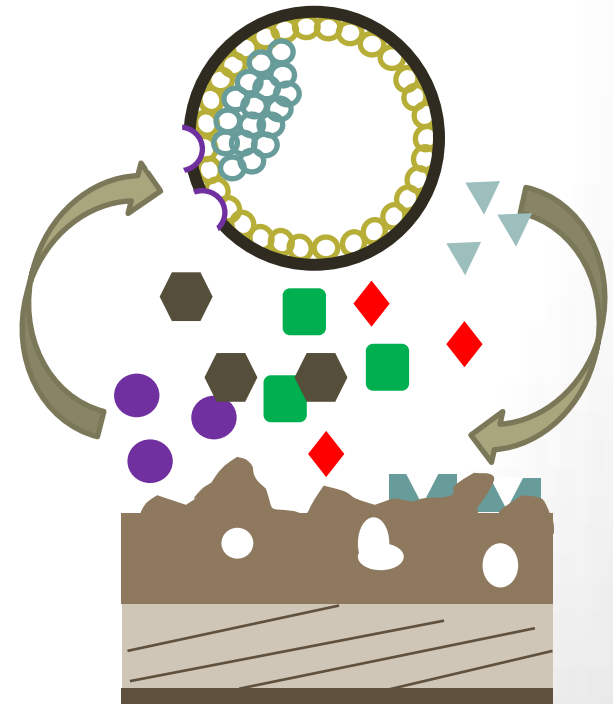


Incremental Advances in Embryo Culture

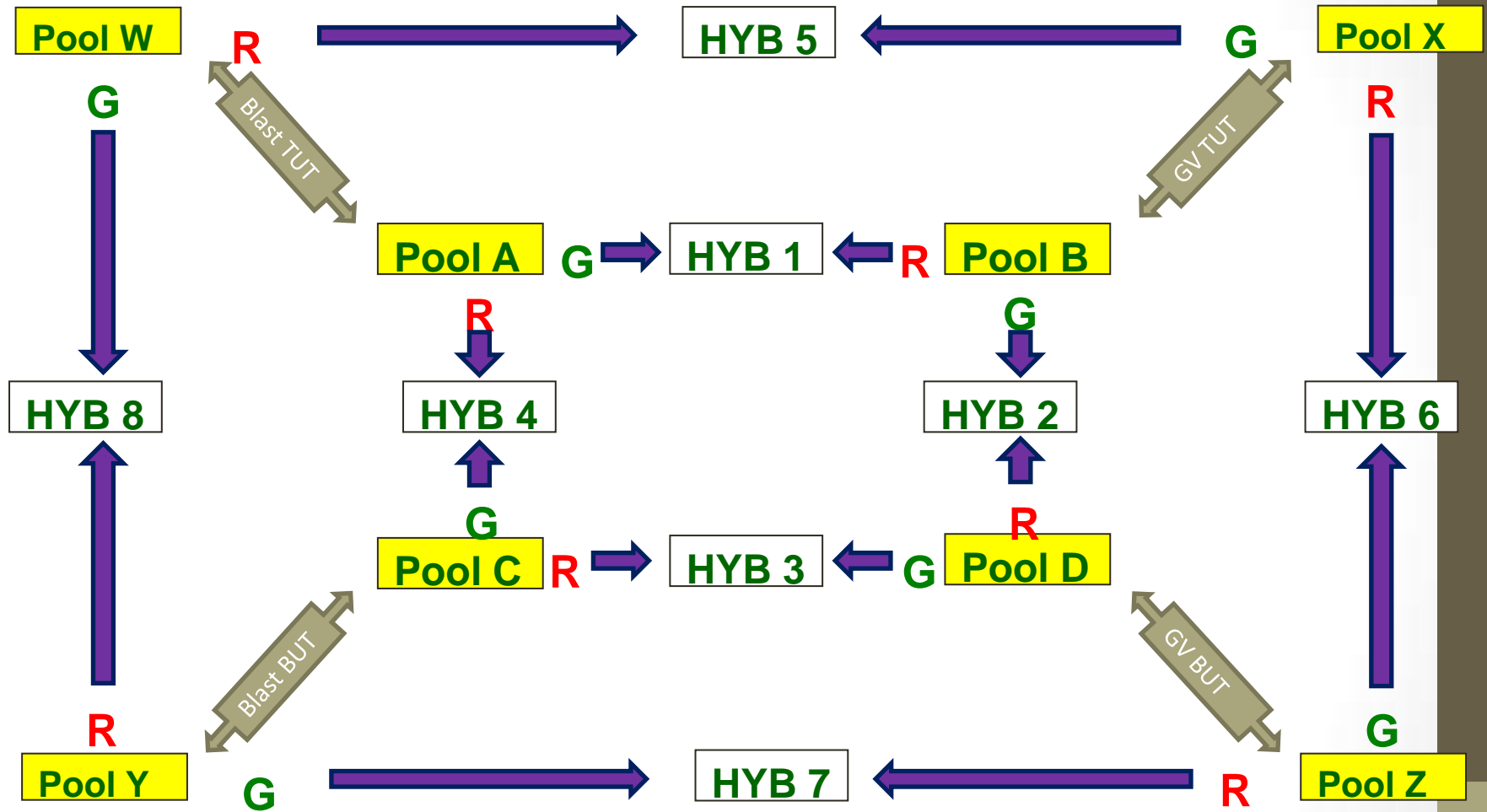
- EGF improves embryo development in a protein-free system - Abeydeera et al. (2000)
- Glutamine and taurine are beneficial for embryo development - Devreker et al. (1999)
- Oviductal glycoprotein improves development in a serum-free system - McCauley et al. (2003)
- Hyaluronic acid is better than BSA, which is better than no protein - Gardner et al. (1999)

A Biomimetic Approach

- Using high throughput methodologies, the key factors in the in vivo environment affecting embryonic development can be identified and reproduced in culture
 1. Candidate approach
 2. 2D proteomics
 3. Microarray



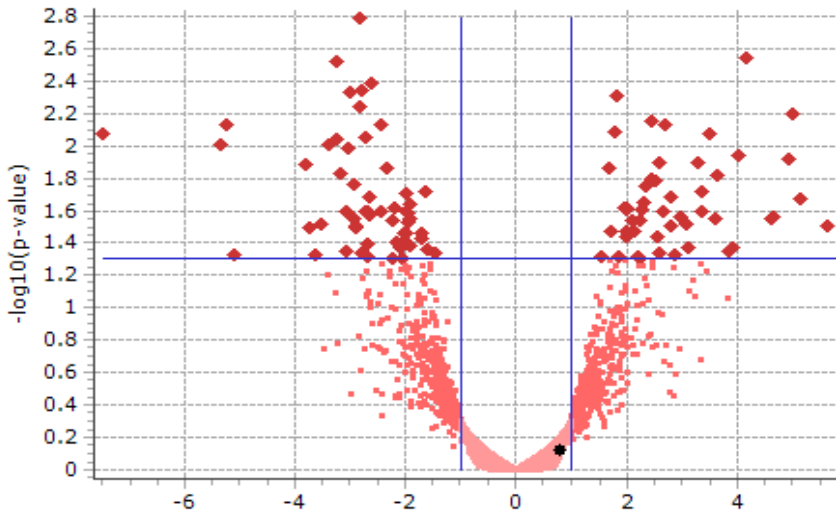
Uterine Experimental Design



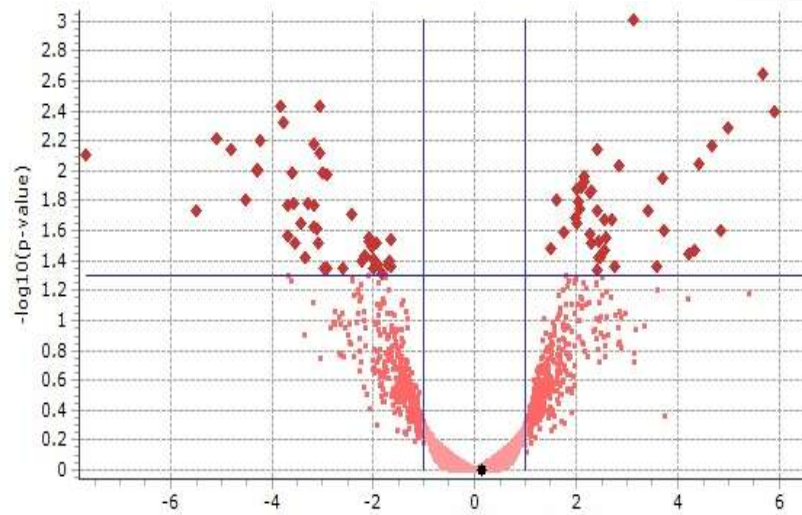
- Extraction, Amplification, Labeling and Hybridization via SRDP standard protocol

Initial Results

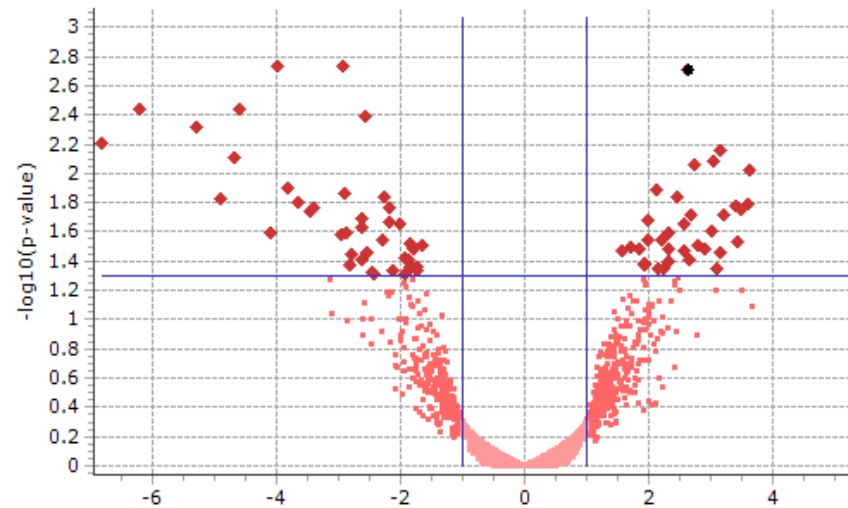
Blast BUT vs GV BUT



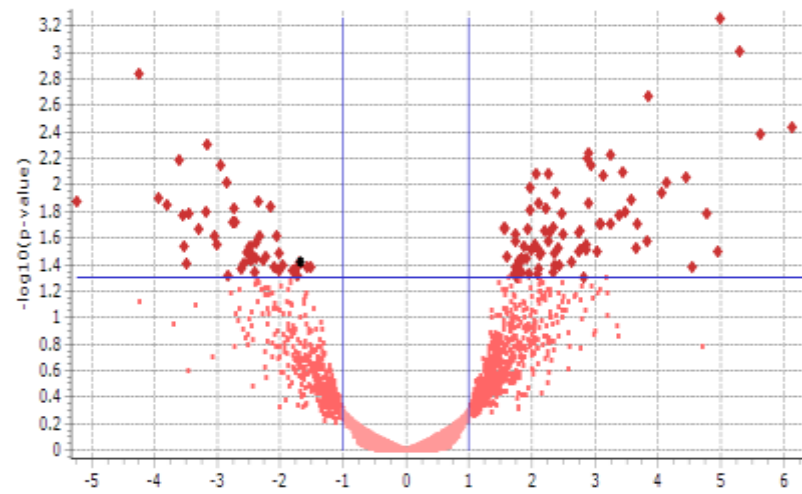
Blast TUT vs Blast BUT



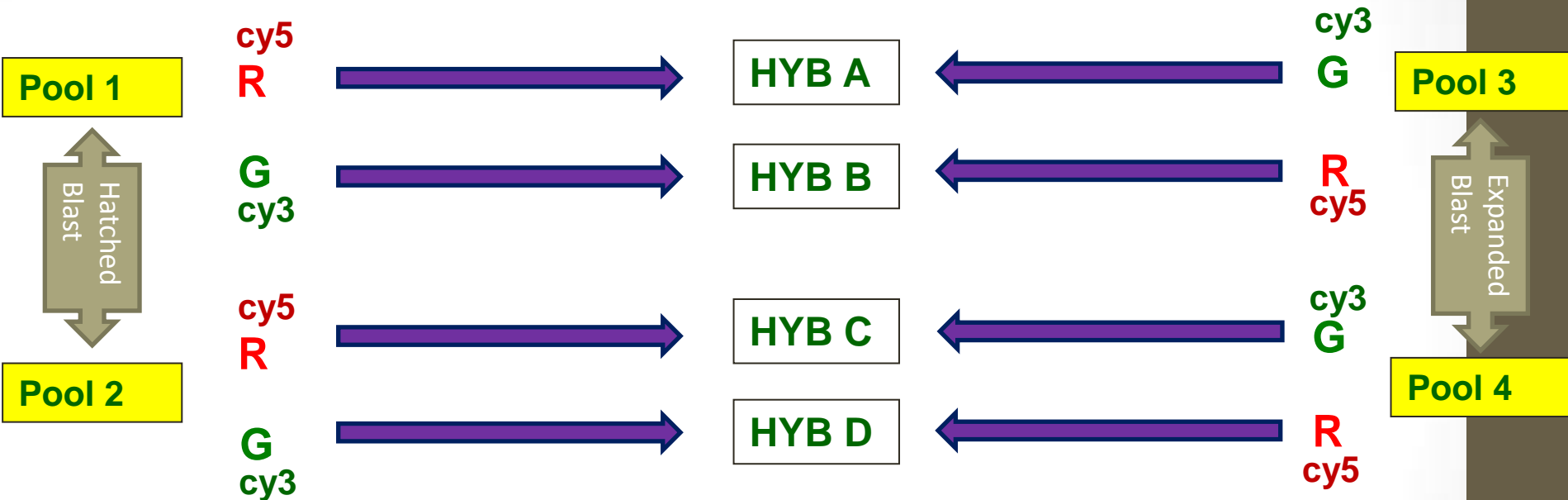
Blast TUT vs GV TUT



GV TUT vs GV BUT

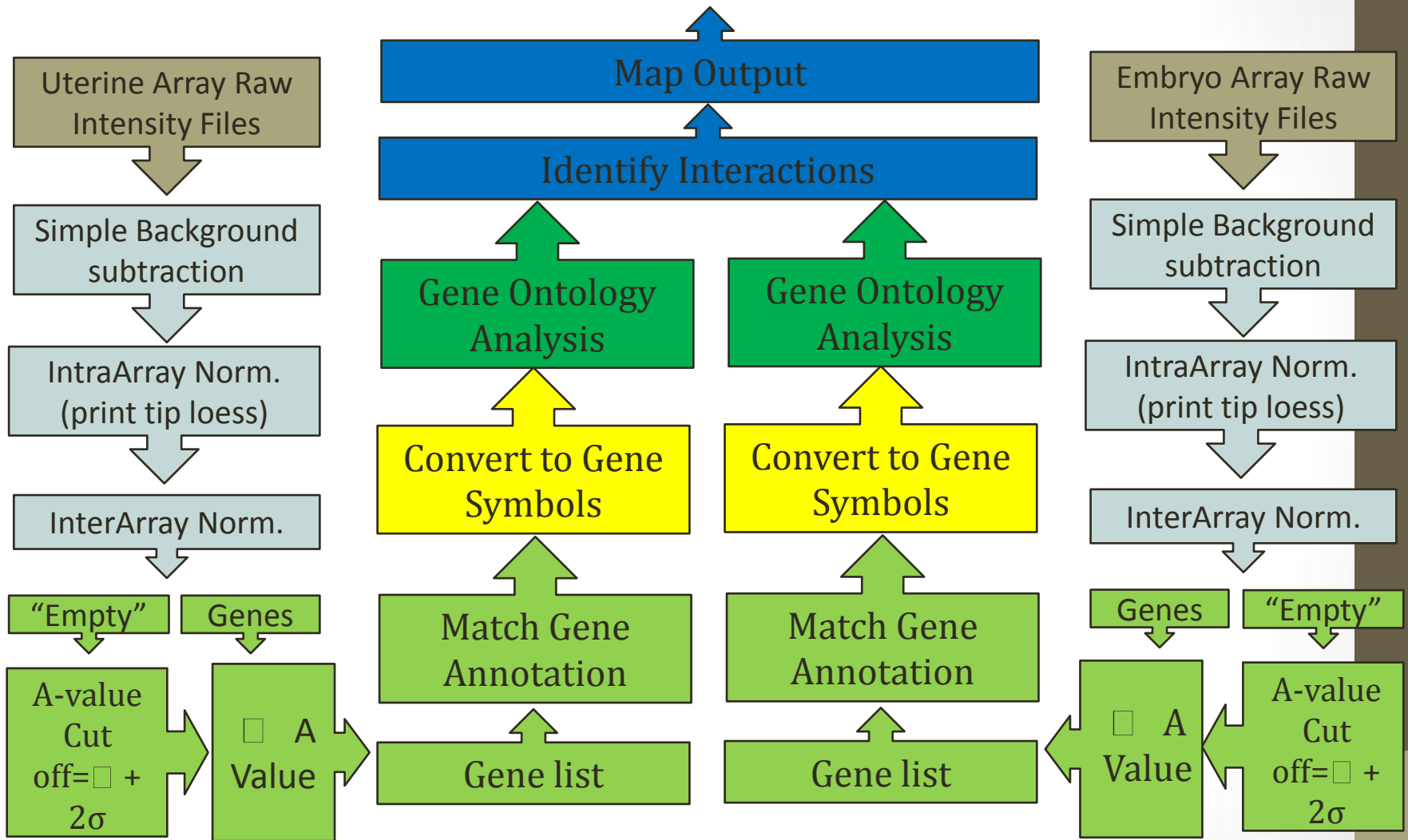


Embryo Microarray Design



- RNA extracted from pools of 8-10 blastocyst stage embryos
- Amplified, Labeled and Hybridized via EmbryoGENE Protocol

Bioinformatics Workflow



Microarray Annotation

- 14,562 genes expressed in the endometrium and 9,306 expressed in the embryo.
- Only 8,648 (59.3%) and 5,476 (58.8%) were annotated to an acceptable level for gene ontology

Contig ID	Oligo Sequence	Best Match Gene ID
40495_41142820	AGACTGGAG...	ENSG00000163995
TC199479:f	GAGCAACTG...	TC199479:f
NM_001001533.1	AAGCCCAA...	NM_001001533.1
45360_74379627	GCCTGTTGAT...	ENSDART00000052478
45360_CL1320Contig1	GGCAGTAAA...	NEWSINFRUT00000172677
5734_49343223	TGGAATATAC...	No Match

STRING

- STRING (*Search Tool for the Retrieval of Interacting Genes/Proteins*)
- A database of known and predicted protein interactions
- The database currently covers 5'214'234 proteins from 1133 organisms.

Genomic
Context



High-throughput
Experiments



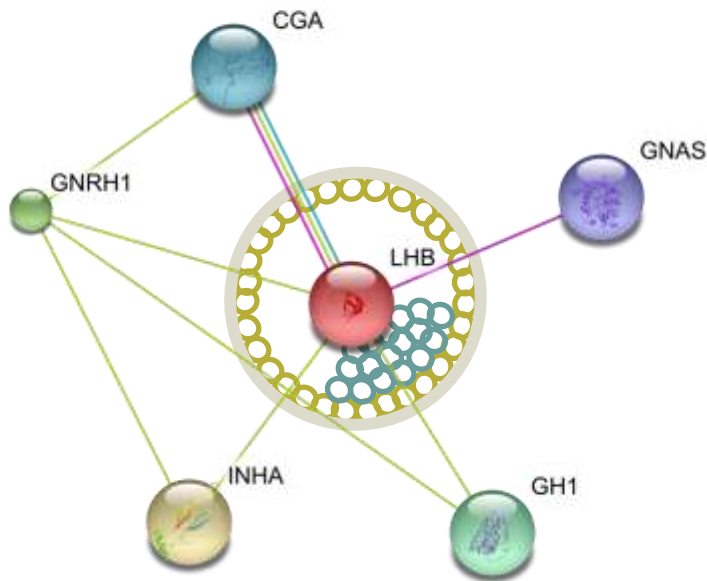
(Conserved)
Coexpression



Previous
Knowledge

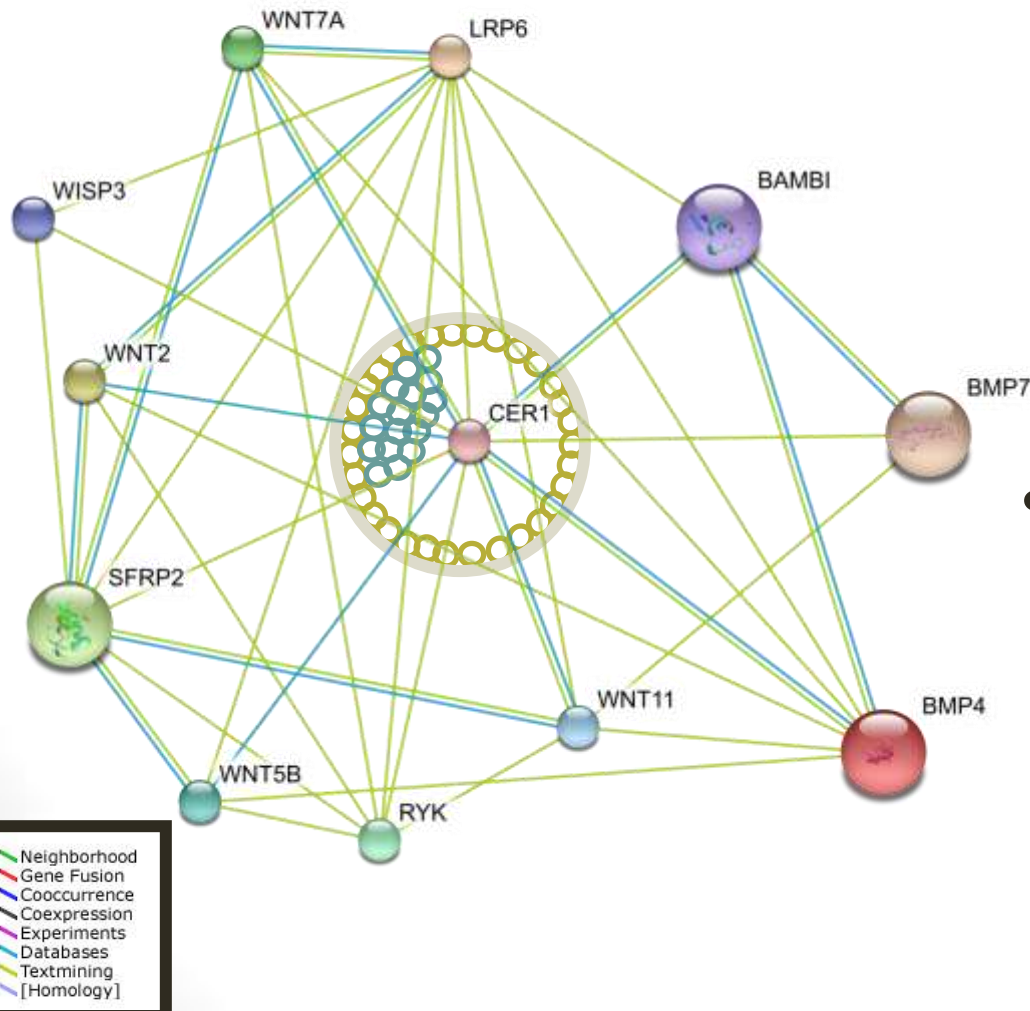


Results: Embryonic Luteinizing Hormone



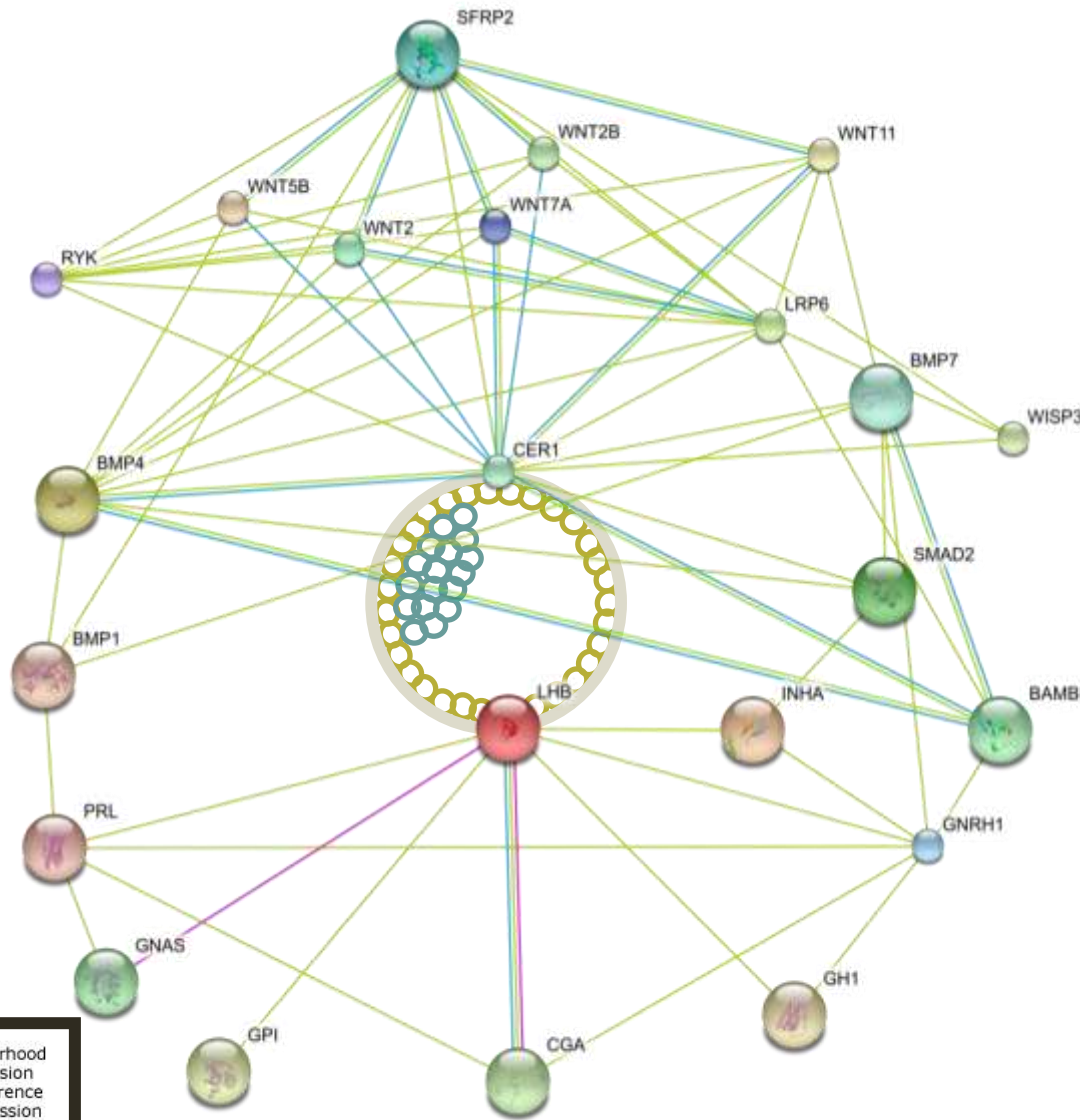
- The gene encoding the Beta Chain of LH (LHB) is expressed in the embryo alone, whereas the Alpha chain CGA is produced only in the uterus
- LH interacts with a number of uterine receptors including GH1, which modulates the release of IGF1

Results: Embryonic CER1



- The Cerberus 1 (CER1) gene is expressed in the embryo and is known to act as a BMP antagonist
- CER1 also interacts with BAMBI, a gene known to negatively regulate TGF β signalling

Results: LHB-CER1 Interplay



While CER1 and LHB do not interact with each other directly, they do interact through intermediaries

Conclusions

- **Further analysis of paired embryo-endometrial gene expression is an ideal method to elucidate key factors involved in their interaction**
- **Up-to-date, complete and accurate annotation is vital to ensure full array coverage during in-depth microarray analysis**
- **Higher stringency expression cut offs should be used to increase reliability of identified interactions**
- **Additional refinements are required to narrow gene interactions to those that exist between embryo and endometrium alone**
- **Gene analysis with the EmbryoGENE SEST Microarray may reveal additional genes important for embryo-uterine interactions.**