# Nuclear Replacement of In Vitro-Matured Porcine Oocytes by a Serial Centrifugation and Fusion Method

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## Abstract

The objective of the present study was to establish a method for nuclear replacement in metaphase-II (M-II) stage porcine oocytes. Karyoplasts containing M-II chromosomes (K) and cytoplasts without chromosomes (C) were produced from in vitro-matured oocytes by a serial centrifugation method. The oocytes were then reconstructed by fusion of one karyoplast with 1, 2, 3 or 4 cytoplasts (K + 1C, K + 2C, K + 3C and K + 4C, respectively). Reconstructed oocytes, karyoplasts without fusion of any cytoplast (K) and zona-free M-II oocytes (control) were used for experiments. The rates of female pronucleus formation after parthenogenetic activation in all groups of reconstructed oocytes (58.2–77.4%) were not different from those of the K and control groups (58.2% and 66.0%, respectively). In vitro fertilization was carried out to assay the fertilization ability and subsequent embryonic development of the reconstructed oocytes. The cytoplast : karyoplast ratio did not affect the fertilization status (penetration and male pronuclear formation rates) of the oocytes. A significantly high monospermy rate was found in K oocytes ($p < 0.05$, 61.6%) compared with the other groups (18.2–32.8%). Blastocyst formation rates increased significantly as the number of the cytoplasts fused with karyoplasts increased ($p < 0.05$, 0.0–15.3%). The blastocyst rate in the K + 4C group (15.3%) was comparable with that of the control (17.8%). Total cell numbers in both the K + 3C and K + 4C groups (16.0 and 15.3 cells, respectively) were comparable with that of the control (26.2 cells). Our results demonstrate that a serial centrifugation and fusion (Centri-Fusion) is an effective method for producing M-II chromosome transferred oocytes with normal fertilization ability and in vitro development. It is suggested that the number of cytoplasts fused with a karyoplast plays a critical role in embryonic development.