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Data in Brief

# Transcriptome analysis of primary bovine extra-embryonic cultured cells

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ABSTRACT

The dataset described in this article pertains to the article by Hue et al. (2015) entitled "Primary bovine extra-embryonic cultured cells: A new resource for the study of in vivo peri-implanting phenotypes and mesoderm formation" [1]. In mammals, extra-embryonic tissues are essential to support not only embryo patterning but also embryo survival, especially in late implanting species. These tissues are composed of three cell types: trophoblast (bTCs), endoderm (bXECs) and mesoderm (bXMCs). Until now, it is unclear how these cells interact. In this study, we have established primary cell cultures of extra-embryonic tissues from bovine embryos collected at day-18 after artificial insemination. We used our homemade bovine 10K array (GPL7417) to analyze the gene expression profiles of these primary extra-embryonic cultured cells compared to the corresponding cells from in vivo microdissected embryos. Here, we described the experimental design, the isolation of bovine extra-embryonic cell types as well as the microarray expression analysis. The dataset has been deposited in Gene Expression Omnibus (GEO) (accession number GSE52967). Finally, these primary cell cultures were a powerful tool to start studying their cellular properties, and will further allow in vitro studies on cellular interactions among extra-embryonic tissues, and potentially between extra-embryonic vs embryonic tissues.

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| Specifications | |
|---|---|
| Organism/cell line/tissue | Primary extra-embryonic cells isolated from Day-18 bovine [*Bos taurus*] conceptuses |
| Sex | N/A |
| Sequencer or array type | Homemade bovine 10K array (GPL7417) |
| Data format | Normalized data (log-transformed and mean-centered) |
| Experimental factors | 3 extra-embryonic cell types : trophoblast (bTCs), endoderm (bXECs) and mesoderm (bXMCs) |
| Experimental features | Microarray gene expression profiling of 3 independent primary cultures of the 3 extra-embryonic cell types after 1 week of culture compared to in vivo microdissected cells |
| Consent | N/A |
| Sample source location | France |

## 1. Direct link to deposited data

http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE52967.

## 2. Experimental design, materials and methods

### 2.1. Embryo collection

Bovine conceptuses were collected at Day 18 post-insemination (or dpi; day of insemination as day 0) [2]. For each conceptus, the embryonic disc was dissected out and the extra-embryonic tissues stored in DMEM supplemented with 10% fetal calf serum (FCS), on ice, until tissue digestion. Animal use and care were performed in accordance with the International Guiding Principles for Biomedical Research involving Animals at the INRA experimental farm (registered under No. FRTB910 in the national registry) and the protocols for these studies were approved by the local Ethics Committee (Comité d'Ethique en Expérimentation Animale du Centre INRA de Jouy-en-Josas et AgroParisTech (or COMETHEA), registered as 12/084 and 12/086 in the National Ethics Committee registry.

### 2.2. Isolation of bovine extra-embryonic cell types: bTCs, bXECs, and bXMCs

For the microarray analyses, 3 independent isolation and culture procedures were performed, each starting from 10 to 12 D18 embryos. Each derivation included the three cell types which were grown for up

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to 1 week. bTCs, bXECs, and bXMCs were isolated as previously described for human trophoblast cells [3]. Briefly, the extra-embryonic tissues were digested with a sterile 0.05% (*w*/*v*) collagenase/trypsin solution (Gibco Life Sciences) at 37 °C. The digested tissues were centrifuged and the supernatant was discarded. The pelleted cells were resuspended in DMEM and filtered through a sterile 70-μm filter (BD Biosciences) prior to separation on a 55%–20% Percoll density gradient. All fractions were collected and cell viability was determined with Trypan blue dye (Invitrogen). bTCs were grown either on collagen IV- or Matrigel-coated culture dishes, whereas bXECs and bXMCs were grown directly on plastic culture dishes in DMEM (supplemented with 10% FCS, 2 mM glutamine, 100 IU/ml penicillin, and 100 μg/ml streptomycin). bTCs, bXECs, and bXMCs were scraped off the culture dishes, put into 1.5 ml tubes, and centrifuged, and the cell pellets were snap-frozen and stored at −80 °C until used.

### 2.3. Micro-dissection of the three in vivo extra-embryonic cell populations

To isolate the three extra-embryonic cell populations, frozen D18 bovine conceptuses were sectioned into 10-μm slices. From these, the trophoblast, extra-embryonic endoderm, and extra-embryonic mesoderm cells were micro-dissected, using the laser pressure catapulting technique adapted from a previous study [4].

### 2.4. Total RNA extraction and RNA amplification

Total RNA was isolated using the RNeasy Mini Kit with in-column DNase digestion (Qiagen) and T7 linear amplification was performed using the MessageAmp aRNA kit (Ambion) [5] after bias assessment [6]. Briefly, 1 μg of total RNA was incubated with 500 ng of an anchored T7-(dT) primer in 12 μl (water) at 70 °C for 10 min. The 1st cDNA strand was synthesized by the addition of 2 μl first-strand buffer, 1 μl RNAse inhibitor, 4 μl dNTP mix and 1 μl reverse transcriptase mix and incubation at 42 °C for 2 h. The second strand synthesis was performed by the addition of 63 μl DEPC-treated water, 10 μl second-strand buffer, 4 μl dNTP mix, 2 μl DNA polymerase, 1 μl RNAse H and incubation at 16 °C for 2 h. DNA was extracted with phenol:chloroform:isoamyl alcohol and precipitated in ethanol with 20 μg glycogen (Ambion). In vitro transcription was carried out at 37 °C for 10 h in a 20 μl reaction volume. 1 μl DNAse was added and incubated at 37 °C for 30 min. RNA was purified on Mini Quick Spin RNA columns (Roche Diagnostic) and its quality verified on RNA 6000 lab-chips (BioAnalyser 2100; Agilent Technologies).

### 2.5. Array hybridization, image acquisition and quantification

Array hybridization was also described in [5,7]. Briefly, 500 ng of amplified RNA (aRNA) was labeled with [$\alpha^{33}$P] dATP by reverse-transcription and hybridized to an INRA bovine 10K array (GPL7417) using ExpressHyb™ Hybridization Solution (Clontech) at 68 °C overnight. Arrays were washed four times in 2× SSC, 1% SDS and once in 0.1× SSC, 0.5% SDS at 68 °C for 30 min each. They were then exposed to phosphor-screens for 7 days. The hybridization signals were quantified with Imagene 5.5 software (BioDiscovery) on the PICT/ICE platform. These datasets are available in the Gene Expression Omnibus database (http://www.ncbi.nlm.nih.gov/geo, GSE52967). Data were log-transformed and mean-centered.

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