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Improvement of Cloned Embryos Development by Co-Culturing with Parthenotes using Microdrop Culture System

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Editorial

In our recent report "Improvement of cloned embryos development by co-culturing with parthenotes: a possible role of exosomes/ microvesicles for embryos paracrine communication" (Saadeldin et al. 2014), we showed that parthenogenetic (PA) embryos improved cloned (NT) embryos development in co-culture conditions in terms of cleavage and blastocyst formation. We used the transwell system for culturing big groups of embryos with embryo/medium density 1/10 µL. Herein, we show the co-culture of zona-free PA embryos with NT embryos in microdrops for supporting cloned embryos development with using few cloned embryos (Fig. 1). For zona removal, oocytes have been subjected to pronase 3% (w/v in PBS) for 90 seconds to digest the zona pellucida and washed 3 times with TALP. Normally, the zona-free PA blastomeres aggregate during the in vitro culture period (Figure 1).

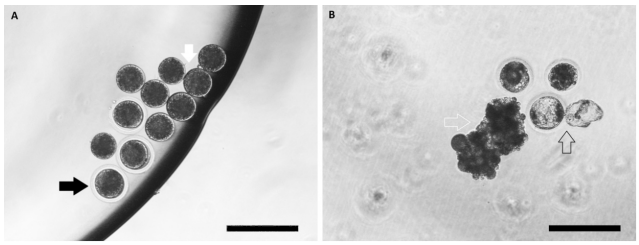

Figure 1: Co-culture of cloned embryos with zona-free parthenogenetic embryos. A. Cloned embryos (black arrow) are co-cultured with pronase-treated zona-free parthenogenetic embryos (white arrow) on day 0 of *in vitro* culture. B. Cloned blastocyst formation (black empty arrow) on day 6 of *in vitro* culture with PA aggregation (white empty arrow). Scale bar = 200 µm.

Three cloned embryos were cultured with 6-8 zona-free PA embryos in 15 µL microdrops of PZM5 medium overlaid with mineral oil at 39°C in a humidified atmosphere of 5 % CO_2, 5 % O_2 and 90 % N_2. For control group, 9-11 cloned embryos (zona intact) were cultured in the same conditions above. For more details about the parthenogenetic activation and nuclear transfer methods please see our previous report (Saadeldin et al. 2014). The experiment was repeated 7 times. The cleavage and blastocyst formation rates were compared using Pearson's chi-square test. In results, co-cultured NT embryos showed 95.23 % (n=20/21) cleavage vs. 75.71 % (n=53/70) in control group, $P < 0.05$. Blastocyst formation rate was 33.3 % (n=7/21) in co-cultured NT embryos vs. 12.85 % (n=9/70) in control groups, $P < 0.05$. These results confirm our previous report and we assume the same proposed mechanism of interaction between the embryos as we discussed in our report (Saadeldin et al. 2014). Blastomeres aggregation of IVF and/or PA embryos has been reported early for production of chimeric animals in some species (Pinyopummin et al. 1994; Suzuki 2001) however in this method we co-cultured different embryos without generation of chimera. This result will be helpful for improvement of porcine cloning efficiency when few embryos are available and will be a cost-effective method. Also, it might be beneficial when the in vivo matured oocytes derived from ovum pick-up (OPU) are used for cloning of elite/endangered animals, the abattoir-derived oocytes might be used as PA embryos feeders.

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