UNRAVELLING THE MOLECULAR MECHANISMS IN THE EARLY STEPS OF THE IMPLANTATION PROCESS

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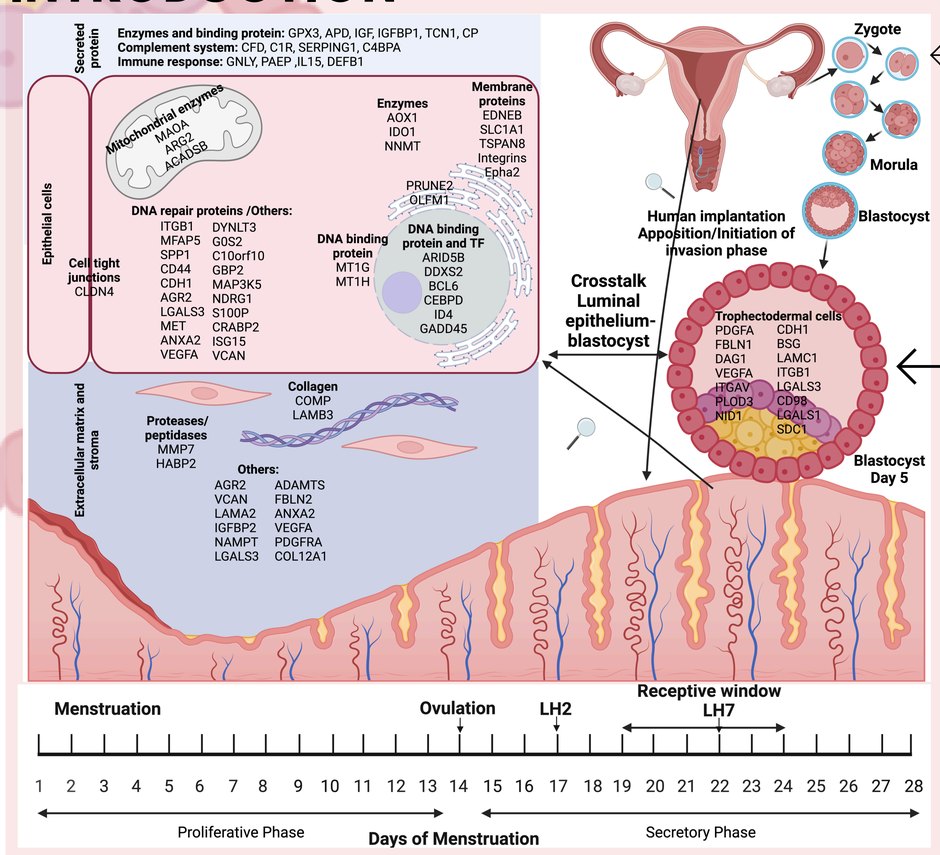
INTRODUCTION



- Endometrium and a competent embryo
- Endometrium function
  - Eliminate hostile
  - Nourish the embryo
- Mid-secretory phase (LH+7-LH+9)

Successful embryo implantation

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Mammalian reproduction

Poorly understood in humans

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Highly complex as it involves a cascade of tightly regulated molecular mechanisms

For ethical reasons the process is largely unknown

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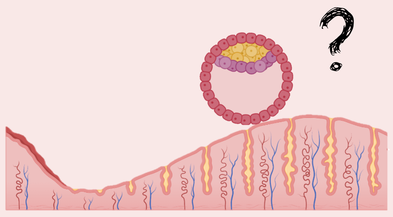
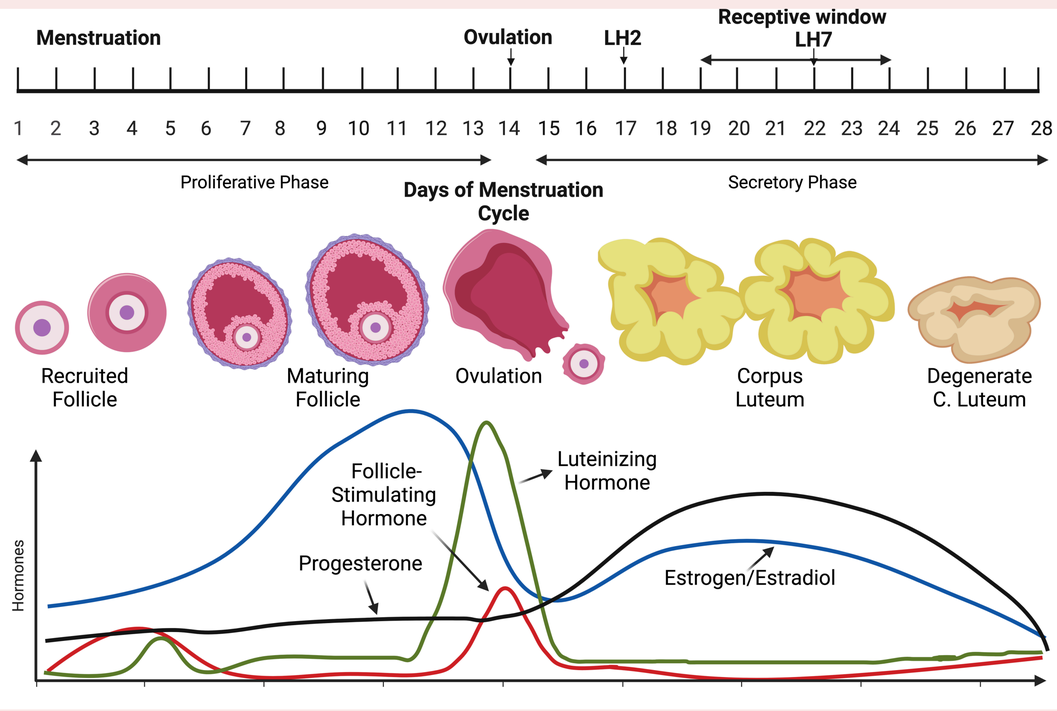
The interactions identified are imprecise



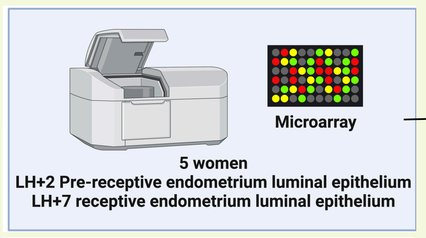


- Complex mucosa inner part of the uterus
- Self-regulating capacity
- Hosts of earliest stages of embryonic development
- Regulation by ovarian hormones

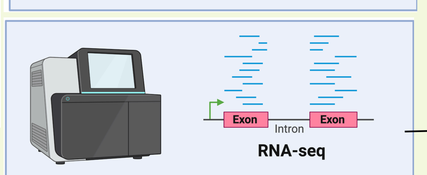
- Chromosome copy number in the embryo reduces fertility/viability
- Euploid --> 46 chr
- Aneuploidy deviation in the number of copies AND affected implantation failure, pregnancy loss and congenital conditions

MATERIAL AND METHODS

Experimental design

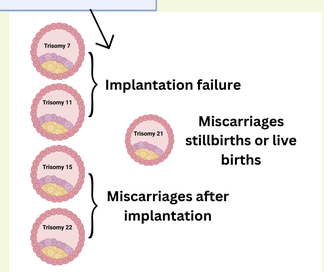


1. Cell isolation
2. RNA extraction
3. Monocatenary cDNA
4. Microarray Agilent 4x44
5. Bioinformatics analysis



1. Cell isolation
2. RNA extraction
3. Library generation
4. Sequencing on NextSeq 500
5. Bioinformatics analysis

GitHub



1. Quality analysis
2. Limma Bioconductor Package
   a. Background subtraction
   b. Normalisation quantile method
   c. Design based on patient and sample type
   d. DEG Adjusted P-value <0.05
   e. Log2FC to measure expression change

↓

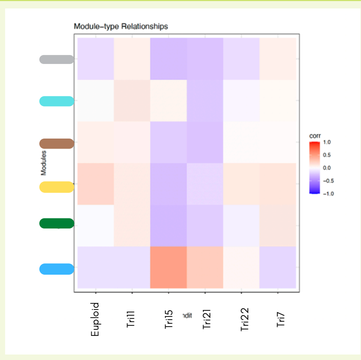
1. Replicability of the samples
2. Functional enrichment with clusterProfiler
   a. Gene Ontology
   b. KEGG

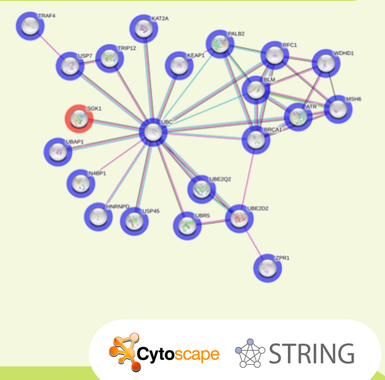
GENEONTOLOGY Unifying Biology

1. miARma-seq
   a. Quality analysis
   b. Alignment
   c. Quantification
2. Limma Bioconductor Package
   a. Deletion of genes with low expression
   b. Normalisation
   c. Design based on batch and sample type
   d. DEG FDR <0.05
   e. Log2FC to measure expression change

WGCNA

1. Biological networks based on pairwise correlations between variables
2. Detailed study of the modules detected from the corrected and normalised data matrix using the ComBat package
3. Functional enrichment of the genes present in each module





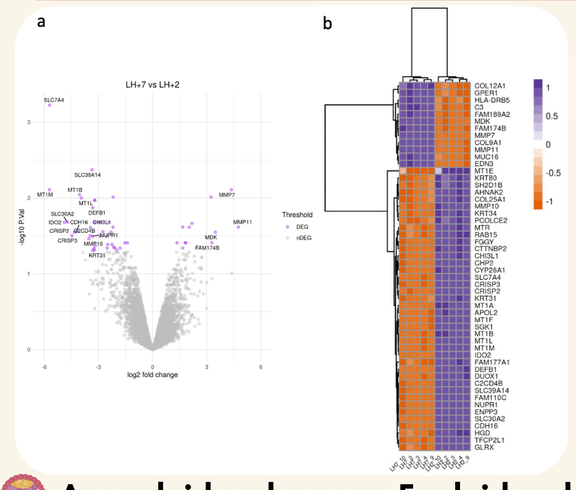
Cytoscape STRING

1. Single set for each endometrium-embryo combination
2. Text mining interactions were removed
3. Interactions with an overall mean score (<0.4) were removed
4. Networks clustering
5. Functional enrichment

RESULTS AND DISCUSSION

Endometrium LH+7 vs LH+2

- 52 DEGs most of them down-regulated
- 401 identified by Evans et al.
- Signature of 57 genes involved in endometrial receptivity (Altmäe et al)--> 3 coincide



Possible quiescence or arrest of cell growth and, therefore, less biological activity in the receptive phase, favouring successful embryo implantation

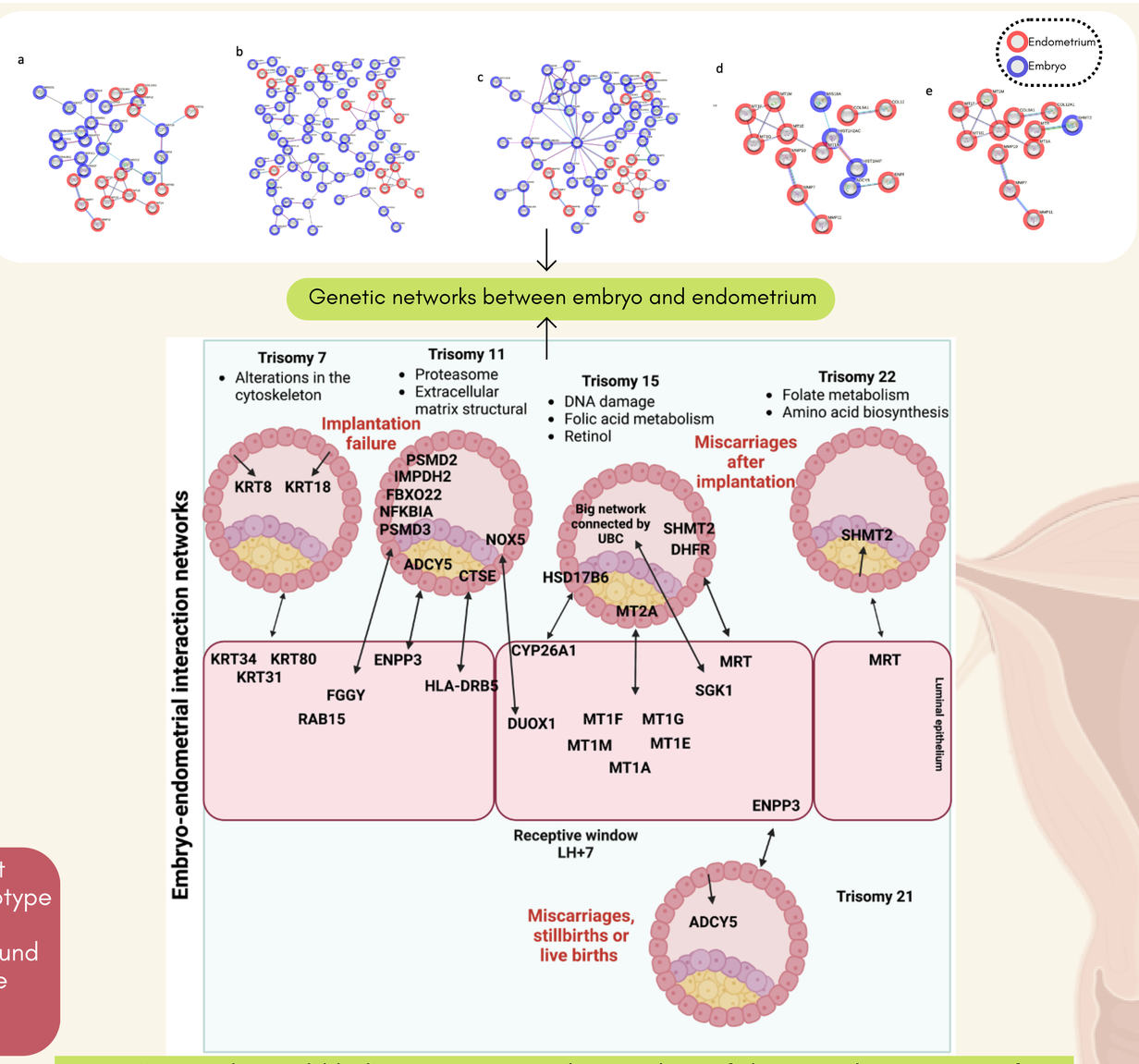
- Regulation of the inflammatory response
- Response to external stimuli
- Regulation of the defence response
- Regulation of the epithelial cell apoptotic process
- Cellular response to inorganic substances
- Detoxification processes
- Regulation of growth

Aneuploid embryos vs Euploid embryos

a) Trisomy 7--> 113
b) Trisomy 11--> 221
c) Trisomy 15--> 215
d) Trisomy 21--> 19
e) Trisomy 22--> 21

- Different alterations in the trophoblast cells can lead to the same final phenotype
- No fundamental altered pathways in embryonic development have been found
- Importance of coordination of multiple independent pathways for successful implantation

They are not implicated in the occurrence of implantation failure or miscarriage in trisomy 21



Our study would help to improve understanding of the complex process of embryo implantation in humans and, hopefully, lead to the generation of new prognostic and diagnostic biomarkers and therapeutic approaches targeting both infertility and fertility

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