

GERMINAL VESICLE TRANSFER AS A RESCUE TOOL

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We examined whether germinal vesicle transfer (GVT) can help to produce matured oocytes derived from freeze-dried oocytes, and to improve maturation rate and developmental competence of growing oocytes. In order to explore the possibility of using GVT to support freeze-drying of pig oocytes, nuclear materials of freeze-dried oocytes were injected into enucleated fresh oocytes. In an attempt to reduce the size of GV to increase survival rate following GVT, oocytes were treated with resveratrol briefly after *in vitro* maturation (IVM). Although resveratrol did not reduce the size of GV ($p = 0.094$), it did significantly enhance the survival rates following GV transfer ($p < 0.001$). Following transfer of freeze-dried GVs into enucleated fresh oocytes, there were three out of 126 reconstructed oocytes reached the metaphase-II stage ($2.4 \pm 1.4\%$). Although this proportion was significantly lower ($P < 0.05$) than that of the IVM control group ($83.2 \pm 2.5\%$), it was comparable with the GVT control group ($7.4 \pm 2.7\%$). Oocytes in IVM control group were matured *in vitro* without any treatment or manipulation, whereas oocytes in GVT control were not introduced to freeze-drying but were exposed to GVT. In order to improve the maturation rate and developmental competence of growing oocytes collected from early antral follicles in pigs, we used two methods 1) fusion of a growing oocyte with the cytoplasm from a fully-grown oocyte (CFR group), and GVT (GVTR group). The maturation rate of GVTR oocytes was significantly improved ($18.8 \pm 3.5\%$) compared with that of growing oocytes (0.0%). The proportion of oocytes that reached the metaphase-II (M-II) stage in the CFR group ($37.8 \pm 2.0\%$) was significantly higher ($P < 0.05$) than that in the GVTR group, although still lower than that in the control group ($75.2 \pm 4.4\%$). No blastocyst was derived from growing oocytes. Among *in vitro* fertilized GVTR oocytes, $3.0 \pm 1.9\%$ developed into blastocysts; however, this percentage showed an insignificant increase compared with the GR group. On the other hand, the percentage of CFR embryos that developed into blastocysts ($12.0 \pm 4.3\%$) was significantly higher than that of GR embryos (0.0%), although still lower than that of control embryos ($27.0 \pm 5.5\%$). Total cell number in blastocysts in the GVTR group (23.3 ± 6.9) was significantly lower ($P < 0.05$) than that in the control group (50.4 ± 5.0). Meanwhile, the total cell number in blastocysts derived from CFR oocytes (36.3 ± 4.8) was comparable to that of the control group. In summary, the use of GVT helped to produce matured oocytes derived from freeze-dried GVs although with rather low rate, and significantly improve maturation rate and developmental competence of growing oocytes.