



Supplemental Figure S1. Relationship between the contact area and time of culture medium on a cover glass, Zn concentration in the culture medium, and developmental rate of mouse preimplantation embryos.

(A) Pronuclear-stage embryos were cultured for 4 days in 5- μ L medium with different contact areas to a cover glass. (B) Relationship between drop area of medium and blastocyst formation rate on day 4. Each dot represents the blastocyst formation rate of 12 embryos in a 5- μ L drop. The line and the number of dots indicate the logistic curve and replicates, respectively. The experiments were repeated four times independently. (C) Zn concentrations (the mean \pm SEM) in culture medium on glass-bottom dish (Company F) from day -1 (8 hours after the start of equilibration) to day 4. Ctrl, untreated medium before addition to the glass-bottom dish. Sample preparations for Zn analysis were independently replicated thrice using pooled culture media collected from two dishes at each time point. (D) Two-cell block rates and blastocyst formation rates in the medium on glass-bottom dishes or ZnCl₂-added medium (3 μ M and 6 μ M) on plastic dishes. Two-cell block, embryos at the two-cell stage until 48 h post-insemination; Ctrl, culture medium on plastic dish; glass bottom, same batch of dishes analyzed in (C). Experiments were independently replicated twice. *P*-values were calculated using two-tailed Fisher's exact tests and adjusted using the Holm procedure.