

## Brilliant cresyl blue staining and incubation media can affect the cortical granules migration of swine oocytes

○ Elisa Caroline da Silva SANTOS<sup>1,2</sup>, Elisângela Mirapalheta MADEIRA<sup>2</sup>, Bruna MION<sup>2,6</sup>, Mirianê Mendes PEREIRA<sup>5,7</sup>, Janaina Fadrique DA SILVA<sup>5,6</sup>, Jorgea PRADIEÉ<sup>4</sup>, Rafael Gianella MONDADORI<sup>2,3</sup>, Arnaldo Diniz VIEIRA<sup>2</sup>, Lígia Margareth Cantarelli PEGORARO<sup>5</sup>, Thomaz LUCIA Jr<sup>2</sup>

<sup>1</sup>Doctorate Student of Biotechnology, Universidade Federal de Pelotas, <sup>2</sup>Repropel, College of Veterinary, Universidade Federal de Pelotas, <sup>3</sup>Biology Institute, Universidade Federal de Pelotas, <sup>4</sup>Post Doctoral Student of INIA, <sup>5</sup>EMBRAPA Temperate Climate, <sup>6</sup>Graduation Student in Veterinary, Universidade Federal de Pelotas, <sup>7</sup>Student of Technician in Agropecuary, CAVG, Brazil

The polyspermy blockage failure is one of the major problems for swine IVP. The efficient block is dependent to adequate exocytosis of the cortical granules (CG) into the perivitelline space after fertilization. Wherever, this phenomenon is dependent to adequate oocyte IVM ability. Considering this, the oocyte selection by Brilliant Cresyl Blue (BCB) could be a useful tool. In this context, the objective of this research was to compare a new media produced from PZM (PZMm), with the most commonly used D-PBS for the BCB staining. Both media was used without BCB (controls) and with 13  $\mu\text{M}$  of BCB for oocyte incubation during 60min prior to IVM. After incubation period, the treated oocytes was classified as stained (+) or no staining (-), constituting the following groups: PBS control (PBSc; n=19); PZMm control (PZMmc; n=20); PBS+ (n=23); PBS- (n=21); PZMm+ (n=20) and PZMm- (n=20). IVM was performed in NCSU-23 (24 hours with AMP-c, PFF, eCG and hCG) and more 24 without hormones and AMP-c. After IVM all oocytes were fixed prior staining with FIT-LCA (10  $\mu\text{g}/\text{mL}$ ) and photographed under confocal microscopy. The CG distribution was estimated using the software ImageJ, i.e. Lower area of CG distribution in ooplasm, means higher migration to the periphery. The results were analyzed by Chi-Square. The CG ooplasm lower area ( $P < 0.05$ ) was observed in PZMmc (34.7 %  $\pm$  7.9). A higher CG area ( $P < 0.05$ ) was observed in PZMm- and PBSc (80.7 %  $\pm$  7.9 and 77.6 %  $\pm$  7.6, respectively), that was similar ( $P > 0.05$ ) to others groups. In conclusion, oocytes incubated with PZMm (without BCB) had better CG migration, indicating possibility to be a good maintenance media.