Tricarboxylic Acid Cycle Metabolites as Mediators of DNA Methylation Reprogramming in Bovine Preimplantation Embryos

Figure S1. (A) Total number of cells in fast (FBL) and slow (SBL) blastocysts; (B) Fluorescence intensity for 5-methylcytosine and 5-hydroxymethylcytosine of fast and slow blastocysts of cells from Trophoectoderm (TE) or inner cell mass (ICM). Fluorescence intensity for 5-methylcytosine of cells from the ICM or TE in blastocysts cultured with (C) dimethyl-succinate or (D) dimethyl-α-ketoglutarate. Statistical significance is identified by different letters.
Figure S2. Experimental design.
Table S1. Selected genes related to metabolism and epigenetic mechanisms from RNA-Seq analysis of bovine blastocysts (slow vs. fast). Genes in blue represent upregulation in slow blastocysts, genes in red represent upregulation in fast blastocysts.

| Gene  | log2FoldChange | p-value | p-Adj  |
|-------|----------------|---------|--------|
| PDHB  | -1.425         | 0.000   | 0.000  |
| MDH1  | -1.206         | 0.000   | 0.000  |
| APEX1 | -1.193         | 0.000   | 0.000  |
| OGDHL | -3.417         | 0.000   | 0.002  |
| PGK1  | -0.942         | 0.000   | 0.002  |
| GLS2  | 1.493          | 0.000   | 0.002  |
| AICDA | 1.171          | 0.001   | 0.005  |
| ACO2  | 0.693          | 0.002   | 0.011  |
| CS    | -0.660         | 0.002   | 0.011  |
| SLC25A1 | 1.181      | 0.007   | 0.032  |
| IDH3A | -0.728         | 0.008   | 0.035  |
| GSS   | 1.039          | 0.013   | 0.053  |
| TET3  | 0.662          | 0.026   | 0.093  |
| GLUD1 | -0.450         | 0.032   | 0.108  |
| SDHD  | -0.619         | 0.049   | 0.143  |
| FH    | -0.547         | 0.054   | 0.149  |
| OGDH  | 0.316          | 0.133   | 0.287  |
| ACO1  | -0.364         | 0.141   | 0.297  |
| SDHC  | -0.335         | 0.149   | 0.311  |
| LIG3  | 0.338          | 0.165   | 0.334  |
| SUCLG | -0.332         | 0.174   | 0.349  |
| SDHA  | 0.297          | 0.210   | 0.396  |
| SUCLA2| -0.324         | 0.248   | 0.439  |
| DNMT1 | 0.266          | 0.279   | 0.486  |
| IDH3B1| -0.269         | 0.296   | 0.503  |
| SDHB  | -0.213         | 0.339   | 0.544  |
| DNMT3B| 0.181          | 0.386   | 0.598  |
| APOBEC1| 0.629        | 0.386   | 0.598  |
| TDG   | 0.427          | 0.398   | 0.611  |
| IDH3G | 0.237          | 0.468   | 0.675  |
| NEIL2 | 0.509          | 0.572   | 0.720  |
| IDH2  | 0.298          | 0.571   | 0.720  |
| DNMT3L| 1.306          | 0.590   | 0.722  |
| GLS   | 0.120          | 0.706   | 0.821  |
| XRCC1 | 0.108          | 0.793   | 0.887  |
| TET1  | -0.028         | 0.879   | 0.919  |
| DNMT3A| 0.029          | 0.893   | 0.920  |
| MBD4  | -0.056         | 0.885   | 0.920  |
| PDHX  | 0.033          | 0.890   | 0.920  |
| SMUG1 | 0.053          | 0.936   | 0.954  |
| TET2  | -0.002         | 0.991   | 0.991  |
Table S2. Validation of RNA-Seq results by correlation with RT-qPCR analysis of genes related to epigenetic mechanisms, mitochondrial apoptosis, and embryonic pluripotency.

| Pathway                        | Gene                          | Gene ID               | TaqMan Code       | Uniprot B# | RT-qPCR FBL | RT-qPCR SBL | RT-qPCR P value | RNA-Seq FBL | RNA-Seq SBL | RNA-Seq P value | Relation |
|--------------------------------|-------------------------------|-----------------------|-------------------|-----------|-------------|-------------|----------------|-------------|-------------|----------------|----------|
| Epigenetic                     | DNA methyl-transferase 1      | DNMT1                 | Bt03224737_m1     | BT.108052 | 0.21 ± 0.02 | 0.20 ± 0.02 | 0.3693         | 10.46 ± 0.18 | 10.17 ± 0.13 | 0.258           | Equal    |
|                                | DNA methyl-transferase 3A     | DNMT3A                | Bt01027164_m1     | BT.64560  | 1.21 ± 0.26 | 0.86 ± 0.11 | 0.1438         | 13.26 ± 0.09 | 13.19 ± 0.20 | 0.806           | Equal    |
|                                | DNA methyl-transferase 3B     | DNMT3B                | Bt03259810_m1     | BT.22977  | 0.41 ± 0.04 | 0.50 ± 0.04 | 0.1032         | 12.87 ± 0.02 | 12.65 ± 0.18 | 0.321           | Equal    |
|                                | H1 histone member O           | H1FOO                 | Bt03228652_g1     | BT.36838  | 0.14 ± 0.02 | 0.12 ± 0.00 | 0.2472         | 6.48 ± 0.68  | 6.85 ± 1.06  | 0.329           | Equal    |
|                                | H3 Histone, Family 3A         | H3F3A                 | Bt03278804_g1     | BT.60099  | 0.21 ± 0.01 | 0.26 ± 0.01 | 0.0149         | 11.82 ± 0.13 | 13.28 ± 0.28 | 3.03         | Equal    |
|                                | H3 Histone, Family 3B         | H3F3B                 | Bt04319377_g1     | BT.15474  | 0.27 ± 0.01 | 0.26 ± 0.01 | 0.1404         | 4.79 ± 0.42  | 5.22 ± 0.87  | 0.471           | Equal    |
|                                | Heterochromatin protein 1     | HP1                   | Bt03246076_m1     | BT.22333  | 0.20 ± 0.01 | 0.21 ± 0.00 | 0.2995         | 12.20 ± 0.14 | 12.02 ± 0.12 | 0.401           | Equal    |
|                                | H2A.Z Variant Histone        | H2AFZ                 | Bt03216346_g1     | BT.2515   | 1.59 ± 0.33 | 1.32 ± 0.21 | 0.2700         | 11.29 ± 0.26 | 12.83 ± 0.40 | 3.44         | Different |
| Mitochondrial apoptosis        | Histone Deacetylase 2         | HDAC2                 | Bt03244871_m1     | BT.27729  | 0.23 ± 0.00 | 0.23 ± 0.00 | 0.4848         | 10.08 ± 0.21 | 10.57 ± 0.13 | 0.065           | Different |
|                                | Caspase 3                     | CASP3                 | Bt03250954_g1     | BT.10884  | 0.14 ± 0.01 | 0.15 ± 0.01 | 0.3283         | 9.45 ± 0.61  | 9.49 ± 0.17  | 0.802           | Equal    |
|                                | Caspase 9                     | CASP9                 | Bt04282453_m1     | BT.66332  | 0.19±0.00   | 0.18±0.00     | 0.0088         | 8.97 ± 0.22  | 7.90 ± 0.10  | 0.003           | Equal    |
|                                | BCL2 Associated X, Apoptosis  | BAX                   | Bt03211777_g1     | BT.109788 | 0.66 ± 0.01 | 0.56 ± 0.04 | 0.0272         | 8.68 ± 0.41  | 8.25 ± 0.35  | 0.273           | Different |
|                                | BH3 Interacting Domain Death  | BID                   | Bt03241255_m1     | BT.87470  | 0.15 ± 0.00 | 0.17 ± 0.01 | 0.0348         | 9.79 ± 0.17  | 8.96 ± 0.41  | 0.022           | Opposite  |
|                                | Embryonic pluripotency        | Nanog Homeobox        | NANOG             | Bt03220541_m1 | 0.15 ± 0.00 | 0.19 ± 0.00 | 0.0016         | 8.84 ± 0.33  | 9.64 ± 0.06  | 0.031           | Equal    |
|                                | Nanog Homeobox 1              | POUF5I                | Bt03223846_g1     | BT.92603  | 0.85 ± 0.08 | 0.57 ± 0.06 | 0.0173         | 11.87 ± 0.26 | 11.66 ± 0.28 | 0.444           | Different |
|                                | Nanog Homeobox 2              | SOX2                  | Bt03279318_s1     | BT.103364 | 0.25 ± 0.03 | 0.25 ± 0.00 | 0.4821         | 8.11 ± 0.19  | 7.31 ± 0.60  | 0.195           | Equal    |
|                                | Nanog Homeobox 3              | CDX2                  | Bt03469157_m1     | BT.46244  | 0.33 ± 0.01 | 0.27 ± 0.01 | 0.0100         | 10.30 ± 0.38 | 9.07 ± 0.48  | 0.001           | Equal    |

(1) The genes PPIA (Bt03224617_g1; Bt.43626), ACTB (PA5-16914; BT.14186), and GAPDH (Bt03210912_g1; Bt.87389) were also analyzed as reference genes and submitted to Normfinder evaluation. PPIA was used for data normalization; (2) From the 17 genes analyzed, 70.58% (12) presented the same pattern in both experiments, while 23.5% (4) were statistically different only in one of the analysis. Only one gene (5.9%) had the opposite pattern between the two techniques.