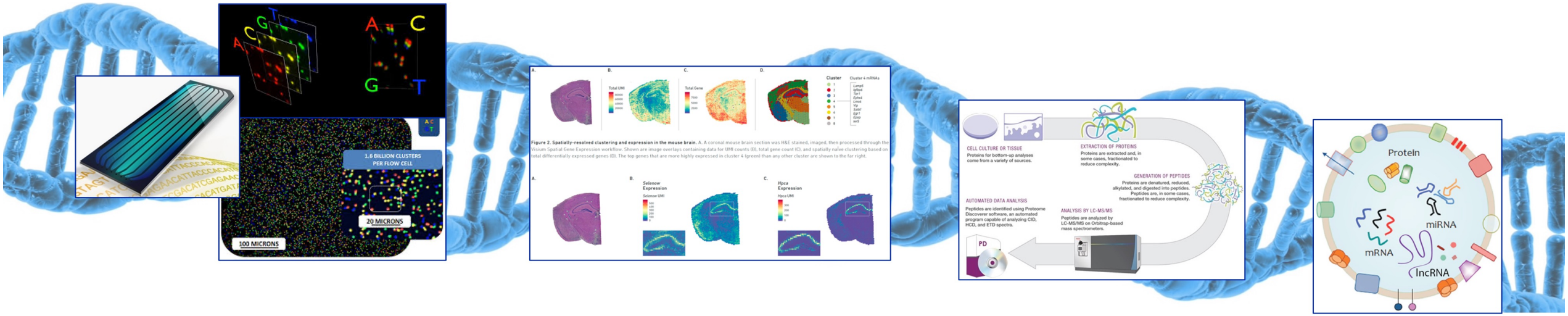
Overview of ongoing research projects



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Research topics and approaches of ongoing projects

Gene expression alterations at RNA and protein level as drivers of impaired fertility:

- Effects of uterine microbiome composition on uterine receptivity in the mare
- Embryo-oviduct interactions: Effects of heat stress on the interaction of bovine embryos and oviduct epithelium spheroids
- Molecular content of boar seminal plasma extracellular vesicles

The uterine Microbiota and Fertility in the mare: Why the bacterial composition matters?

Supported by the Swiss National Science Foundation, Project 310030_200534 (2021-2025)

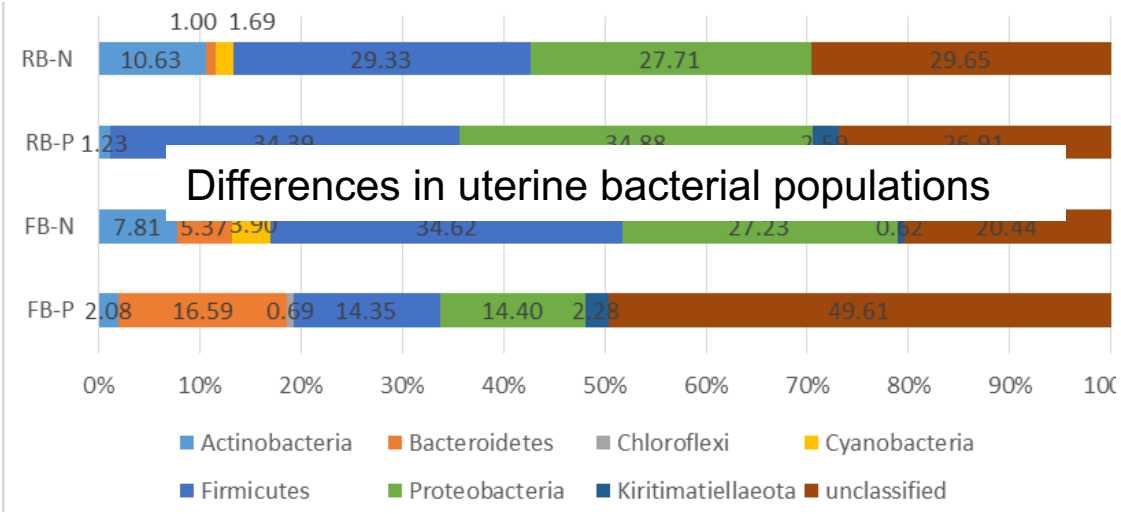
Objectives

1. To determine the relation of uterine microbiota composition and mare fertility by comparing the uterine microbiota in fertile and subfertile mares.

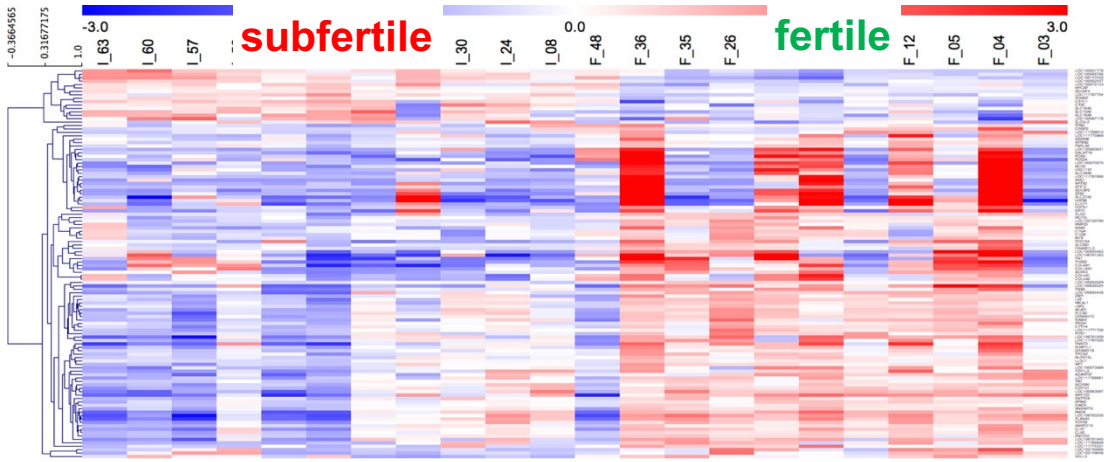
2. To determine the functional impact on the endometrium by examining mechanisms of action of uterine microbiota-host interaction (alterations in endometrium gene expression, epithelial barrier integrity, and extracellular vesicles as novel drivers of infertility).

Research approaches

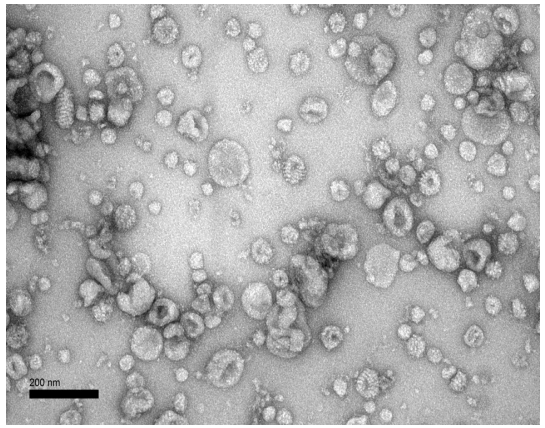
Microbiome analysis using 16S rRNA gene sequencing



RNA sequencing – uterine cytobrush and spatial transcriptomics of endometrial biopsies

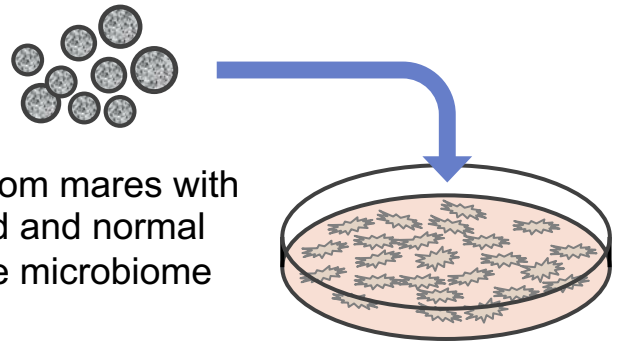


Uterine EVs RNA and protein cargo



- Low-input RNA-seq
- Proteomics

Primary equine endometrial epithelial cell (eEEC) culture



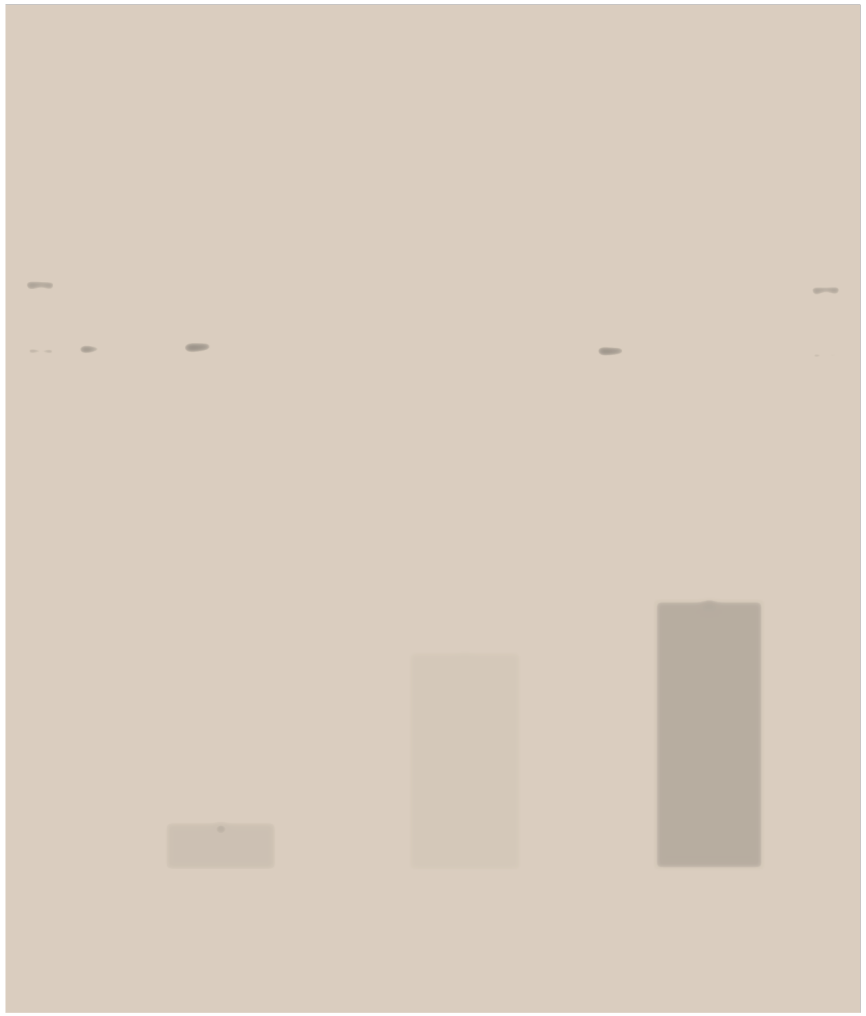
EVs from mares with altered and normal uterine microbiome

- Analysis of EVs uptake
- Response to EVs in eEECs

Microbiome analysis – establishing 16S rRNA V3/V4 amplicon generation from uterine cytobrush samples

- DNA isolation kits
- Test of various PCR kits and PCR conditions
- Blocking unspecific byproducts
- DNA or cDNA (RNA) as template

Bacterial DNA contamination in PCR kits



Unspecific product from mito 12S rRNA

Sample collection in collaboration with Prof. Canisso
(University of Illinois Urbana-Champaign)

- Sampling from research mares and client mares
- Estrus, diestrus, day 8 of pregnancy
- Young and old mares
- Mares with fertility problems (group 1: persistent breeding-induced endometritis; group 2: without clinical reason for subfertility)
- Samples: uterine cytobrush, uterine lavage, endometrial biopsies

doubleguarded cytobrush

Uterine fluid

Biopsy

Oviductal organoids to study the embryo-maternal dialog and develop strategies to alleviate climate change impact on cattle breeding (ORGALOG)

Lead Agency process, a joint application of researchers in Switzerland and France

Swiss National Science Foundation, Project 310030E_205507 (2022-2026)

FNSNF
Swiss National Science Foundation

Objective

ORGALOG aims at increasing our understanding of the impact of (heat) stress on oviduct physiology and on the embryo-oviduct dialog to develop strategies to boost embryo survival in cattle

Approach:

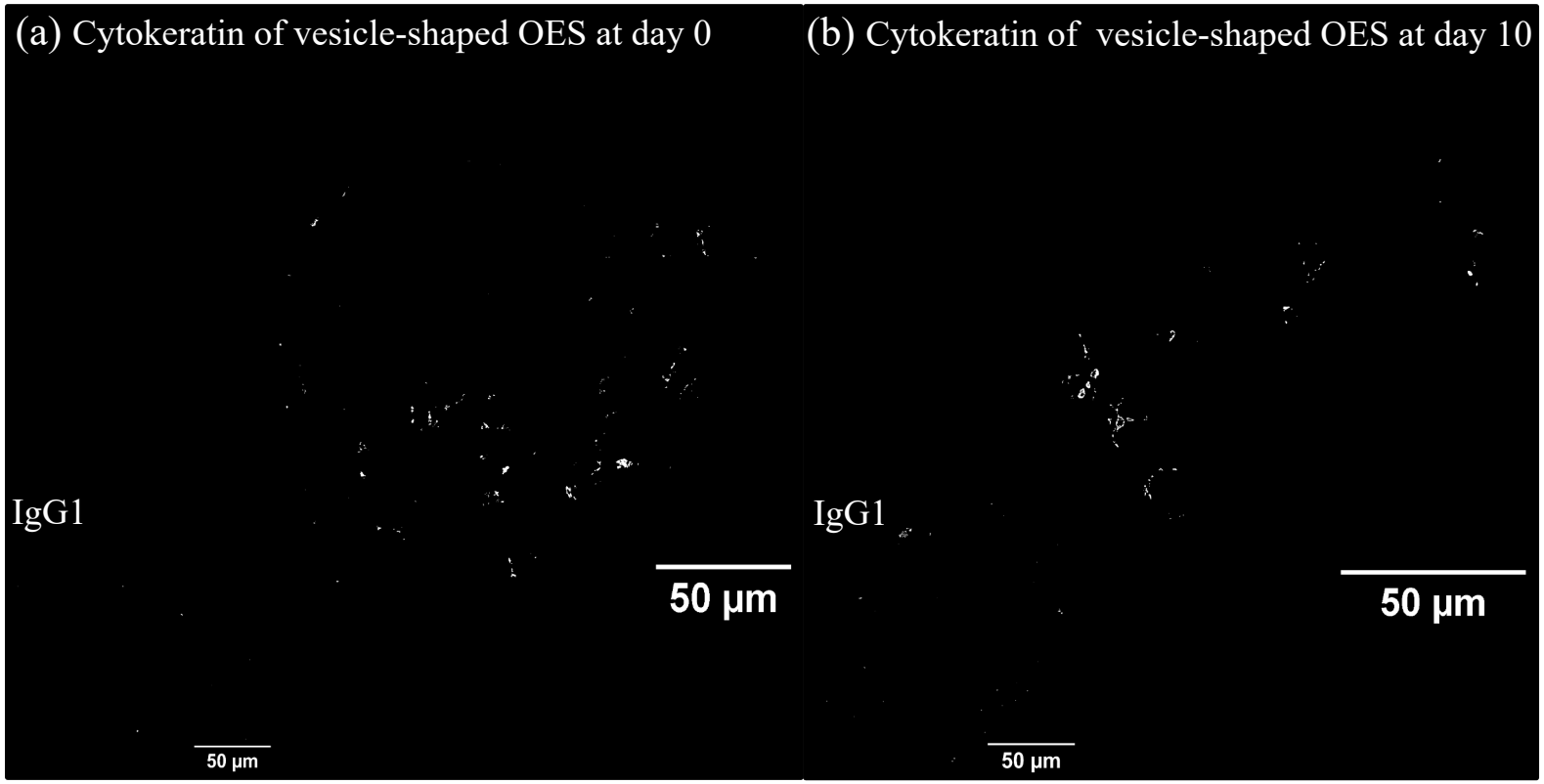
ORGALOG is using 3D cell culture models based on **oviduct spheroids and organoids** and a combination of different **omics technologies** to study the impact of (heat) stress on stem cells, ciliated and secretory cells composing the oviduct epithelium, and on the embryo-oviduct dialog via extracellular vesicles

Oviductal epithelial spheroids

Bovine oviduct epithelial spheroids selected on day 0. Vesicle-shape spheroids containing a cavity, homogeneous in form and size -100 to 200-µm diameter and displaying outward ciliary beating were selected in this study.

Pranomphon T. et al. under review

Oviductal epithelial spheroids



Expression of cytokeratin in OES. Vesicle-shaped OES at day 0 (a) and day 10 (b) were stained positively for anti-cytokeratin (green signal). The nuclei appear in blue (stained with Hoechst) while actin appears in red (stained with Texas Red™-X Phalloidin). Inserts in (a-b) show the negative controls incubated with the immunoglobulin isotype (IgG1) of the primary antibody.

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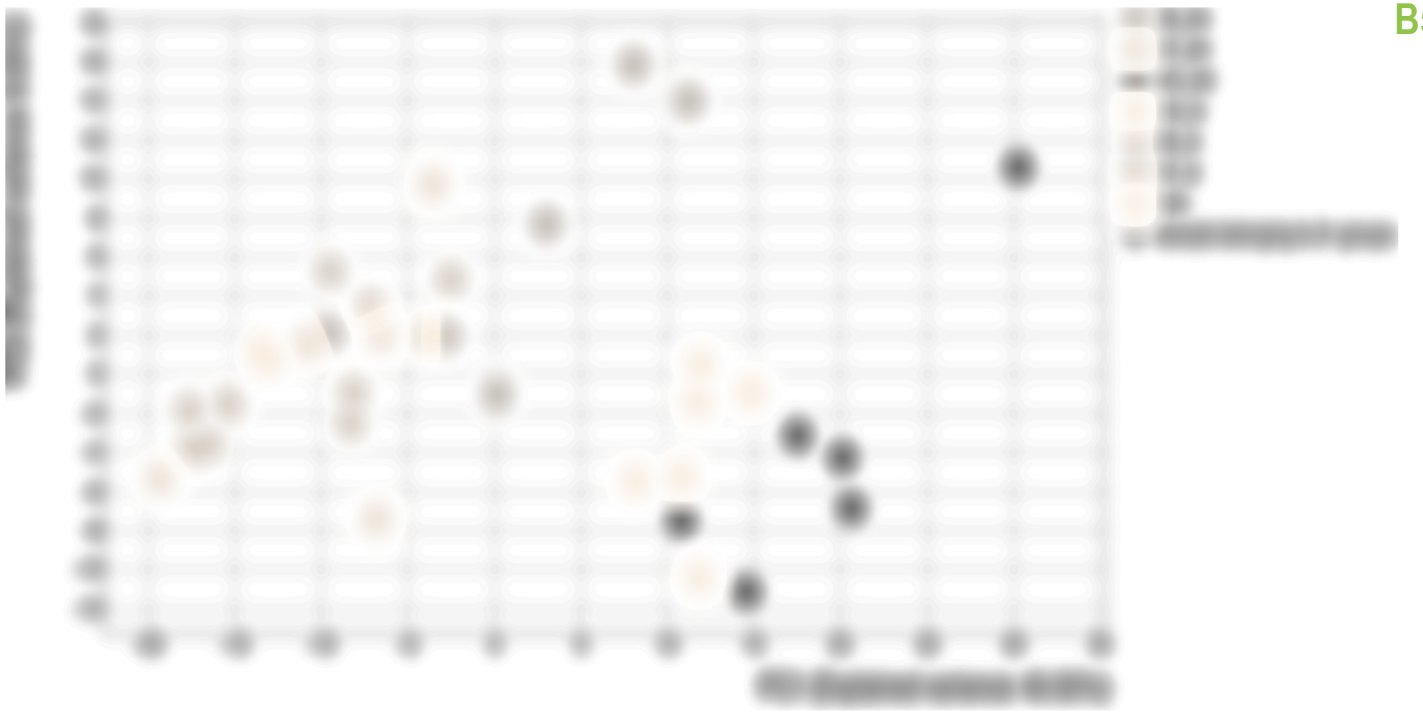
Co-culture of bOES and IVP embryos under 5% and 20% O_2

Experimental design:

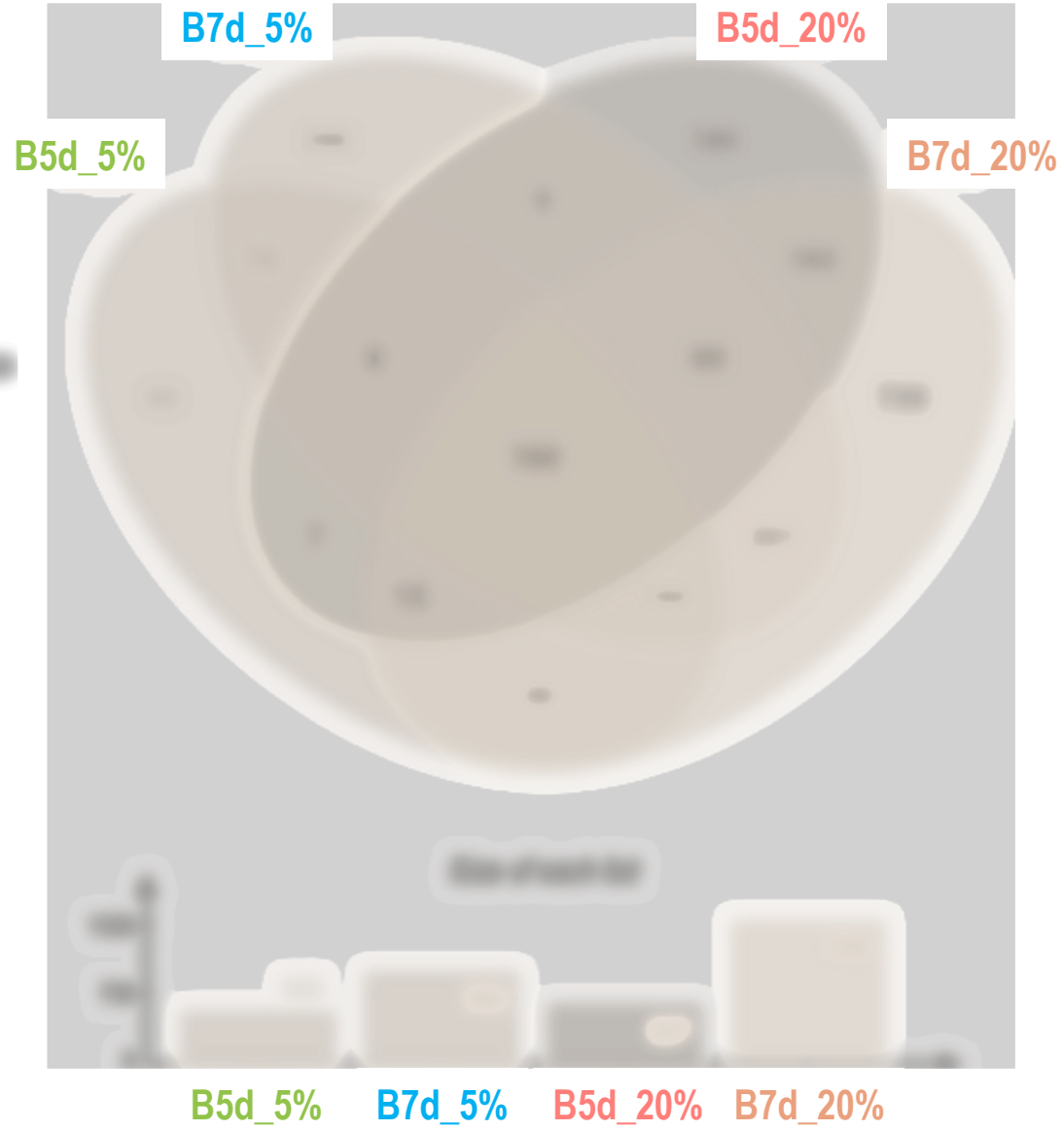
B5_5	co-culture for **5** days under **5**% O_2
B7_5	co-culture for **7** days under **5**% O_2
B5_20	co-culture for **5** days under **20**% O_2
B7_20	co-culture for **7** days under **20**% O_2
CO_5	embryos alone under **5**% O_2
CO_20	embryos alone under **20**% O_2

Results of low-input total RNA-seq

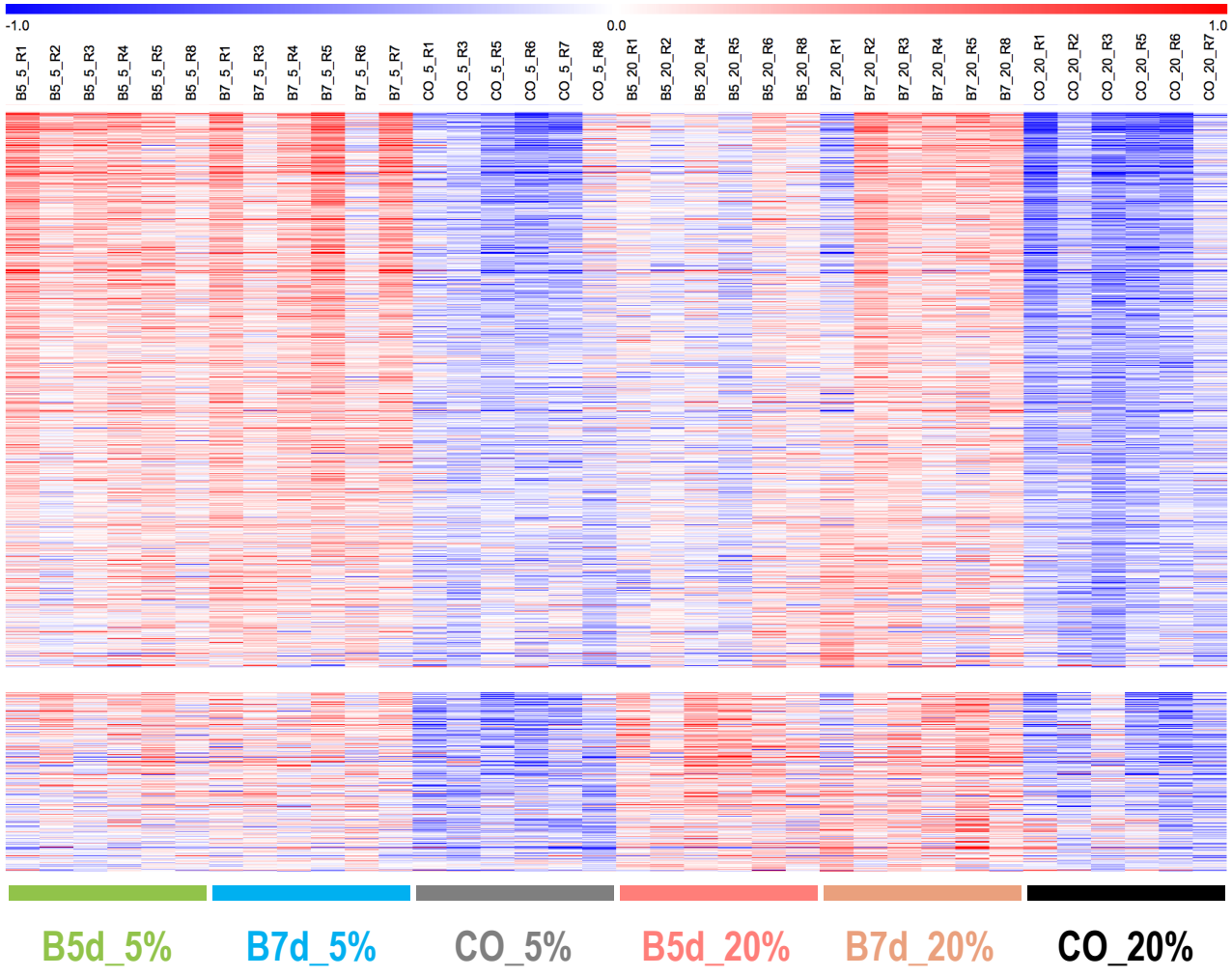
PCA based on all DEGs (FDR 5%)

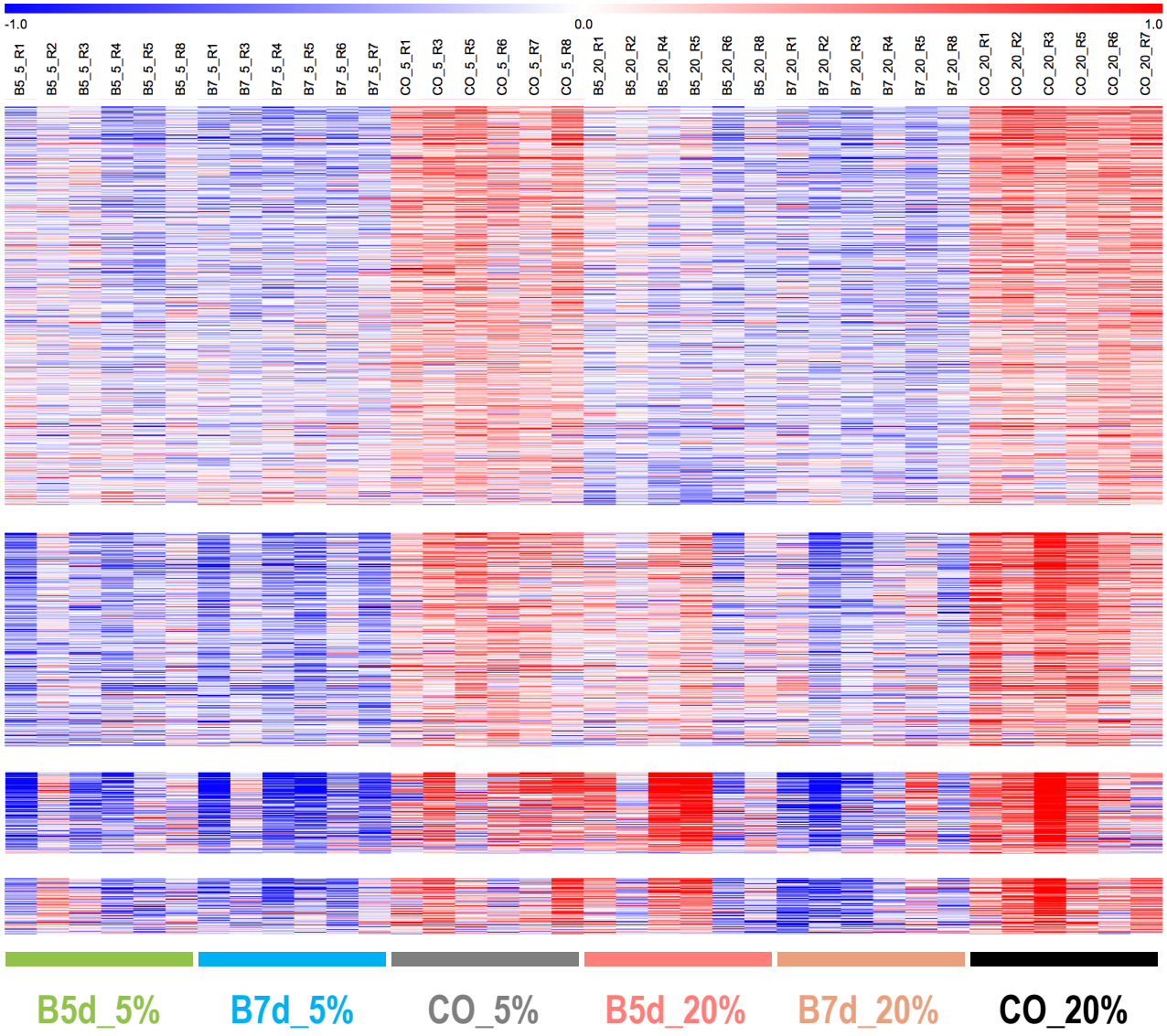


Overlap of DEGs (FDR 5%) co-culture vs. control



All DEGs – SOM analysis (genes with similar profiles)



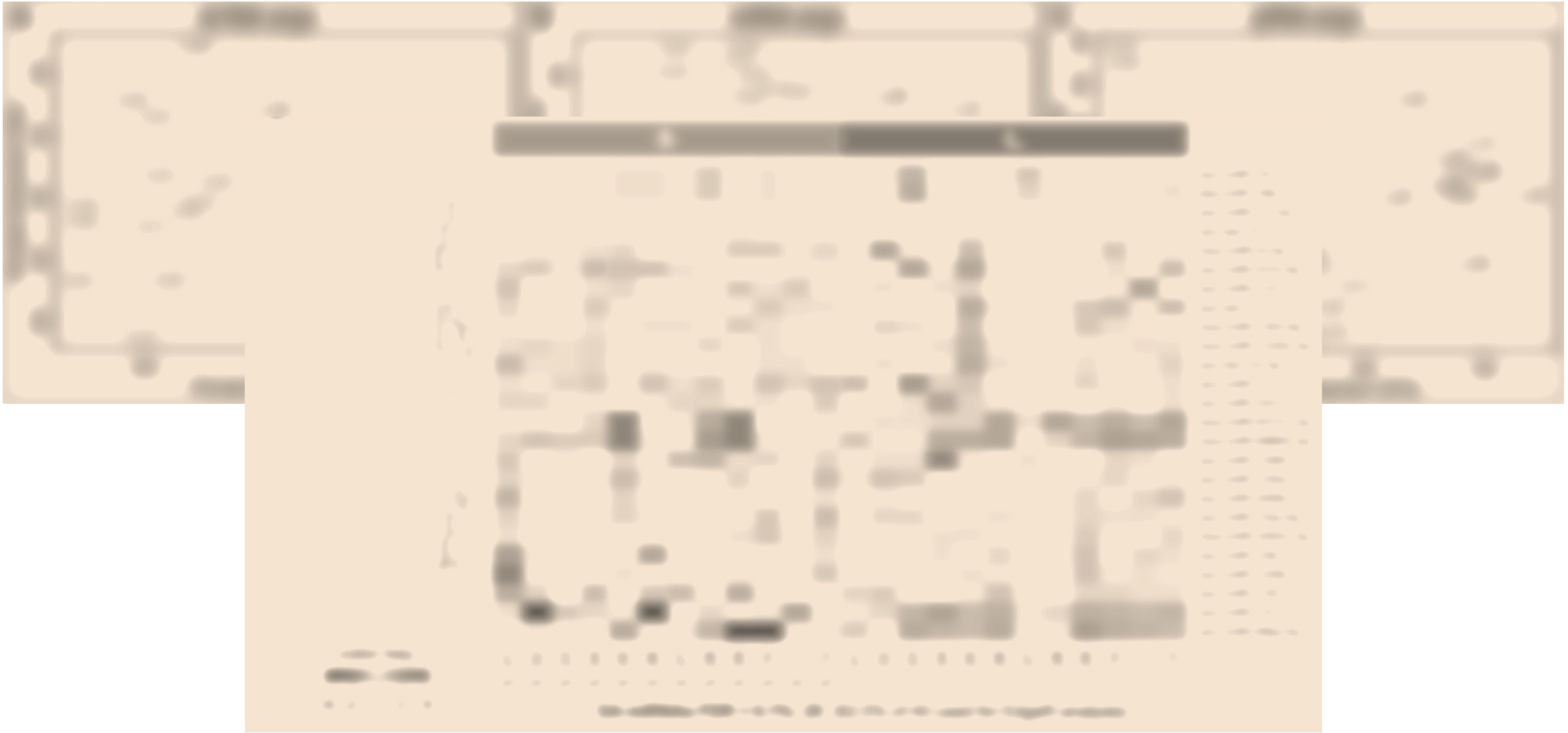


Analysis of RNA cargo of seminal plasma extracellular vesicles from boars with different fertility and farrowing rates

(Collaboration: Prof. Jordi Roca, University of Murcia, Spain)

- Analysis of subpopulations of EVs with different size range
- Hypothesis: EVs subpopulations have different functional roles

Differences in RNA cargo between L and S EVs



Prediction of specific functional impact of DA miRNAs

Research Station AgroVet-Strickhof

Thank you for your attention!!!