



Supplementary Figure 2. GV transplantation. Primary oocytes were released from ovaries by follicle puncture (Eppig & Telfer 1993) in medium M2 (Wood *et al.* 1987) modified to contain 1 mM glucose, 1 mM glutamine, 0.2 mM pyruvate, 0.01 mM EDTA, and 2 mg/mL BSA (Probumin 81-068, Millipore). This medium also contained 50 µg/mL (0.225 mM) of 3-isobutyl-1-methylxanthine (IBMX) (Sigma, I7018) to inhibit GVBD (Magnusson & Hillensjo 1977). On release from ovaries, residual granulosa cells on oocytes were removed by gentle repeated aspiration with a glass pipette of bore slightly less than the diameter of an oocyte. Oocytes were then cultured in medium CZB (Chatot *et al.*, 1989)—modified as described above for medium M2, and containing 50 µg/mL IBMX (2 h, 37°C, 5% CO₂ in air). This allowed for the development of a perivitelline space, required for slitting of the zona pellucida. GV transplantation was carried out in medium M2-modified containing 50 µg/mL IBMX and 1 µg/mL cytochalasin D (Sigma, C8273): **(A)** To slit the zona pellucida, the oocyte was held and a needle passed through the perivitelline space. The oocyte was released and the zona rubbed against the holding pipette until the oocyte fell off the pipette. **(B)** To enucleate, a bevelled transplantation pipette was pushed through the slit, then the GV, surrounded by a small amount of ooplasm and plasma membrane (karyoplast) was aspirated. **(C)** The pipette was retracted until the plasma membrane pinched off. The karyoplast was discarded or used for renucleation. **(D)** To renucleate, the karyoplast was moved to a drop of inactivated Sendai virus and a small amount aspirated in front of the karyoplast. **(E)** The virus followed by karyoplast were ejected under the zona of an enucleated oocyte. **(F)** The pipette was withdrawn. **(G)** Manipulated oocytes were washed free of IBMX and cytochalasin D for 5 min then cultured in medium CZB-modified (37°C, 5% CO₂ in air). **(H)** Fusion of the karyoplast occurred soon after. Shown are the same oocytes in (G) after culture (45 min, 37°C in 5% CO₂ in air)—for purpose of demonstration only, these particular oocytes were left in IBMX to ensure continued visibility of the GV.

Supplementary references

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