

Supplementary Materials and Methods

RNA extraction from pig-Bovine iSCNT embryos

Briefly, samples were lysed in 100ml of lysis/binding buffer (Dyna. 100 mM Tris-HCl pH 7.5; 500 mM LiCl; 10 mM EDTA pH 8.0; 1% LiDS; 5mM DTT). Then, 1 pg of rabbit *alfa-GLOBIN* mRNA (Gibco BRL, Milan, Italy) per embryo equivalent was added to each tube as an internal standard. The rabbit *alfa-GLOBIN* mRNA is a mixture of the alpha- and beta-chains derived from poly-ribosomes of reticulocytes purified by oligo-dT cellulose chromatography. *alfa-GLOBIN* mRNA did not generate amplification products when the transcripts for the genes of choice were analyzed. After a brief centrifugation, samples were incubated at room temperature for 10 min. A total of 20 ml of pre-washed Dynabeads oligo d(T)₂₅ were then added to each samples. Binding of poly(A)⁺RNAs to oligo(dT) was allowed for 5 min at room temperature, then beads were separated from the binding buffer using the Dynal magnetic separator and washed twice with Buffer A (Dyna: 10 mM Tris-HCl pH 7.5; 0.15 mM LiCl; 1 mM EDTA; 0.1% LiDS) and three times with Buffer B (Dyna: 10 mM Tris-HCl pH 7.5; 0.15 mM LiCl; 1 mM EDTA). Poly(A)⁺ RNA was eluted from the beads by incubation in 8.5 ml of diethylpyrocarbonate-treated water at 65°C for 2 min.

FISH

Briefly, embryos without zona pellucida were washed in CSK buffer (100mM NaCl, 300mM sucrose, 10mM PIPES, pH 6.8, 3mM MgCl₂ and 1.2mM PMSF) and placed on a SuperFrost slide (VWR International, Leuven, Belgium). After a short incubation in 0.5% Triton X-100 in CSK with 10 mM VRC (vanadyl ribonucleoside complex) that destroyed and eliminated the cytoplasm, embryos were fixed in 4% paraformaldehyde in CSK, rinsed and stored in 70% ethanol. Before hybridisation, slides were washed and rehydrated in 2x SSC. Embryos were covered with hybridisation buffer - 25% formamide, 1 mg/ml E.coli tRNA (Roche, Prague, Czeck Republic), 0.005% BSA (Roche, Prague, Czeck Republic), 10% Dextran Sulfate (Qbiogene, MP Biomedicals) in 2x SSC (Saline sodium citrate buffer) - containing 1ng/μl of oligo dT probe, then covered with cover slide and incubated in a humidified chamber at 37°C in the dark overnight. Slides were then washed in 4x SSC, 2x SSC with 0.1% Triton X-100, and 2x SSC and mounted in Cytifluor with DAPI.