



Mare aging as young as 10 years old alters gene expression and structure of term placenta without affecting foal growth

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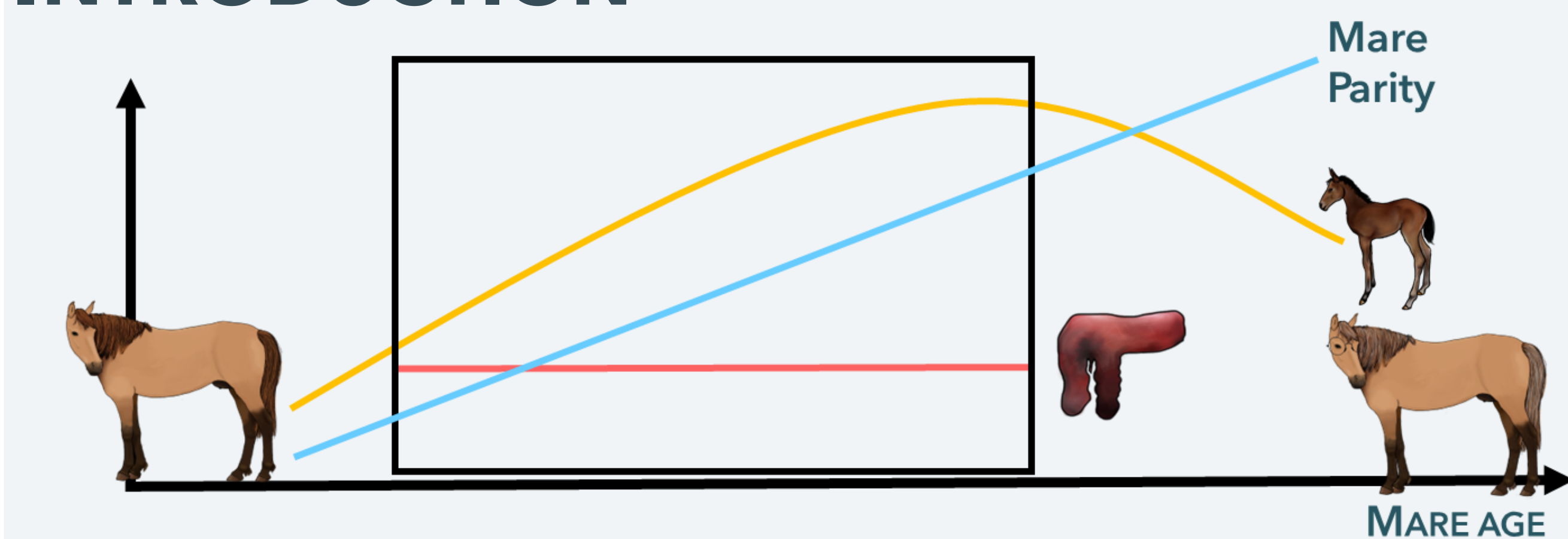
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INTRODUCTION



Smaller & lighter foals at birth + until 510 days of age in very young & old
(Hintz et al., 1979)

↗ Foal weight with ↗ mare age
(Wilsher & Allen, 2003; Elliott et al. 2009; Fernandes et al 2014; Meirelles et al. 2017)

Same placental weight but structural alterations
(Wilsher & Allen, 2003; Elliott et al. 2009; Ousey et al., 2012; Meirelles et al. 2017; Neto da Silva et al., 2022)

Alteration of placental development including vascularization during gestation
(Bracher et al., 1996; Hendricks et al., 2009; Willmann et al., 2011; Klewitz et al., 2015)

BIOINFORMATICS & STATISTICAL ANALYSIS

RNAseq differential analysis: DESeq2 package on R software with gestation length, foal sex and placental weight as cofactors; 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)

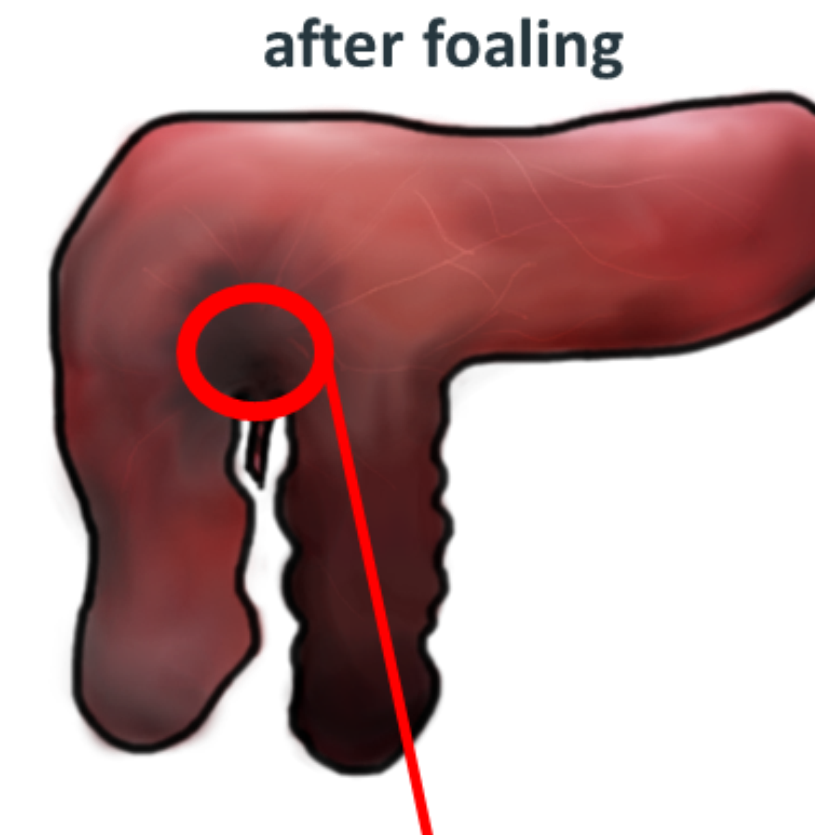
Statistics: linear model with permutations with R software, including gestation length and/or mare weight as cofactors

OBJECTIVES: ANALYZE THE EFFECT OF MARE AGE, WHILE EXCLUDING A PARITY EFFECT, ON PLACENTAL STRUCTURE & GENE EXPRESSION AND FOAL GROWTH AND HEALTH.

MATERIALS & METHODS 10 **Young (6-year-old)** & 12 **Old (≥ 10-year-old)** multiparous saddlebred mares inseminated with one stallion

PLACENTA ANALYSIS

Term placentas collected after foaling

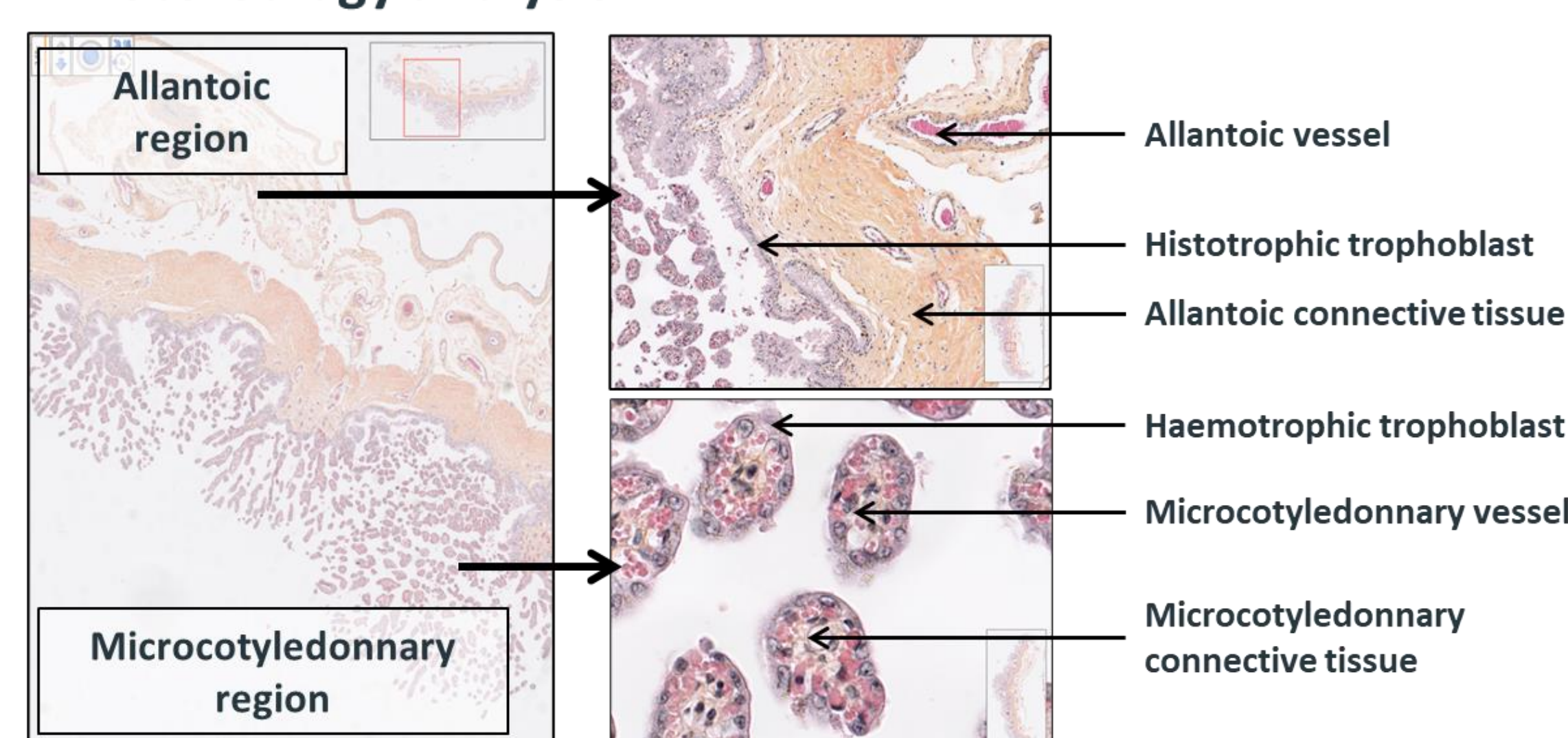


Samples in the zona close to the cordon insertion

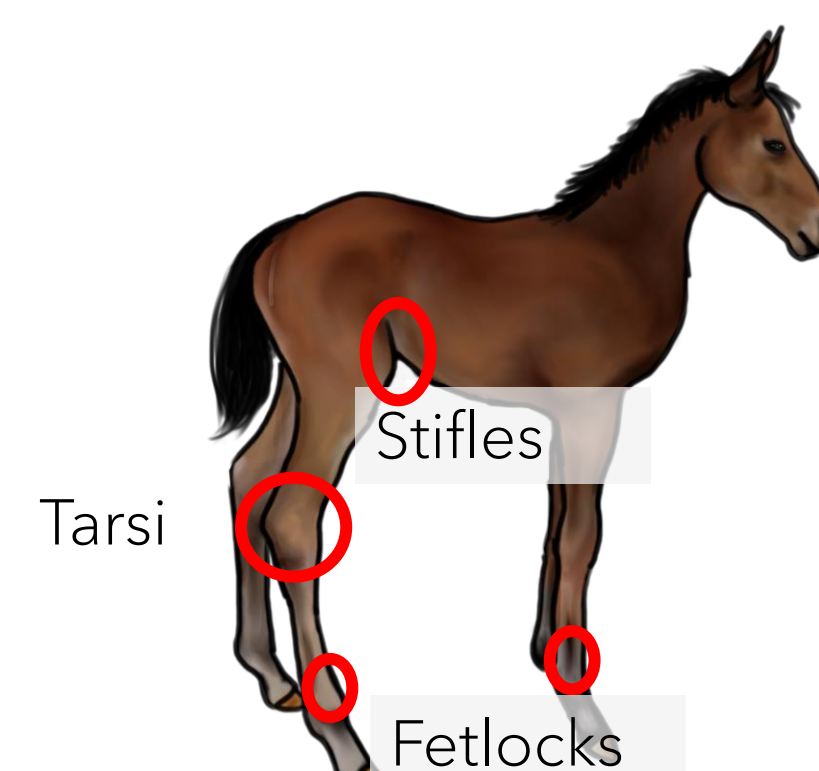
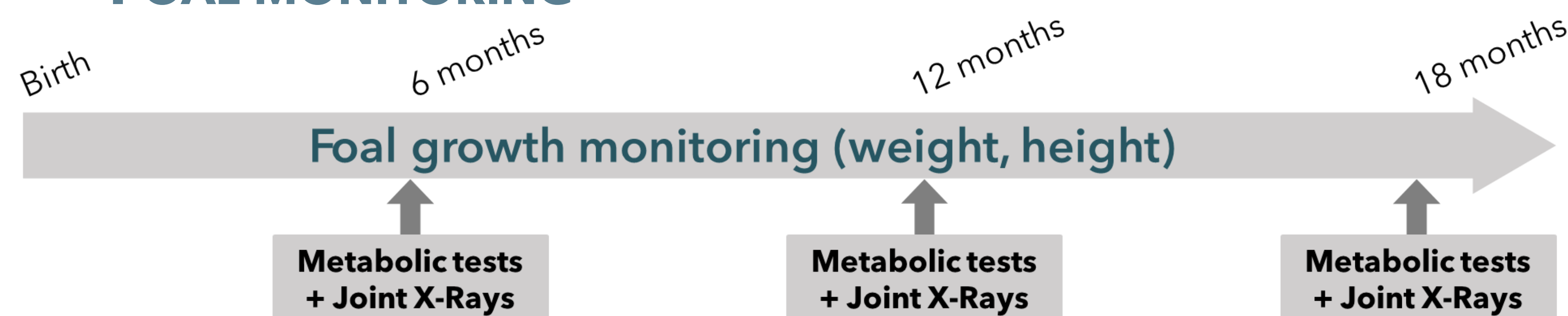
ARN extraction & sequencing
(n = 6 placentas/group)

Morphology measure
(weight, volume, surface)

Paraffin embedded & HES stained
→ **Stereology analysis**



FOAL MONITORING

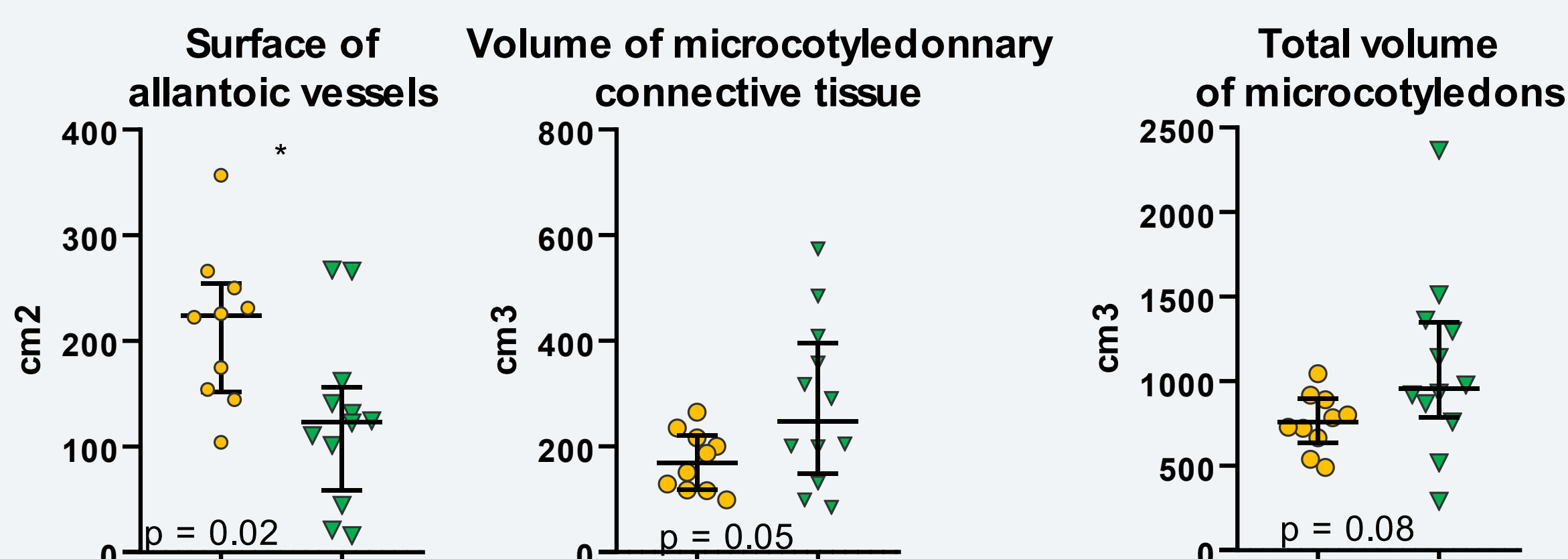


Metabolic tests → adapted from bergmann et al., 1989
18 months analysis still on going

10 X-rays/foal according to Denoix et al., 2013
Positive: irregularity (osteochondrosis) or bone fragment in at least one X-ray
Negative: no irregularity nor bone fragment in all joints

RESULTS

PLACENTAL MORPHOLOGY & STEREOLOGY



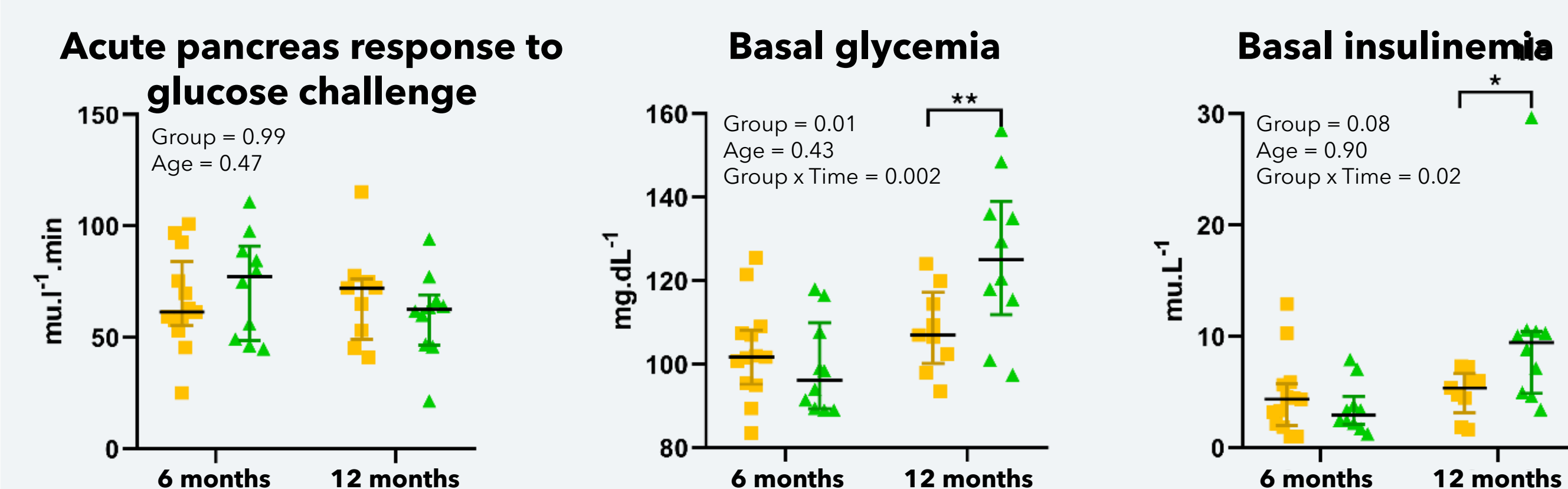
No variation in weight, volume and surface according to maternal age
BUT

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

FOAL MONITORING

No variation in weight, height & osteochondrosis status according to maternal age over the 18 months of post-natal study
BUT

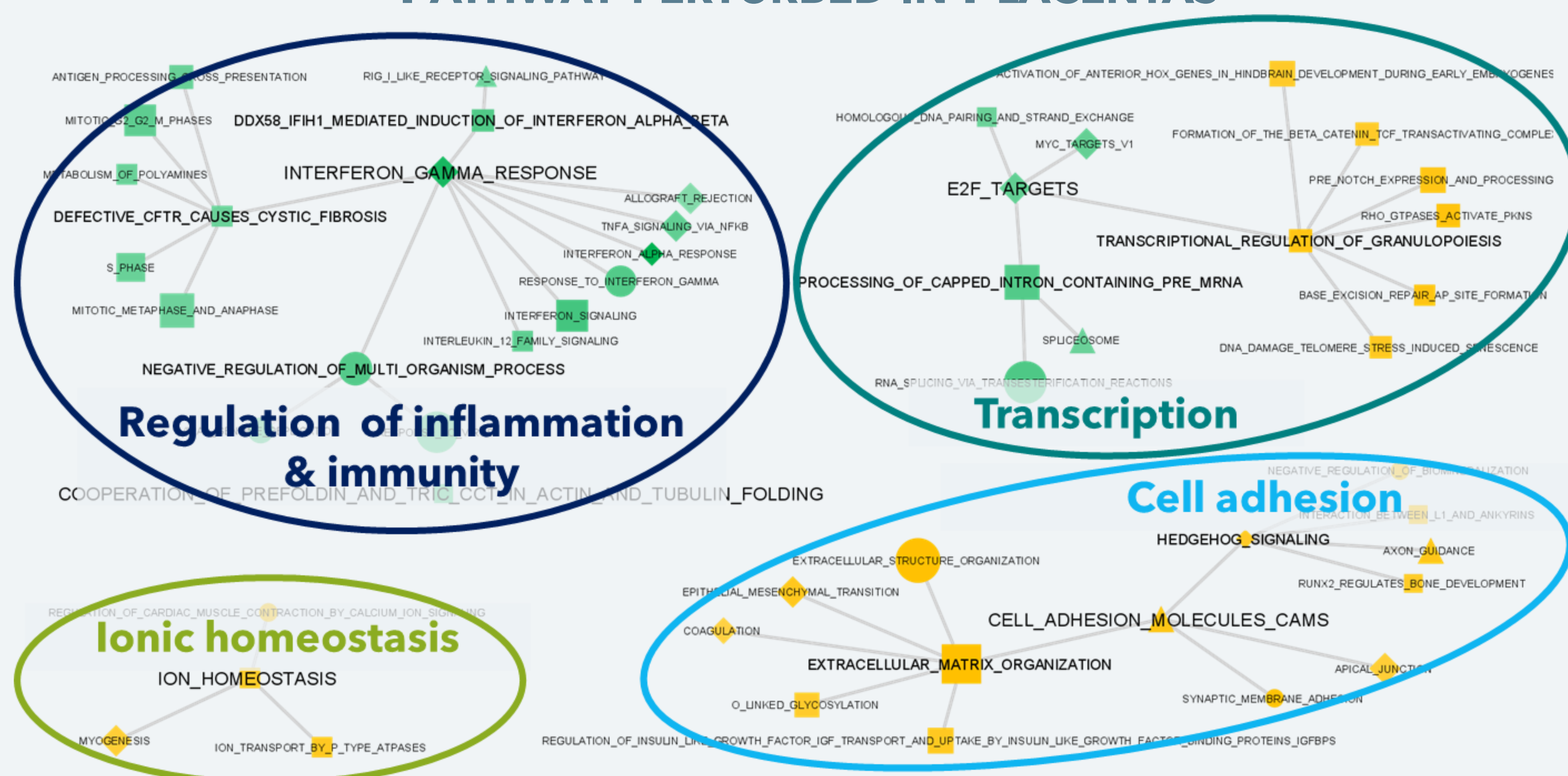
Few alterations of carbohydrate metabolism



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 PLACENTAS



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

Gene expression & histology of placentas are affected by maternal age but no gross morphology

Possible adaptations to uterine degradation observed in old mares to maintain the foal growth

Foal growth morphology is not affected by maternal aging but slight long-term modifications of carbohydrate metabolism are observed