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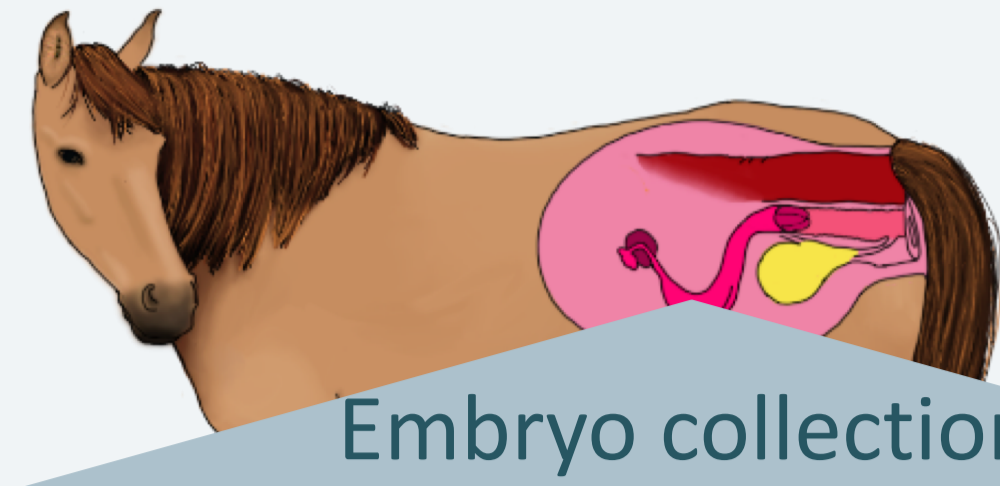
INRAE Equine embryo size does matter!



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INTRODUCTION



In vivo embryo size is very variable at the same age

Embryo collection at 8 days post ovulation (dpo)

Are they the same?

3,9800µm

120µm

Table 1. DIAMETER OF EQUINE EMBRYOS RECOVERED FROM THE UTERINE LUMEN

Day Postovulation	Number of Embryos	Embryo Diameter (mm)	
		Mean	Range
6	121	0.208	0.132-0.756
7	144	0.406	0.136-1.460
8	142	1.132	0.120-3.980
9	41	2.220	0.730-4.520

Adapted from Squires EL, Cook VM, Voss JL: Collection and transfer of equine embryos. Fort Collins, CO, Colorado State University, Animal Reproduction Laboratory, Bulletin no. 1, 1985; with permission.

Vanderwall, Veterinary Clinics of North America: Equine Practice, 1996

Factors affecting embryo diameter in 215 horse embryos.

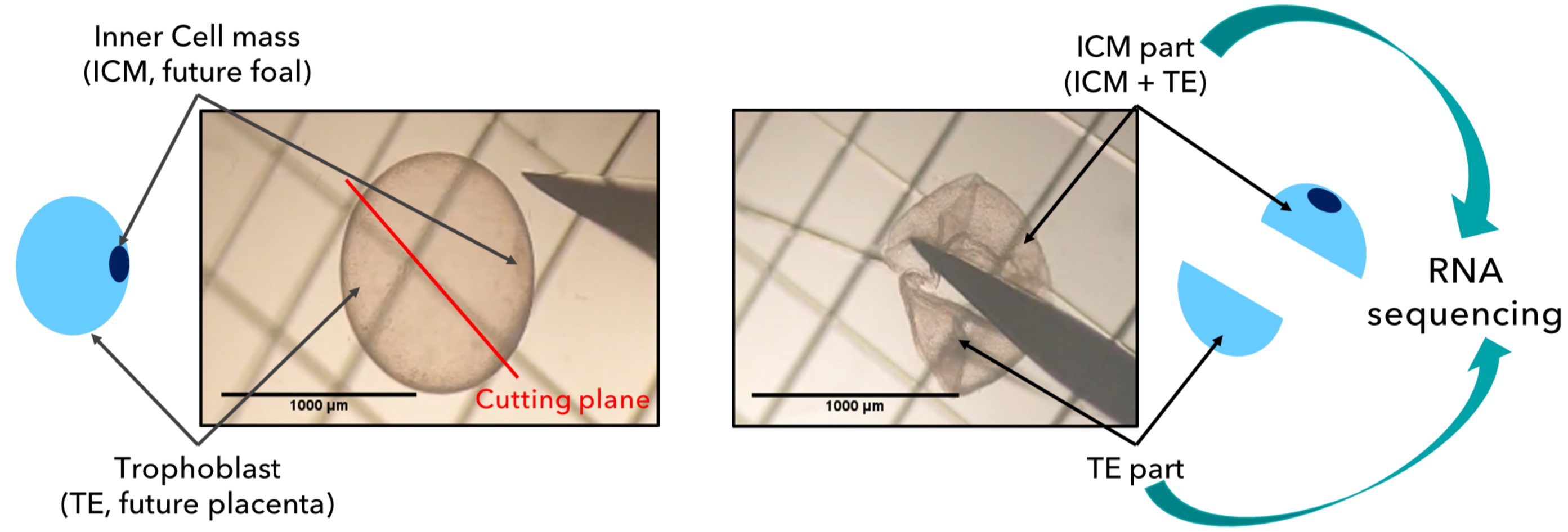
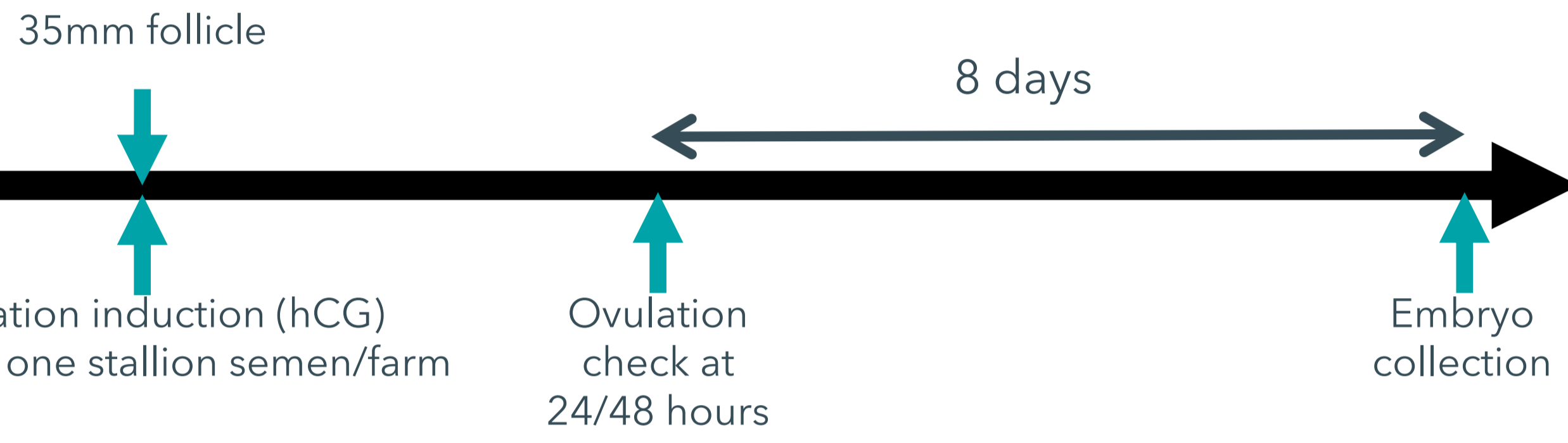
Factors	Embryo diameter (µm, average ± SD)
Day of flushing	
7 (N = 12)	404.91 ± 306.50 ^a
8 (N = 189)	660.29 ± 329.33 ^b
9 (N = 10)	912.39 ± 665.11 ^c
10 (N = 4)	1224.50 ± 821.04 ^d

Panzani et al., Theriogenology, 2014

OBJECTIVE: STUDY THE EFFECT OF EMBRYO SIZE ON GENE EXPRESSION

EMBRYO COLLECTION & PROCESSING

Saddlebred mares
2 farms
one stallion/farm



STATISTICS

Deconvolution: DeMixT package; estimation of ICM and TE cells in ICM + TE part → Deconvolution of gene expression

RNAseq differential analysis: DESeq2 package on R software with farm and embryo sex as cofactors; padj < 0.05 for significance

Pluripotency markers: Nanog Homeobox (NANOG) & SRY-Box Transcription Factor 2 (SOX2); Hormone metabolism: cytochrome P450 aromatase (CYP19A1), 17β-Hydroxysteroid dehydrogenase (HSD17B1), Insulin Like Growth Factors 1 & 2 (IGF1 & IGF2)

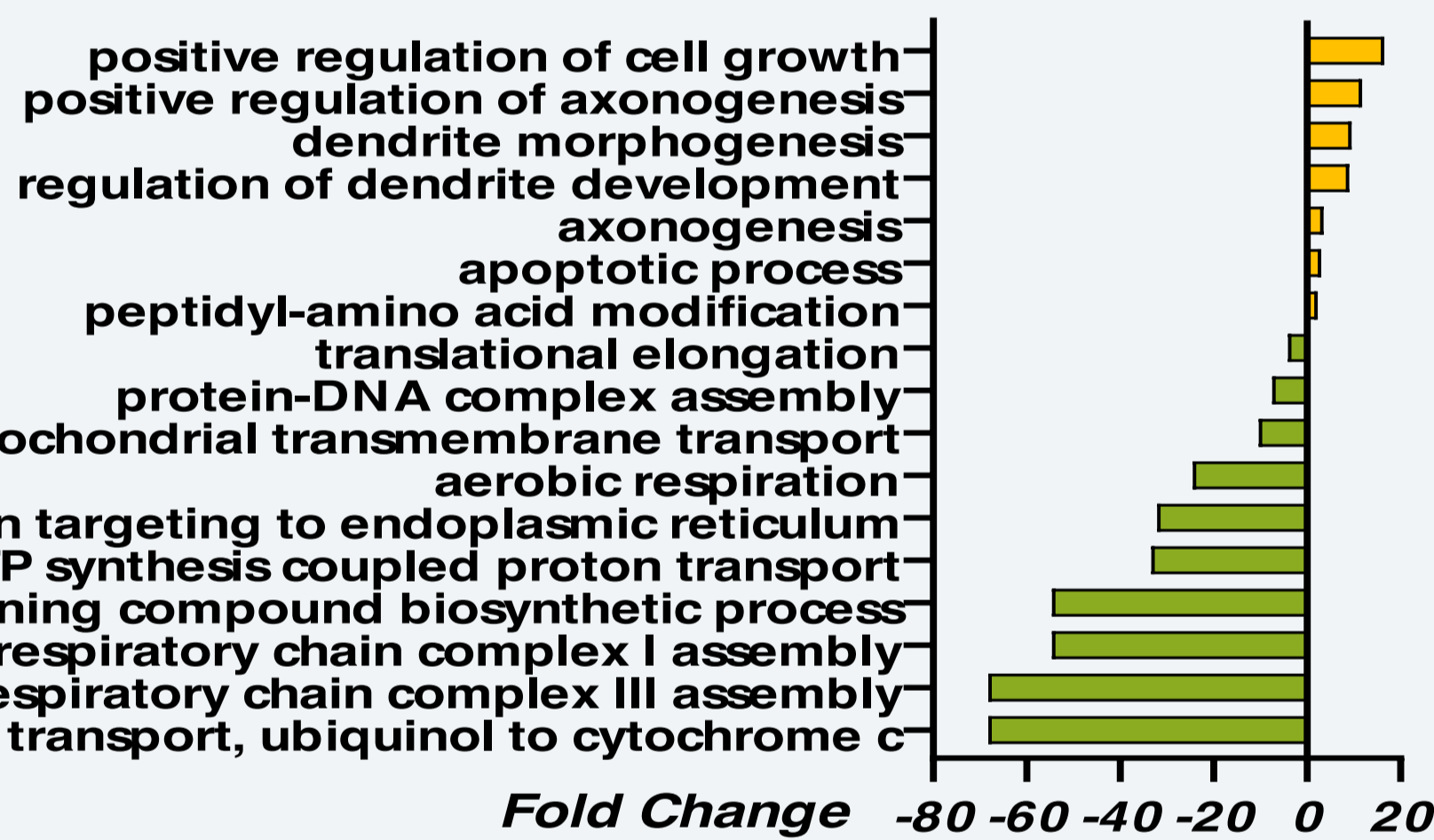
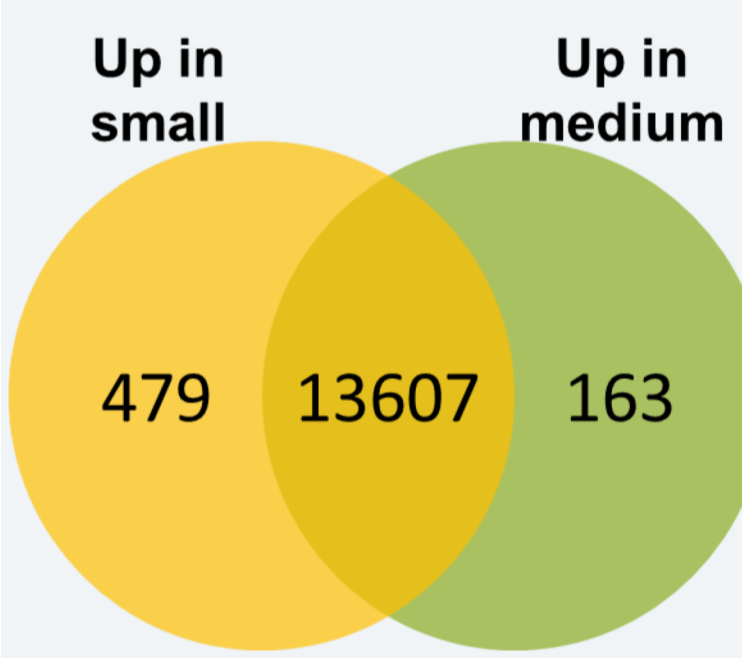
Overrepresentation test (PANTHER web software): on differentially expressed genes using GO BP databases;

Fold change < 0 indicated overrepresentation in medium size embryos

Group size	Number of embryos	Embryo diameter		
		Mean ± SD	Min	Max
Small	9	560 ± 85µm	409	676
Medium	11	886 ± 160µm	703	1169
Large	8	1,719 ± 488µm	1255	2643

RESULTS

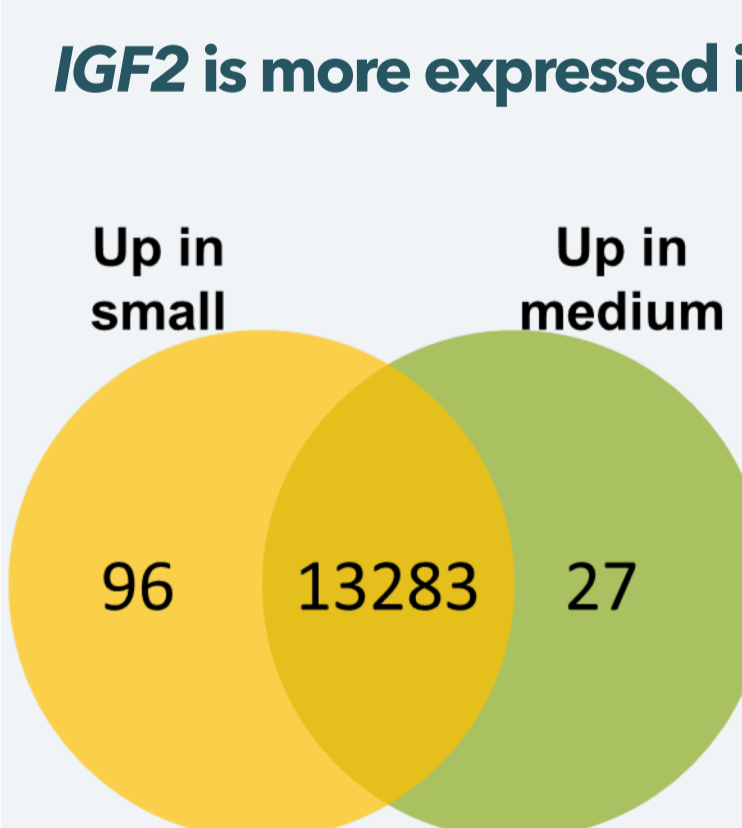
SMALL VS MEDIUM



Cell proliferation and differentiation seem to be more active in small embryos ICM

→ Maybe ICM of medium embryos has already enough cells and is less active in cell proliferation at this stage

Mitochondria and aerobic respiration overrepresented in medium embryo ICM → small embryos do not seem to be able of aerobic respiration



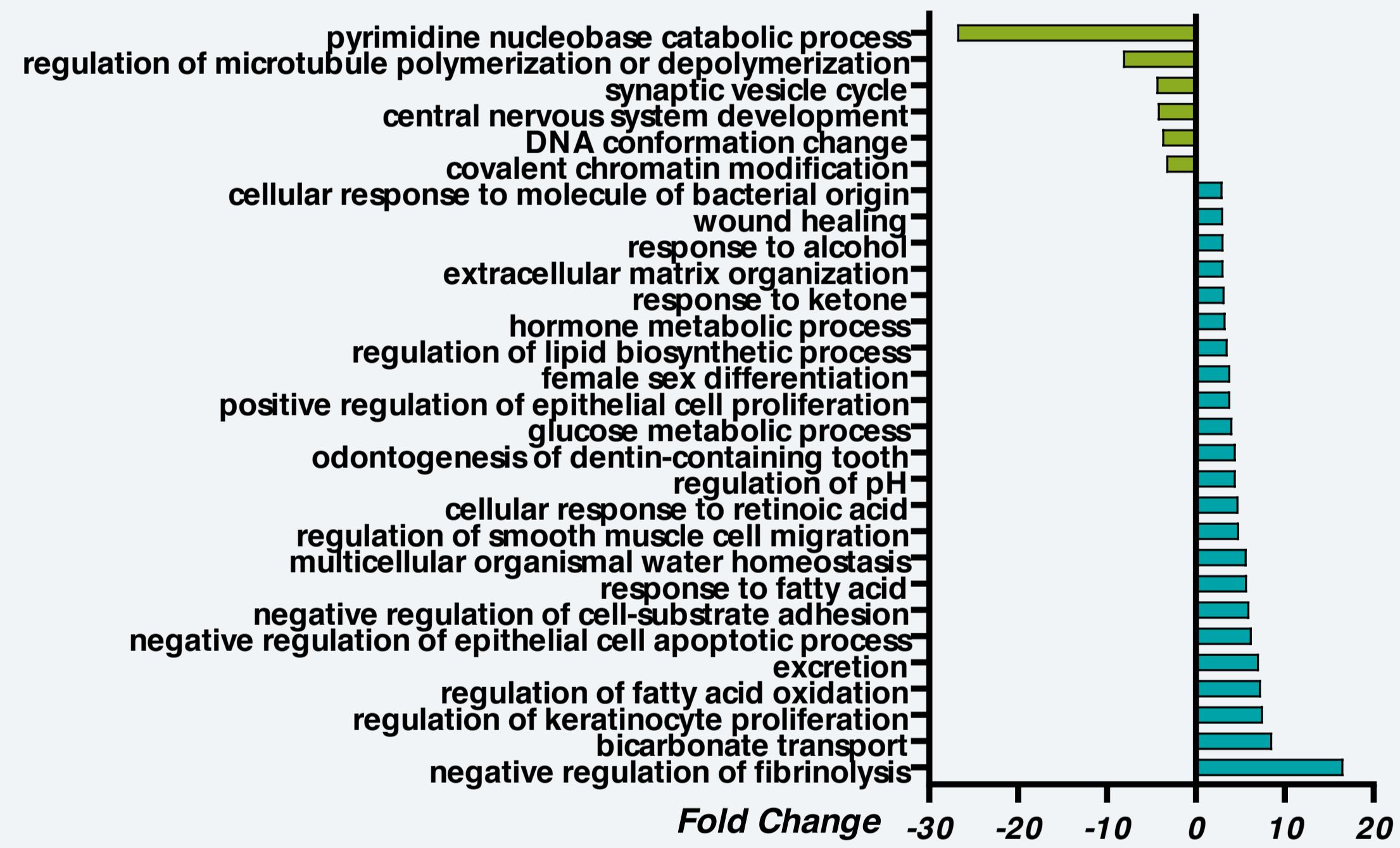
No overrepresented functions

ICM

Gene	Small vs Medium	Large vs Medium
NANOG	No difference	↘ in large embryos
SOX2	No difference	↘ in large embryos
CYP19A1	No difference	↗ in large embryos
HSD17B1	No difference	↗ in large embryos
IGF1	No difference	↗ in large embryos
IGF2	↗ in small embryos	No difference
CYP19A1	No difference	No difference
HSD17B1	No difference	Tended ↗ in large embryos
IGF1	No difference	↘ in large embryos
IGF2	↗ in small embryos	No difference

No overrepresented functions in large embryos but amino acid and nucleic acid metabolism overrepresented in medium embryos → Switch in energy sources?

LARGE VS MEDIUM



Reduced markers of pluripotency

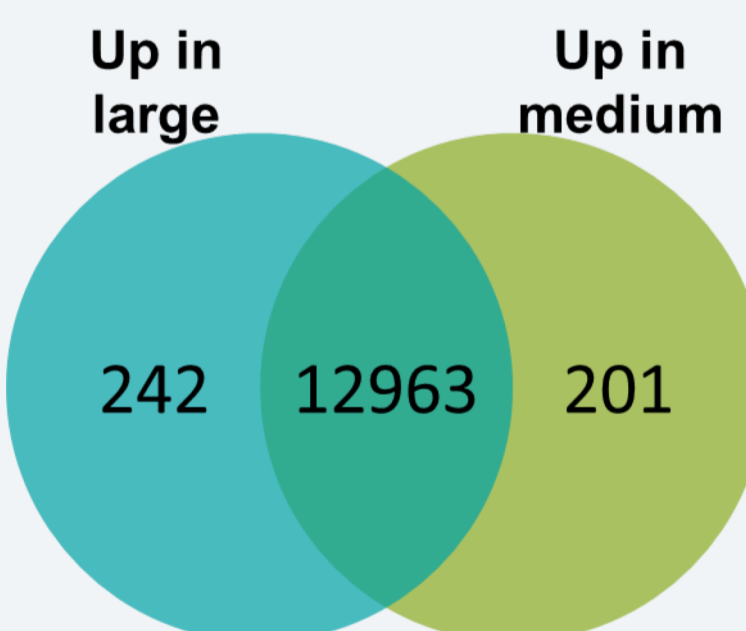
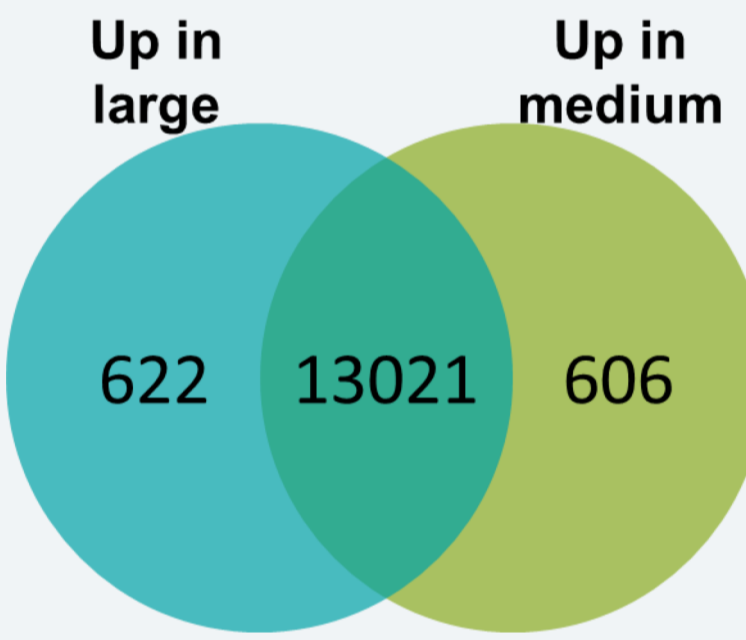
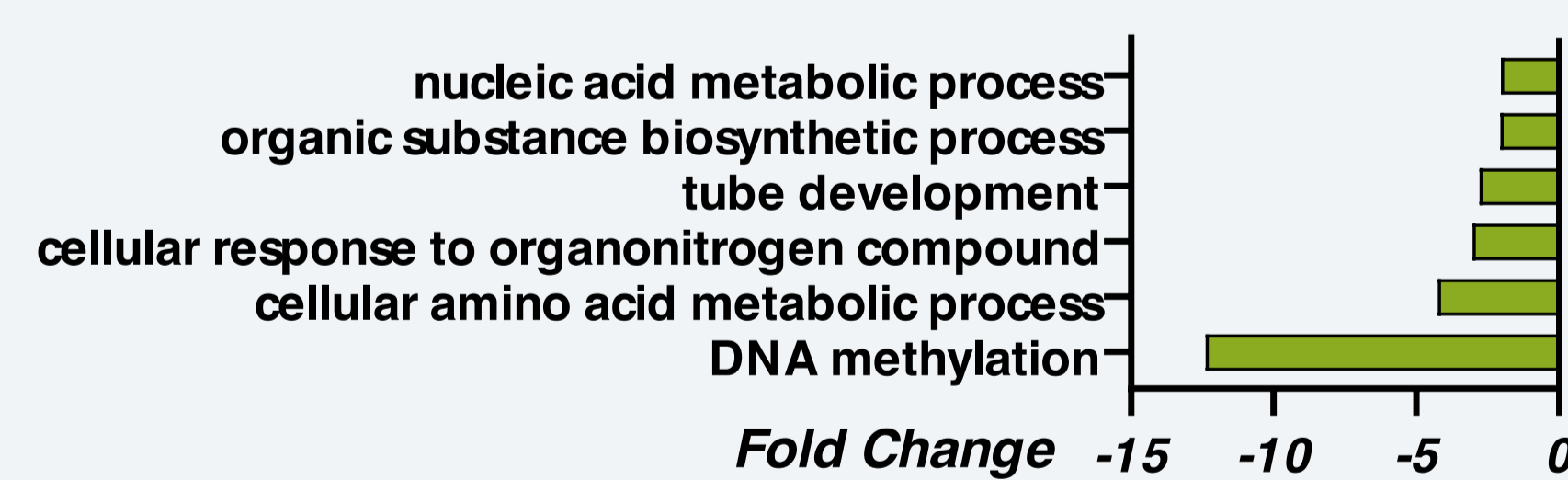
→ End of cell fate commitment?

Estrogen biosynthesis pathways: involved in maternal pregnancy recognition?

IGF1 takes over IGF2 for embryo growth

Modification of metabolic pathways and establishment of regulatory processes

→ glucose metabolism and aerobic respiration seem effective and begin to produce reactive oxygen species, that require regulation pathways to avoid an excess of oxidation in ICM cells



CONCLUSIONS

Gene expression is different between Day 8 small, medium and large embryos, with larger differences in the ICM

Developmental stage appears different

→ Does a reduced speed growth or a later fertilization explain the differences?

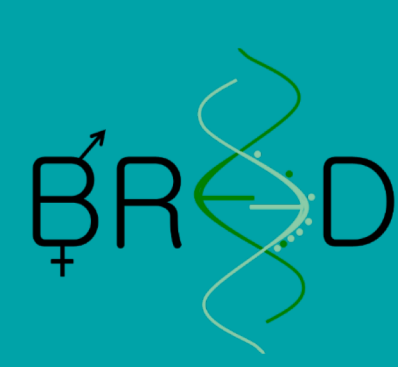
→ What are consequences for further development and implantation?

→ Role of steroid biosynthesis pathways for maternal recognition of pregnancy?

Embryo size is important to consider when studying embryo gene expression

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