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Maternal age alters gene expression in blastocyst trophoblast and term placenta as well as term placental structure

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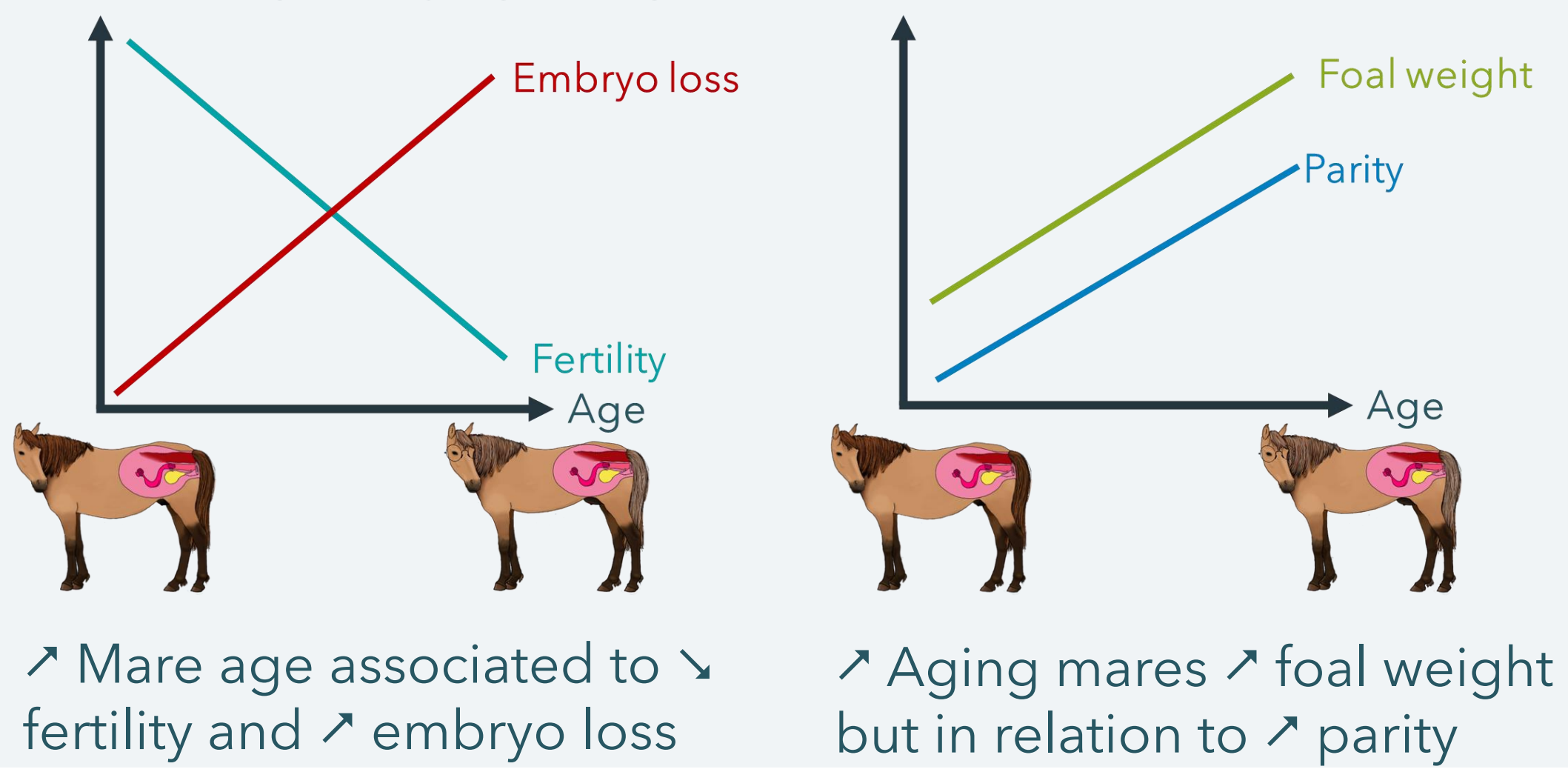
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OBJECTIVES: EVALUATE EFFECTS OF MATERNAL AGE ON GENE EXPRESSION IN BLASTOCYST'S TROPHOBLAST AND IN TERM PLACENTA AS WELL AS ON PLACENTAL HISTOLOGY



INTRODUCTION



BIOINFORMATIC & STATISTICAL ANALYSIS

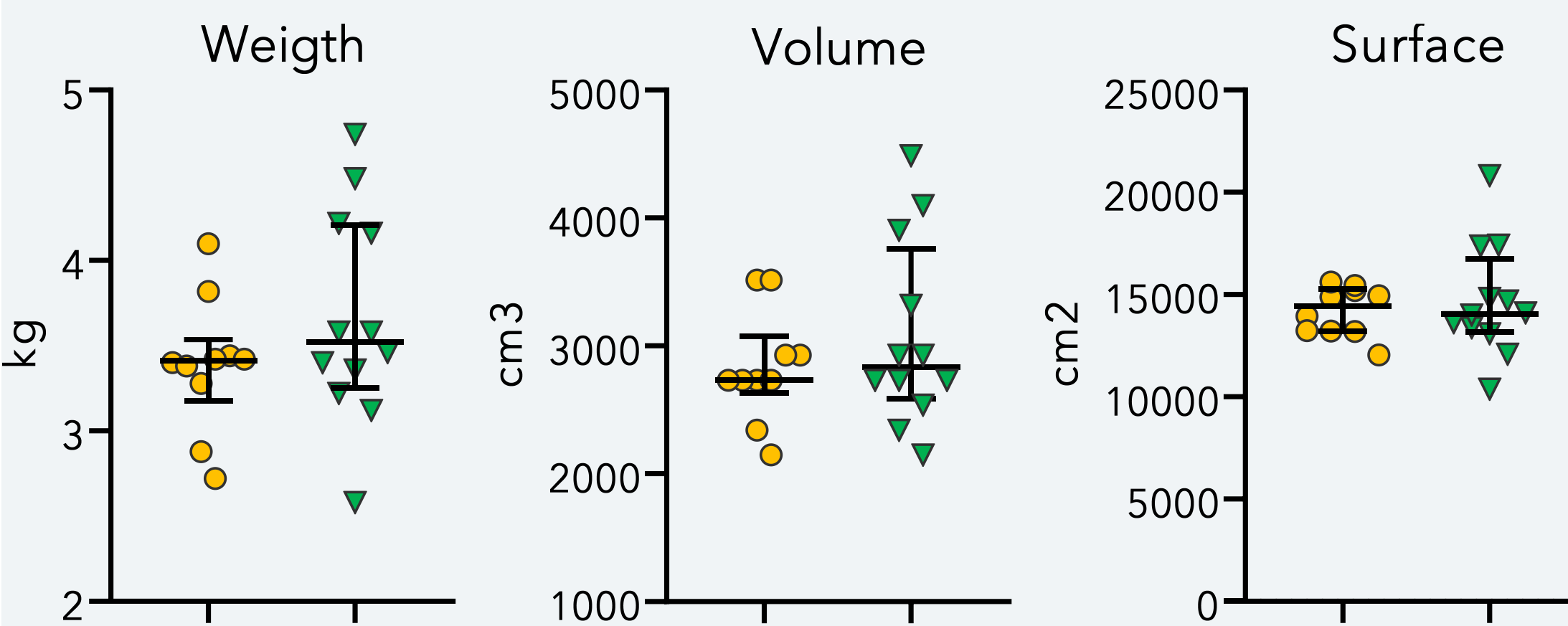
RNAseq differential analysis: DESeq2 package on R software with size and sex as cofactors for embryos; gestation length, foal sex and placental weight as cofactors for placentas; padj<0.05 for significance

Gene Set Enrichment Analysis (GSEA): Broad Institute software using the KEGG and GO biological process (BP) databases
 → Redundancy was reduced using SUMER analysis
 FDR<0.05 for significance; In analyses, "young" is the reference group

Stereological analysis: Microcotyledonary and allantoid relative surface and volume quantification by One Stop Stereology using Mercator® software (ExploraNova)

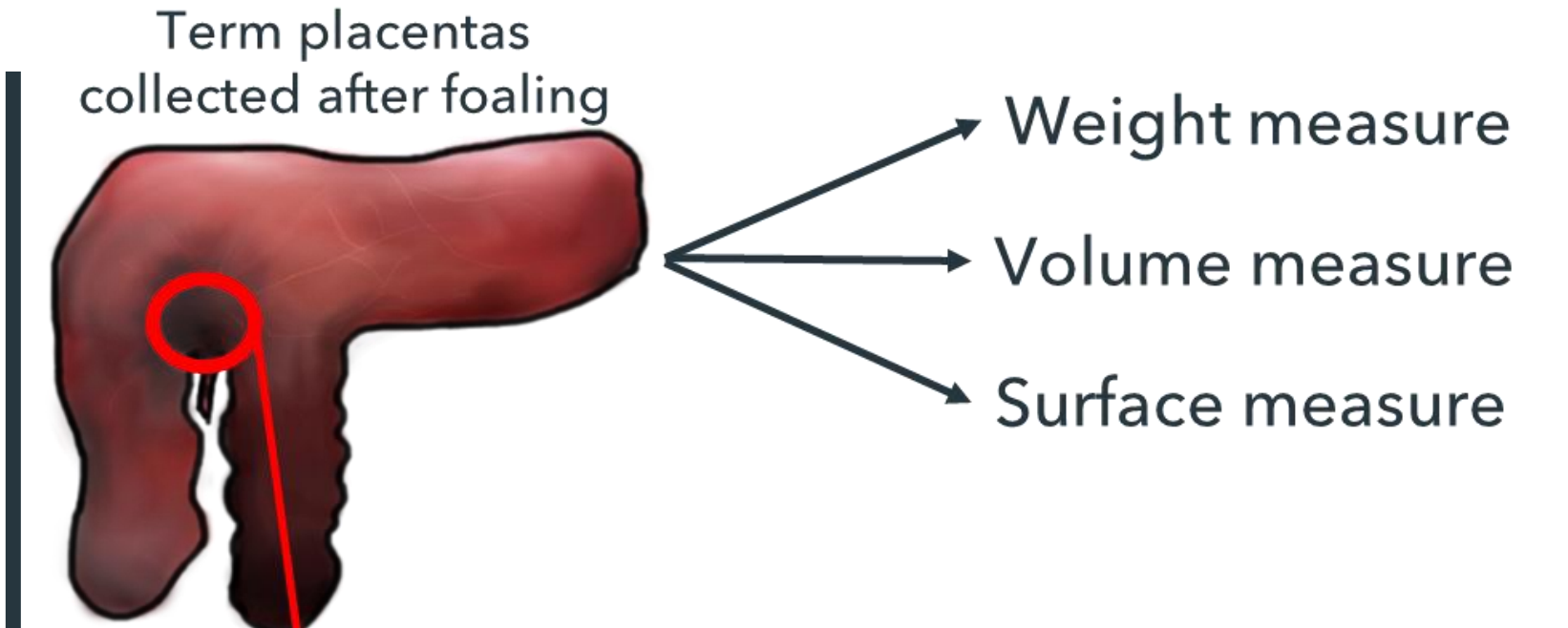
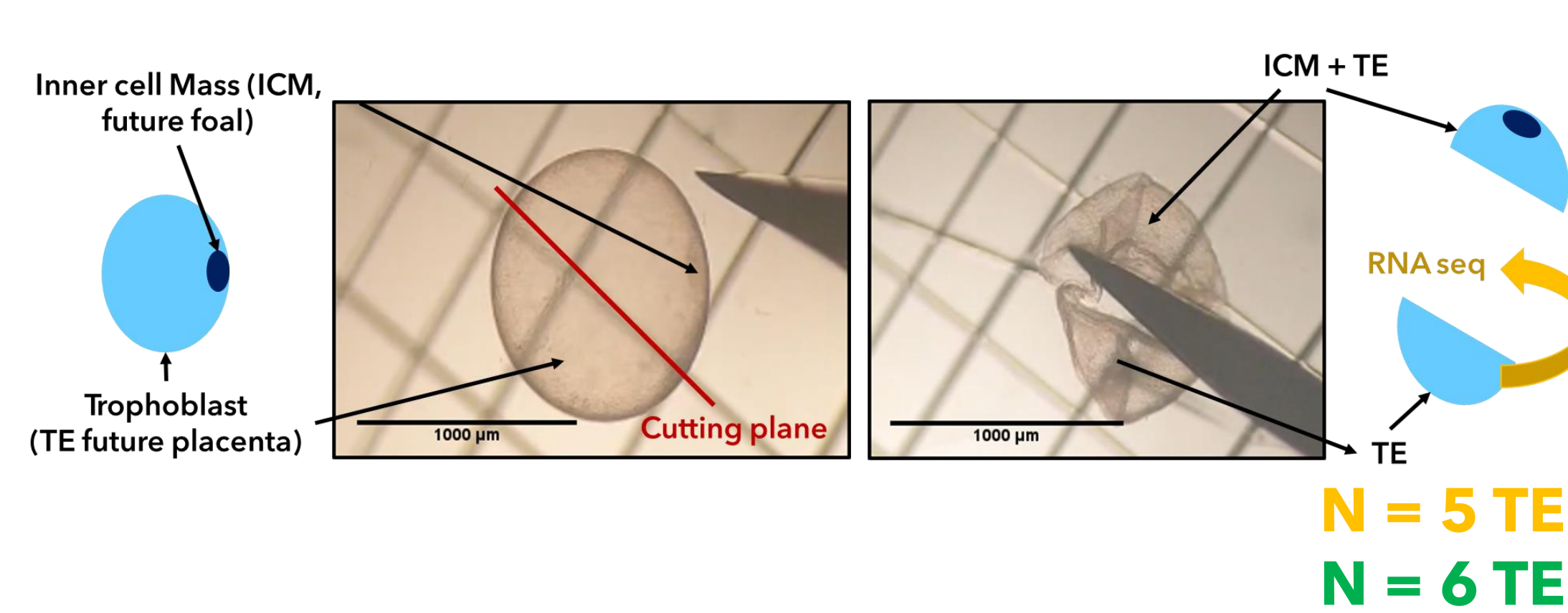
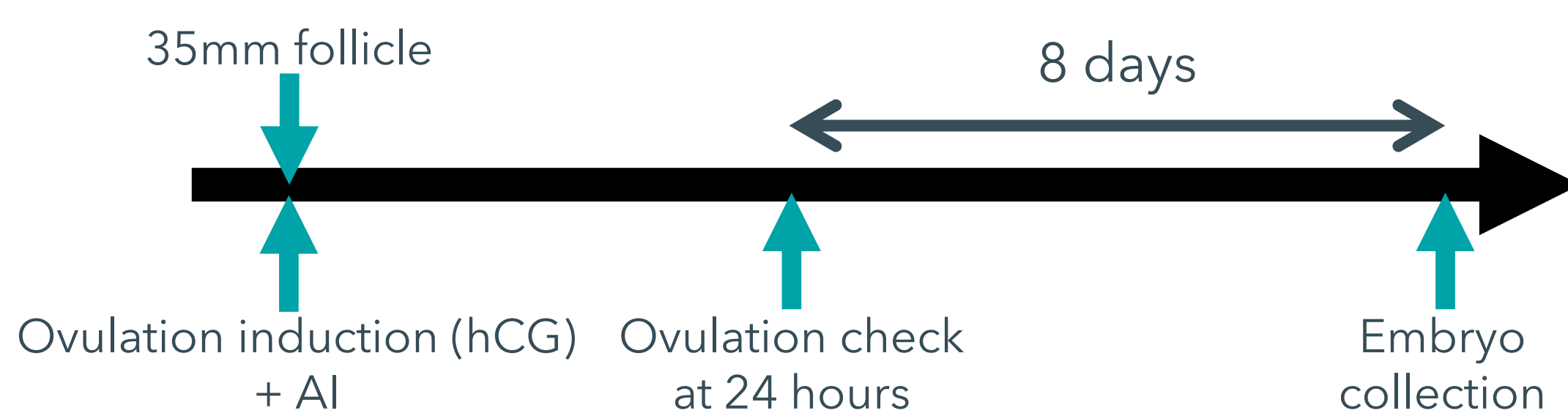
Statistics: linear model with permutations with R software, including gestation length, mare weight and foal sex

PLACENTAL MORPHOLOGY



EMBRYO & PLACENTA COLLECTIONS

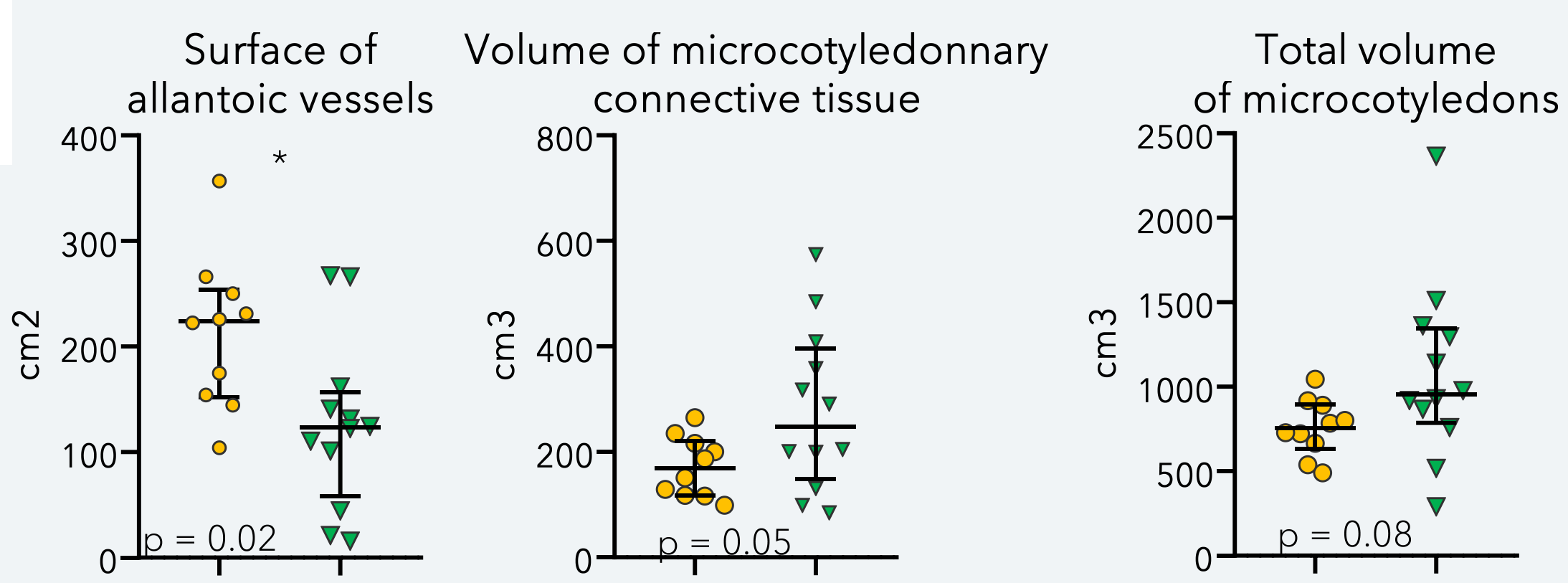
Young (6-year-old) & Old (≥ 10-year-old) multiparous saddlebred mares inseminated with one stallion



N = 6 placentas
 N = 6 placentas
 ARN extraction & sequencing
 N = 10 placentas
 N = 12 placentas
 Paraffin embedded & HES stained

RESULTS

PLACENTAL STEREOLOGY

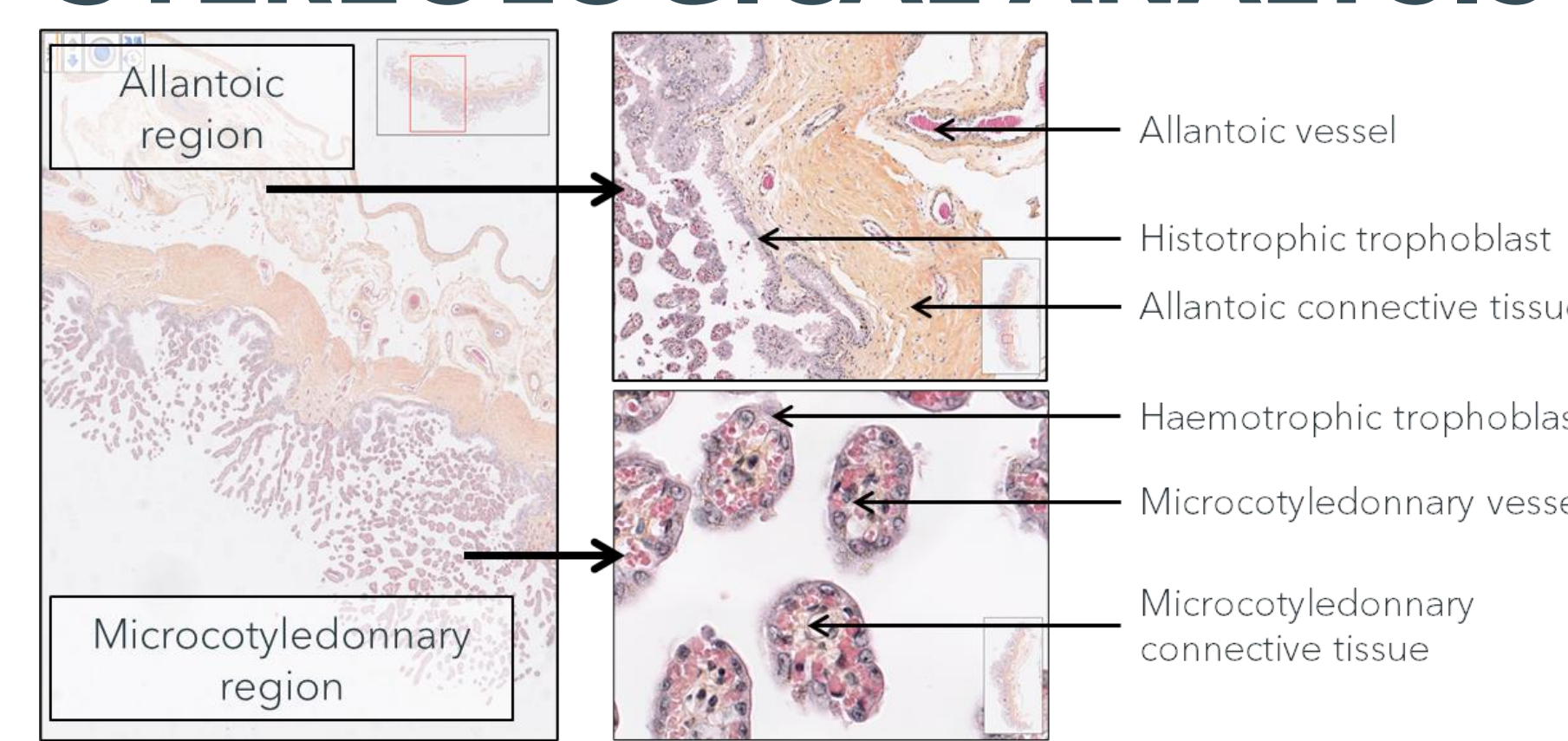


No variation in weight, volume and surface according to maternal age

BUT

↘ Surface of allantoid vessels and tendency to ↗ volume of connective tissue and total microcotyledonary in old mares

STEREOLOGICAL ANALYSIS



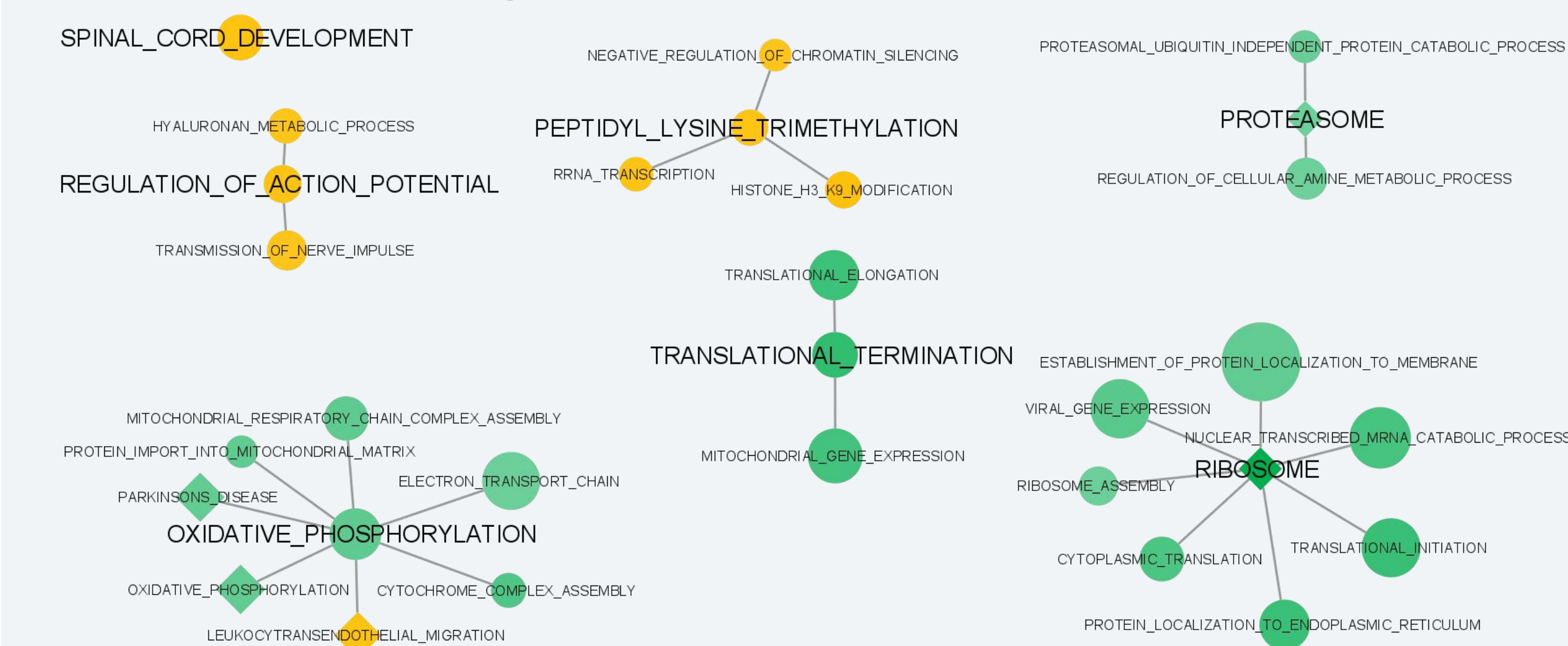
DIFFERENTIALLY EXPRESSED GENES IN TE

Gene name	Description	log2FoldChange	padj
ENSECAG00000022280	Protein coding gene	-2.53	2.62E-04
ENSECAG00000038654	LncRNA	-2.48	1.87E-07
RNASE1	ribonuclease A family member 1, pancreatic	-2.14	3.83E-09
H4C3	H4 clustered histone 3	-1.33	9.83E-10
ATAD2B	ATPase family AAA domain containing 2B	-0.93	2.62E-04
REV3L	REV3 like, DNA directed polymerase zeta catalytic subunit	-0.75	0.019
LUZP1	leucine zipper protein 1	-0.75	0.04
AASS	aminoacidipate-semialdehyde synthase	-0.7	0.013
SETX	senataxin	-0.49	0.011
PFAS	phosphoribosylformylglycinamide synthase	-0.38	0.029
CBS	cystathionine beta-synthase	-0.37	0.017
RPS29	ribosomal protein S29	0.78	7.98E-06
SRGAP3	SLIT-ROBO Rho GTPase activating protein 3	2.52	6.20E-03
CD69	CD69 molecule	5.09	0.024

DIFFERENTIALLY EXPRESSED GENES IN PLACENTAS

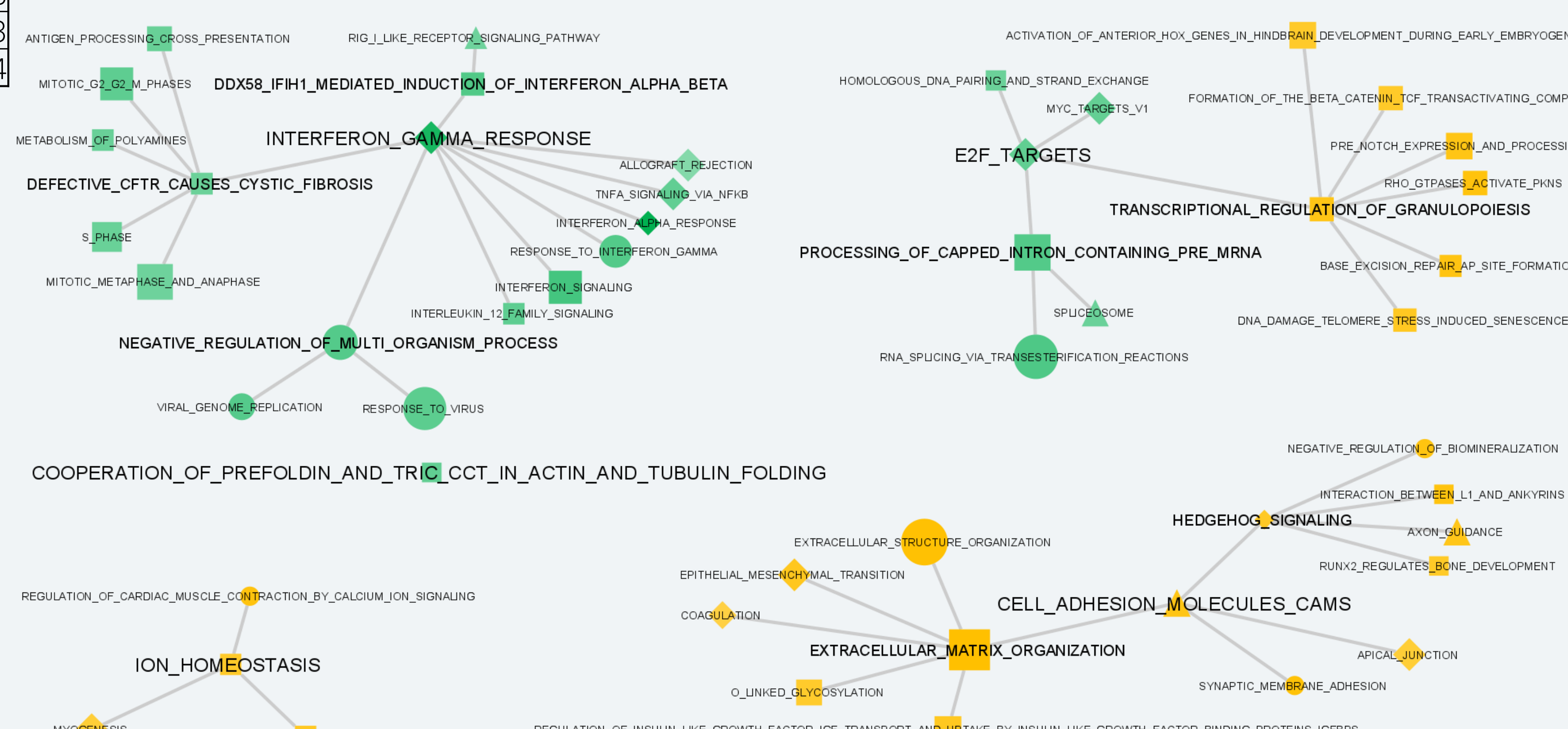
Gene name	Description	log2FoldChange	padj
PDZD7	PDZ domain containing 7	-1.75	0.029
CD55	CD55 molecule (Cromer blood group)	-1.33	1.58E-03
ENSECAG00000040224	LncRNA	-1.07	0.03
ENSECAG00000032286	Protein coding gene	-0.71	2.96E-04
GCA	grancalcin	-0.66	0.014
TDRD7	tudor domain containing 7	0.59	7.92E-03
HELB	DNA helicase B	1.04	0.029
APLN	apelin	1.28	0.023
COMP	Cartilage oligomeric matrix protein	1.5	7.92E-03

PATHWAY PERTURBED IN TE



Genes and functions perturbed by maternal age related to **embryo energetic metabolism, ion transfer and transcription**
 → Perturbations of embryo development

PATHWAY PERTURBED IN PLACENTAS



Genes and functions perturbed by maternal age related to **immunity response, transcription, ion transfer and cell adhesion**

CONCLUSIONS

Gene expression of equine trophoblast is affected by maternal age
 Possibly contribute to the lower fertility in older mares

Gene expression & histology of placentas are affected by maternal age but no gross morphology
 Possibly affects fetomaternal communication/exchanges

Perturbations in gene expression in placentas are already present in the embryo trophoblast