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Electronic Supplementary Information

# Changes in Lipid Profile and SOX-2 Expression in RM-1 Cells after Co-Culture with Preimplantation Embryos or with Blastocyst Deproteinated Extracts

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## Optimization Experiments

### Experiment 1 – Co-culture of RM-1 cells and 2-cell mouse preimplantation in DMEM/KSOM media in droplets under mineral oil

In Experiment 1, RM-1 cells were co-cultured with preimplantation embryos using a mixture of DMEM and KSOM culture media in 100µL droplets under mineral oil. The culture medium traditionally used for RM-1 cells is DMEM + 10% fetal calf serum (FCS). Our group's protocol for mouse embryo culture includes the use of KSOM medium droplets of 100µL under mineral oil to avoid evaporation over time.

For the co-culture preparation, RM-1 cells were removed from T75 flasks and distributed into 12 droplets of 100µL DMEM + 10% FCS under mineral oil in 60mm cell culture dishes (Falcon 353002, Corning Inc., Corning, NY). RM-1 confluence was estimated to be 30%. In parallel, 150 1-cell embryos were recovered from five C57BL/6 females, and these transferred to droplets of 100µL KSOM medium under mineral oil.

After 24h of incubation, the cells reached 50% confluence. A volume of 50µL of DMEM +10% FCS was then removed from the droplets and 100µL of KSOM medium was added, so that each droplet had a final volume of 150µL. Groups of 15 embryos were transferred to 6 droplets containing RM-1 cells (2-cell embryos; total of 90). Six droplets containing only RM-1 cells were left as controls.

After 24h of co-culture, when the exchange of 100µL of KSOM was planned, it was noticed that the mouse embryos were blocked at the 2-cell stage and RM-1 were detaching from the surface of the well, indicating that both embryos and cells were not adaptable to the combination of DMEM and KSOM culture media.

### Experiment 2 - Co-culture of RM-1 cells and 4 to 8-cell embryos in KSOM medium

Based on results from experiment 1, it was decided to allow the embryos to develop beyond the 2-cell stage before starting the co-culture, since the 2-cell stage is a critical period when mouse embryos activate nuclear DNA transcription and can be more sensitive to environmental conditions.

For that, ca. 150 1-cell embryos were recovered from 5 female mice and incubated in KSOM drops under mineral oil. RM-1 cells were plated at round 30% density in 12-well plates containing DMEN + 10% FCS medium. After 48 hours, the cells were at 50% confluence, and mice embryos were at the morula stage. The culture medium of the cells was replaced with KSOM and 120 morulae were split into 4 wells containing cells (30 morulae/well), starting the co-culture condition. After 2 days of co-culture, about 90% of the morulae developed to blastocysts and the RM-1 cells were attached to the bottom of the well and reached confluency.

Experiment 2 indicated that embryos could develop for two days in co-culture with RM-1 cells using just DMEN + 10% FCS.

For experiments 3 and 4, which are described in the text of the manuscript, we extended the co-culture to three days, starting with mouse 2-cell embryos instead of morulae. At the end of the incubation period, the embryos contained within their zona pellucida were removed and RM-1 cells were processed for SOX-2 gene expression and lipid profiling analysis.

**A**

SOX-2 *Primer 1* 5.0 5’-GTACAACTCCATGACCAGCTC-3’

**B**

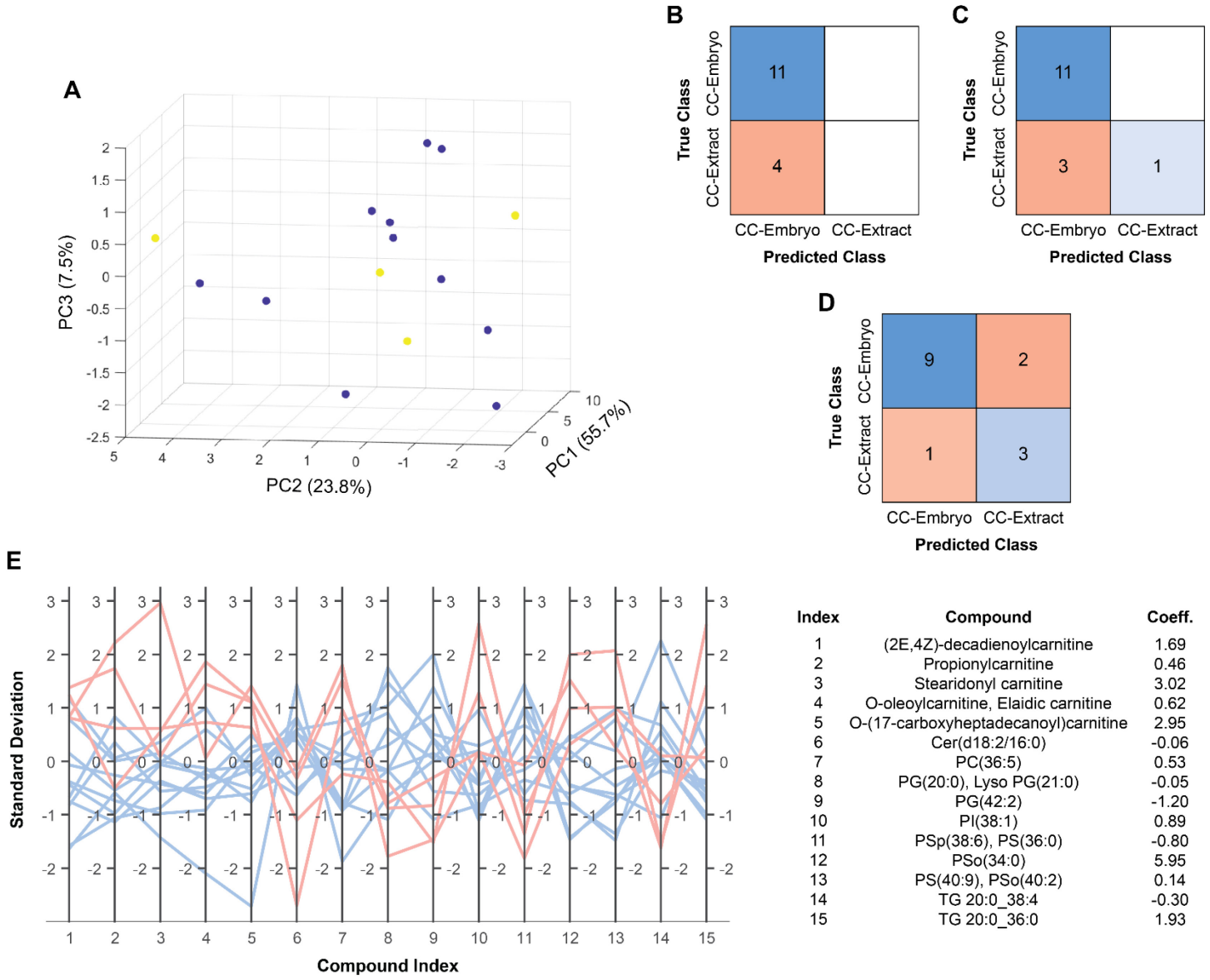
SOX-2 *Primer 2* 5.0 5’-CTTGACCACAGAGCCCAT-3’

**C**

X03205.1: 417-791 18S ribosomal RNA

CAGGGTTCGATTCCGGAGAGGGAGCCTGAGAAACGGCTACCACATCCAAGGAAG
GCAGCAGGCGCGCAAATTACCCACTCCCGACCCGGGGAGGTAGTGACGAAAAAT
AACAATACAGGACTCTTTCGAGGCCCTGTAATTGGAATGAGTCCACTTTAAATCCTT
TAACGAGGATCCATTGGAGGGCAAGTCTGGTGCCAGCAGCCGCGGTAATTCCAG
CTCCAATAGCGTATATTAAAGTTGCTGCAGTTAAAAAGCTCGTAGTTGGATCTTGGG
AGCGGGCGGGCGGTCCGCCGCGAGGCGAGCCACCGCCCGTCCCCGCCCCTTG
CCTCTCGGCGCCCCCTCGATGCTCTTAGCTGAGTGTCCCGCGGGGCC

**Figure S1**. (**A-B**) Primer sequences utilized for SOX-2 gene expression studies. Both forward (SOX-2 *primer 1*, **A**) and reverse (SOX-2 *primer 2*, **B**) primers are shown. (**C**) Amplicon context sequence of the commercial TaqMan Ribosomal RNA Control Reagent (product ID 4308329, Thermo Fisher) used for 18S gene expression. Endogenous 18S was used as housekeeping gene for normalization of SOX-2 response.




| Index | Compound | Coeff. |
|---|---|---|
| 1 | (2E,4Z)-decadienoylcarnitine | 1.69 |
| 2 | Propionylcarnitine | 0.46 |
| 3 | Stearidonyl carnitine | 3.02 |
| 4 | O-oleoylcarnitine, Elaidic carnitine | 0.62 |
| 5 | O-(17-carboxyheptadecanoyl)carnitine | 2.95 |
| 6 | Cer(d18:2/16:0) | -0.06 |
| 7 | PC(36:5) | 0.53 |
| 8 | PG(20:0), Lyso PG(21:0) | -0.05 |
| 9 | PG(42:2) | -1.20 |
| 10 | PI(38:1) | 0.89 |
| 11 | PSp(38:6), PS(36:0) | -0.80 |
| 12 | PSo(34:0) | 5.95 |
| 13 | PS(40:9), PSo(40:2) | 0.14 |
| 14 | TG 20:0_38:4 | -0.30 |
| 15 | TG 20:0_36:0 | 1.93 |

**Figure S2**. Comparison between the CC-Extract and CC-Embryo treatments. (**A**) Preliminary Principal Component Analysis (PCA) of the data associated with both treatment groups after Standard Normal Variate (SNV) normalization. Shown is a 3D plot of the three first principal components (PC), which together explain ca. 85% of the variance. No significant separation is observed in this reduced dimension between the two treatment groups. (**B-D**) Confusion matrixes obtained when attempting classification of the samples using an elastic net model after feature selection (following the same approach as described in the manuscript, **B**), unsupervised agglomerative clustering based on Euclidean distances (**C**), and several machine learning methods on the elastic net-selected features including k-nearest neighbors, decision trees and support-vector machines (**C** and **D**). (**E**) Table and parallel plot including the 15 features selected using the elastic net approach for the classification of the two treatment classes.