COLOcATION

Fetal maturity at the feto-maternal interface: COntribution of fetaL and maternal genOmes and tissue metAbolism perturbaTIONs

Sampling of tissue from pig fetuses

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1 Summary

COLOcATION aims to explore the feto-maternal crosstalk associated to pig fetal maturity, using an integrative omics approach (metabolome, lipidome and transcriptome) and by **simultaneously** questioning **the two adjacent tissues (placenta/endometrium)** that are players of feto-maternal interactions.

The project has taken advantage of the unique collection of placenta and endometrium samples already collected in the PORCINET project (ANR-09-GENM-005-01, 2010–2014). This genetic protocol producing pure and reciprocal crossed fetuses by insemination with mixed semen, using two extreme breeds: Large White and Meishan at 90 and 110 days of gestation (dg) (period of fetal maturity acquisition for pigs).

We describe here the procedures used to sample tissues.

2 Protocol description

2.1 Required reagents and instruments

- o Liquid nitrogen in a storage tank
- o 1 small styroform box (30 x 20 x 15) for temporary storage of liquid nitrogen
- o Cryoprotection gloves
- o box of dry ice
- o cutting board
- o Zip lock bags
- o Sterile clamps with smooth ends, 10cm long and 15cm long
- o Pairs of fine dissecting forceps
- o Scalpels and scissors
- o Latex gloves
- o A permanent marker to label the zip lock bags
- o Paper towels
- o Waste bag
- o Ethanol spray bottle
- o Mixer Mill MM400 (Retsch)

2.2 Preparatory step

The workplace was prepared by putting sterile aluminum foil, paper towel and 70% ethanol on the working bench. One styroform box for liquid nitrogen and one cutting board with dissecting material were placed on each workplace.

2.3 Animal dissection

All experiments on animals were performed under European Union legislation (directive 86/609/EEC) and French legislation of région Midi-Pyrénées in France (Décret n ◦:2001-464 29/05/01; http://ethique.ipbs.fr/sdv/charteexpeanimale.pdf; accreditation for animal housing number C-35-275-32). The technical and scientific staff obtained individual accreditation (Ref: MP/01/01/01/11) from the ethics committee (région Midi-Pyrénées - France; http://comethmp.ipbs.fr/) to experiment on living animals.

Pregnant sows were anesthetized and the fetuses were extracted by cesarean for physiological phenotyping. The placenta of each fetus was identified by the fetal number. The pregnant sows and fetuses were finally euthanized for tissue sampling and the uterus was extracted from the carcass. For each fetus a pre-determined area of the **two adjacent tissues (placenta/endometrium)** was dissected (Figure 1 from Gayrard V. ENVT course).

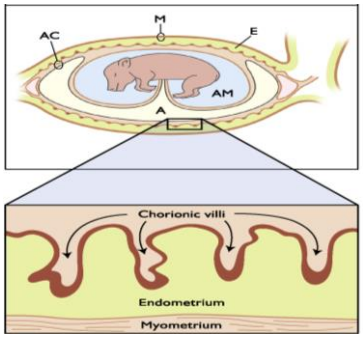

Figure 1

2.3 Tissue processing

The two tissues were dissociated with dissection forceps from each determined area and immediately snapfrozed into liquid nitrogen. Then the tissues were placed in a zip lock bag labelled with animal number and tissue name and stored temporarily on dry ice during the experiment. Between each tissue and between each animal, dissection forceps and scalpel were washed which 70% ethanol.

The tissues are then stored at -80°C at laboratory. Before extraction, the tissues were disrupted, homogenized and ground to a fine powder with the mixer-mill MM400 by rapid agitation for 1 minute at 30Hz in a liquid-nitrogen cooled container with stainless steel beads. Fine powder samples were conserved at -80°C until extraction (for RNAseq, metabolomics, and lipidomics).