

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

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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

• Embryo loss Fertility

✓ Mare age associated to ↘ fertility and ∧ embryo loss

Aging mares / foal weight but in relation to **/** parity

Foal weight

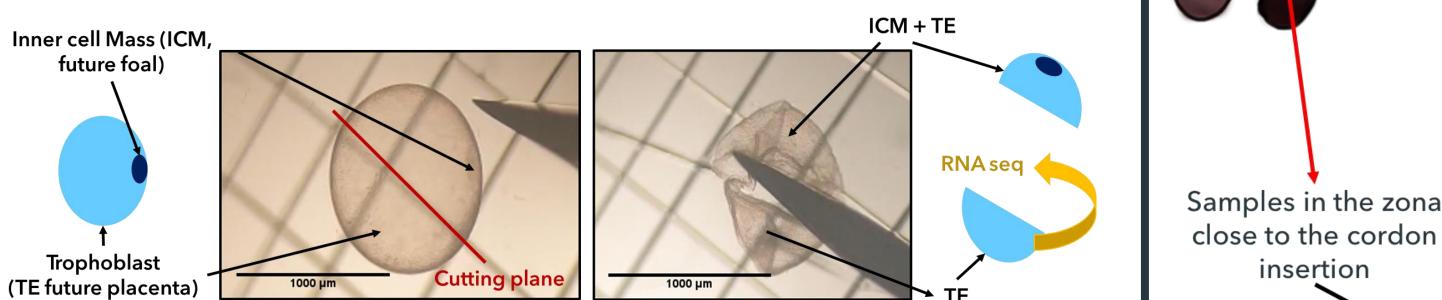
Parity

BIOINFORMATIC & STATISTICAL ANALYSIS

<u>RNAseq differential analysis:</u> 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

EMBRYO & PLACENTA COLLECTIONS

Young (6-year-old) & Old (> 10-year-old) multiparous saddlebred mares inseminated with one stallion Term placentas 35mm follicle 8 days collected after foaling Weight measure Ovulation induction (hCG) Ovulation check Embryo collection at 24 hours + AI



1000 µm

 Volume measure Surface measure

N = 6 placentas N = 6 placentas **ARN** extraction & sequencing

<u>Gene Set Enrichment Analysis (GSEA):</u> Broad Institute software using the KEGG and GO biological process (BP) databases

- \rightarrow Redundancy was reduced using SUMER analysis
- FDR<0.05 for significance; In analyses, "young" is the reference group

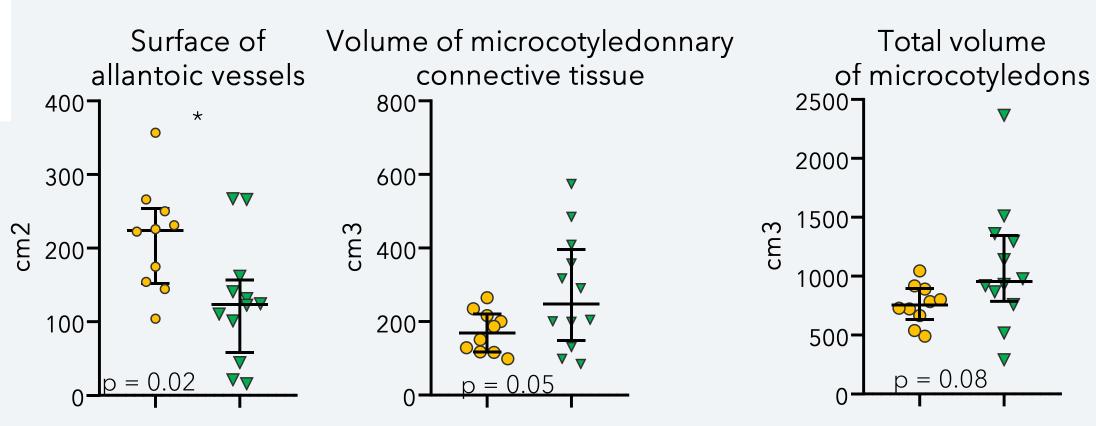
Stereological analysis: Microcotyledonary and allantoid relative surface and volume quantification by One Stop Stereology using Mercator[®] software (ExploraNova)

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

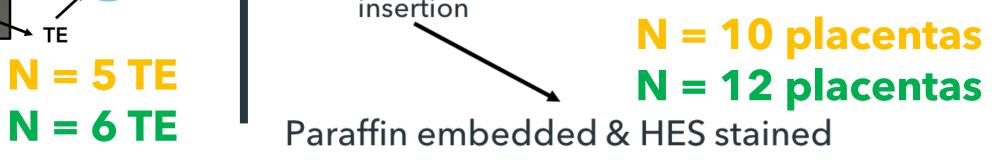
PLACENTAL MORPHOLOGY Surface Weigth Volume 5000-25000-57 20000-4000-N 15000-4-E 3000b V ope 10000-3-2000-5000-1000-

RESULTS

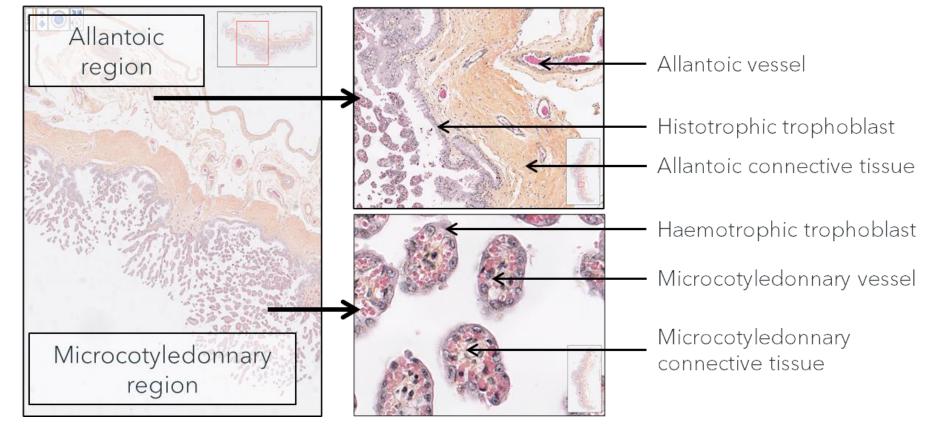
PLACENTAL STEREOLOGY



Cutting plane



STEREOLOGICAL ANALYSIS



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

BUT

Surface of allantoic vessels and tendency to A volume of connective tissue and total microcotyledonary in old mares

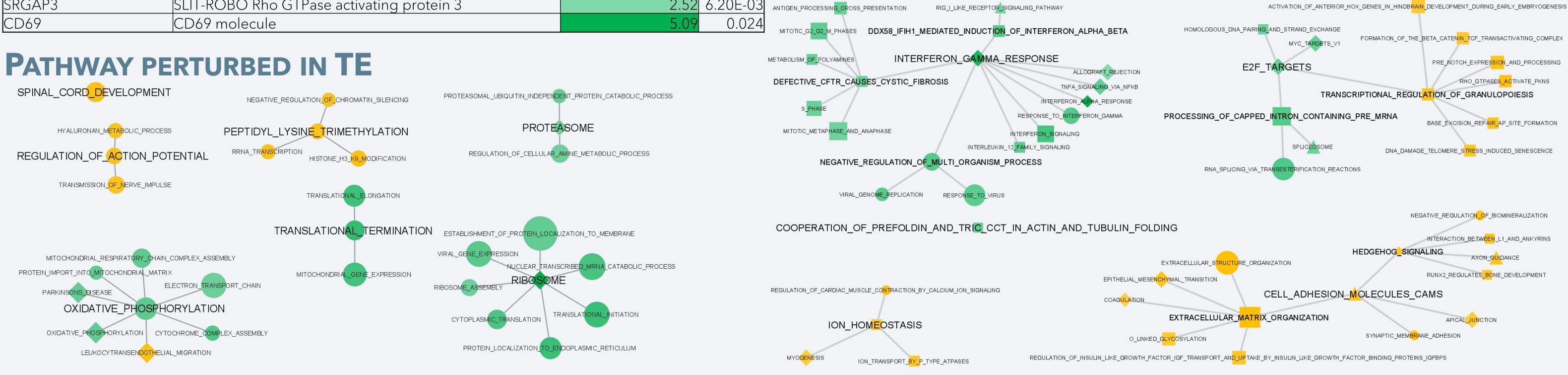
DIFFERENTIALLY EXPRESSED GENES IN PLACENTAS

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
ENSECAG0000032286	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

DIFFERENTIALLY EXPRESSED GENES IN TE

Gene name	Description	log2FoldChange	padj
ENSECAG0000022280	Protein coding gene	-2.53	2.62E-04
ENSECAG0000038654	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	aminoadipate-semialdehyde synthase	-0.7	0.013
SETX	senataxin	-0.49	0.011
PFAS	phosphoribosylformylglycinamidine 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

PATHWAY PERTURBED IN PLACENTAS



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

CONCLUSIONS

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

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





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