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Different media for selection of swine oocytes with Brilliant Cresyl Blue – effects on parthenogenetic embryo development

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Parthenogenetic activation is a crucial step for the establishment of other technologies such as nuclear transfer. However, regarding swine, more effective protocols need to be established to provide best oocyte selection and to increase rates of oocyte parthenogenesis. Considering this, the oocyte selection by the viability dye Brilliant Cresyl Blue (BCB) is a useful tool. The stained oocytes are considered the most suitable for IVM. Within this context, the aim of this study was to compare development rates by parthenogenesis of swine oocytes selected with 13 μ M of BCB, in a richer medium, called modified Porcine Zygote Medium (PZM-m) and in PBS solution, the most utilized medium. Prior to IVM 1,621 oocytes were incubated for 60 min at 39 °C in different media, with BCB. The oocytes were classified as positive (stained) or negative (no staining), except for group 1 which was washed in PFF and were not incubated, being considered the general control. The oocytes were distributed in the following groups: Porcine Fluid Follicular (PFF) (n=62); PBS-control (PBSc) (n=336); PZM-m control (PZMc) (n=371); PBS BCB positive (PBS+) (n=336); PBS BCB negative (PBS-) (n=161); PZM-m BCB positive (PZM+) (n=293); PZM-m BCB negative (PZM-) (n=90). IVM was performed in NCSU-23m with eCG, hCG, hypotaurine, β -mercaptoethanol, cysteine, EGF, AMP-c and PFF, in the first 24 hours, followed by NCSU-23 without eCG, hCG and AMP-c, for additional 24 hours. The oocytes were parthenogenetically activated with 20 μ M ionomycin for 5 minutes prepared in TCM Hepes medium and 2 mM 6-DMAP for 3 hours in PZM-3, the same medium for embryo culture. On day 4, 10 % of fetal calf serum was added to embryo culture. The results were analyzed by Chi-Square using the software Statistix 9.0. The cleavage rate in group PFF was 67.7% (n=42); 45.1% in PBSc (n=139); 52% in PZMc (n=193); 58.6% in PBS+ (n=197); 0% in PBS- (n=0); 55.3% in PZM+ (n=162); 23.3% in PZM- (n=21). The embryo rate on D7 (blastocyst and morulae) was 1.6% in group PFF (n=1); 14.9% in PBSc (n=46); 18.9% in PZMc (n=70); 8.6% in PBS+ (n=29); 0% in PBS- (n=0); 14.7% in PZM+ (n=43); 1.1% in PZM- (n=1). These results showed that cleavage rates in PZM+ were superior ($p<0.05$) to PBS+ and PBSc. However, this rate was not superior to PFF. The embryo results showed no difference between the groups PZMc, PZM+ and PBSc, which obtained the best rates. The groups PZM+ and PFF had the best cleavage rates. However, parthenogenetic activation did not show influence of the medium. At the same time, the PZM+ oocytes showed better embryo development than PBS+. Nevertheless, more research and experiments are underway to confirm these findings.