Supplementary data (Cell cycle analysis)

Control of Nuclear Remodelling and Subsequent In Vitro Development and Methylation

Status of Porcine Nuclear Transfer Embryos

Supplementary Method

Cell Cycle Analysis

Cell cycle phases of porcine foetal fibroblast cells were determined by measuring the DNA content of individual cells (1 \times 10^4 cells) using flow cytometry (EPICS XL-MCL, Beckman Coulter Inc., FL, USA) as described previously (Cheong et al. 2003). Briefly, serum-starved and trypsinised porcine foetal fibroblast cells were resuspended in DPBS and centrifuged at 500\times g for 5 min. Cell pellets were resuspended in 1 ml of 70% (v/v) ethanol (4°C), fixed overnight, and kept at –20°C until analysis. After ethanol fixation, cells were washed once with DPBS and incubated with 200 \mu g/ml RNase A (Boerenger Mannheim GmbH, Mannheim, Germany) at 37°C for 30 min. The cells were stained with 50 \mu g/ml propidium iodide (Sigma) at room temperature for 1 h. Stained cells were filtered through nylon mesh with 50-\mu m pores (Kyoshin Riko, Tokyo, Japan) just prior to flow cytometry. The distribution of cells in various phases of the cell cycle was determined using forward-scatter light. The subsequent calculation of percentages of cells in G0/G1, S, and G2/M was conducted using
Supplementary Figure 1. A typical DNA histogram obtained using flow cytometry of serum-starved porcine foetal fibroblast cells. Histograms allow for the discrimination of cell populations existing in the G0+G1, S, and G2+M phases of the cell cycle.
Supplementary Table 1. Cell cycle phase of porcine foetal fibroblast cells after serum starvation culture.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>G0+G1</th>
<th>S</th>
<th>G2+M</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum starvation</td>
<td>95.4 ± 3.2</td>
<td>0.9 ± 0.3</td>
<td>3.7 ± 2.1</td>
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</table>

Data are the mean ± SD of three replicates.

**Supplementary Reference**