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Ooplasmic tranfer on the development of zona-free IVF bovine embryos

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Ooplasmic transfer (OT) has been mainly used to improve compromised oocytes and SCNT embryos development. The objective of this work was to evaluate the effect of the OT on development and quality of zona-free IVF embryos (ZF-IVF) and their individual blastomeres (IB) after disaggregation on day 1 post-insemination. COCs were aspirated from slaughterhouses ovaries and selected for standard IVM. A group of oocytes was subjected to IVF and presumptive zygotes were denuded and ZP removed. During gametes coincubation, a second group of matured oocytes was subjected to denudation and to ZP removal prior to enucleation. One (1+ group) or two (2+ group) ooplasms were fused to one presumptive zygote (2 pulses of 60V during 30 usec length and 100 msec interval). A ZF-IVF group (without ooplasm fusion) and a standard IVF (ZP-IVF) group were used as controls. For evaluation of IB development, cleaved embryos at day 1 post-insemination from ZF-IVF, 1+ and 2+ were subjected to hard pipetting for blastomeres disaggregation. ZF-zygotes and IB were cultured using the WOW system. Cleavage and cell numbers of ZF embryos were evaluated on Day 2. Blastocysts rates and total cell numbers were evaluated on Day 7 in all groups. Data were analyzed by Fisher´s test ($p<0.05$). The groups 1+ and 2+ showed a higher number of cells (>9 cells) on Day 2 (62/144; 43% and 49/138; 35.5%) than the ZF-IVF embryos (81/318; 25.5%, $p<0.05$). On the contrary, a higher proportion of cleaved ZF-IVF embryos showed 5 to 8 cells on Day 2 (149/318; 47%). The overall cleavage and blastocyst rates were significantly higher in the ZF-IVF (88% and 24%) and the 1+ (82% and 25%) groups than in the 2+ group (61% and 14%). The ZP-IVF group showed the highest blastocysts rates (131/343; 38%). Surprisingly, 1+ and 2+ groups showed blastocyst cell numbers (60.8±16.38 and 56.50±26.00, respectively) similar to the ZP-IVF group (58.26±6.65) and higher ($p<0.05$) than observed in the ZF-IVF control blastocysts (43.94±11.69). Interestingly, the highest percentage of blastocysts was obtained in groups showing an increased proportion of cells on Day 2: 62% of the blastocysts in 1+ group were obtained from embryos that were in 9 to 16-cell-stage, while 50% of the blastocysts of 2+ group had more than 16 cells-stage on Day 2. A total of 20 disaggregated embryos (49 IB) in the ZF-IVF group resulted in 24 blastocysts (120%); significantly higher than the other experimental groups (27% and 0% for the 1+ and 2+ group, respectively). Additional experiments are being carried out to identify the effects of OT in zygotes in terms of transcriptional pattern, pregnancy establishment and post-vitrification survival. In addition, we confirmed in all groups a positive correlation between more advanced stages of development at Day 2 and higher blastocysts rates. However, 1+ and 2+ reconstructed embryos did not improve blastomeres development. In conclusion, OT improved embryo development when 1 ooplasm (1+) was added, but 2 ooplasms transfer (2+) showed to be excessive and harmful to the embryo.